Supporting Online Material

Materials and Methods

Bacterial strains, plasmids, primers and growth conditions

The bacterial strains and plasmids used in this study are listed in Supplemental Table S4, and the primers used in this study are listed in Tables S5-S7. All *Salmonella enterica* serovar Typhimurium strains used in this study were derived from the wild-type strain 14028s. Bacteria were grown at 37°C in Luria-Bertani (LB) broth or in N-minimal media {Snavely, 1991 #475}; pH 7.7 supplemented with 0.1% Casamino Acids, 38 mM glycerol, and 50 μM or 10 mM $MgCl_2$. When necessary, antibiotics were added at the following final concentrations: ampicillin, 50 μg/ml; kanamycin, 25 μg/ml; and tetracycline, 10 μg/ml. *Escherichia coli* DH5α was used as a host for the preparation of plasmid DNA.

Chromatin immunoprecipitation experiments

We carried out these experiments as described {Shin, 2006 #487}.

***In vivo* expression assays**

We carried out these experiments as described {Shin, 2006 #487}.

DNaseI Footprinting assays

Primers A2, A4, B2, C2, D2, E2, F2, G2, H2, I2, I4, J2, K2, L2, M2 and N2 where labeled with 2 units of T4 polynucleotide kinase and 10 picomol of γ-^{32}P-ATP using 10 x polynucleotide kinase buffer in total volume of 25 μl at 37° C for 11 and 2 hrs and unincorporated ^{32}p was removed using ProbeQuant G-50 microcolumns (GE Healthcare). Then PCR fragments to be used as probes were generated by using *enterica* serovar Typhimurium chromosomal DNA as template for each pair of labeled and unlabeled primers A1 and A2, A3 and A4, B1 and B2, C1 and C2, D1 and D2, E1 and E2, F1 and F2, G1 and G2, H1 and H2, I1 and I2, I3 and I4, J1 and J2, K1 and K2, L1 and L2, M1 and M2 respectively. Probes generated from primers A3 and A4 and I3 and I4 were used with SlyA-H6 protein. Then DnaseI footprinting assays were carried out as described in (*42*) using different concentrations of PhoP-His6 and SlyA-His6 proteins for each gene and using 0.02 units of DNaseI (Epicentre).

SI nuclease-protection assay

SI nuclease-protection was performed as described (*43*) with RNA harvested from early exponential (OD600, 0.3-0.4) phase cultures of wild-type (14028s) and *phoP* (MS8953s) *Salmonella* grown in 30 ml of N-minimal medium, pH 7.4, containing either 10 mM $MgCl_2$ or 10 μM $MgCl_2$. Total RNA was isolated using SV Total RNA Isolation System (Promega, Madison, WI) according to manufacturer specifications. *Enterica* serovar Typhimurium chromosomal DNA was used as template. Probes used for *mig-14*, *pagK*,

yobG, *pagP*, *ybjX*, *slyB*, *pdgL*, *pagD*, and *virK* were the same probes used for DNaseI footprinting. Probes for *yrbL*, *ompX*, *pipD* and *mgtC* were generated by labeling C2, J4, K3, M4 and doing PCR as above using labeled and unlabeled primers C3 and C2, J3 and J4, K1 and K3 and M3 and M4, respectively.

Electrophoretic mobility shift assay (EMSA)

The DNA fragments used as probes for this assay were generated as follows: (1) PCR fragments were generated by using plasmid pMS201 as template and primer 9252 (pMS201-GFP-5′) 5′-CCAGTTTACTTTGCAGGGCTTC-3′ with each of following primers 9320, 9322, 9324, 9326, 9328, 9330, 9332, 9334, 9336, 9338, 9340, 9342, 9344, 9346, 9348, 9350, 9352, 9354, 9356, 9358, 9360, 9362, 9364, 9366, 9368, 9370, 9372, 9374, 9376, 9378. The DNA fragments were then gel-purified with QIAquick columns (Qiagen). (2) Second sets of PCR fragments were generated by using plasmid pMS201 as template and primer 7817 (pMS201-GFP-3′) 5′-GCCCATTAACATCACCATC -3′ with each of following primers 9319, 9321, 9323, 9325, 9327, 9329, 9331, 9333, 9335, 9337, 9339, 9341, 9343, 9345, 9347, 9349, 9351, 9353, 9355, 9357, 9359, 9361, 9363, 9365, 9367, 9369, 9371, 9373, 9375, 9377. The DNA fragments were then gel-purified with QIAquick columns (Qiagen). (3) The PCR fragments from steps I and II were then mixed and were used as templates for generating PCR fragments using primers 9252 5′-CCAGTTT ACTTTGCAGGGCTTC-3 and 7817 5′-GCCCATTAACATCACCA

TC -3′. The DNA fragments were then gel-purified with QIAquick columns (Qiagen). (4) 150 ng of DNAs from step III were then labeled with 2 units of T4 polynucleotide kinase and 10 pmol. of γ-^{32}P-ATP and then unincorporated ^{32}p was removed using ProbeQuant G-50 microcolumns (GE Healthcare). Phosphorylation of PhoP-His6 with acetyl phosphate: 1.2 nmol of PhoP-His6 protein was incubated at room temperature for 2.5 hrs. in 50 μl of a buffer consisting of 10 μl of 5 x phosphorylation buffer (250 mM Tris pH 7.5, 250 mM KCl, 100 mM $MgCl_2$) and 5 μl of 100 mM acetyl phosphate (Sigma) and nuclease-free water. Phosphorylated protein was then cleaned up with pre-equilibrated (2 x 500 μl of 50 ml Tris pH 8.0) Micro Bio-Spin 6 (BIO RAD). The gel mobility shift assays were carried out as described in (*28*) with freshly made phosphorylated proteins and samples were then electophoresed on 4%-20% TBE gels Invitrogen) on 0.38 x TBE running buffer at V=100 for 3 hrs.

Estimation of the promoter occupancy of multiple binding sites from apparent affinities

Let *R* be the regulator, *P* the promoter with two *R*-binding sites, and assume that the binding to the two sites are independent, where k_i = dissociation constant for *R* binding to site *i*.

By definition, at equilibrium (omitting brackets):

$$k_1 = \frac{R_f P_{00}}{P_{10}} = \frac{R_f P_{01}}{P_{11}} \qquad (1)$$

$$k_2 = \frac{R_f P_{00}}{P_{01}} = \frac{R_f P_{10}}{P_{11}} \qquad (2)$$

where R_f = concentration of free R.

From (1), $$P_{10} = \frac{R_f P_{00}}{k_1} \qquad (3)$$

and $$\frac{P_{00}}{P_{10}} = \frac{P_{01}}{P_{11}} \qquad (4)$$

From (2), $$P_{01} = \frac{R_f P_{00}}{k_2} \qquad (5)$$

Parenthetically, from Eqn 3 and 5: $\frac{P_{01}}{P_{10}} = \frac{k_1}{k_2}$ as shown before.

From (3-5), $$P_{11} = \frac{P_{10} P_{01}}{P_{00}} = \frac{R_f^2 P_{00}}{k_1 k_2} \qquad (6)$$

Let the concentration of the promoter be

$$P = P_{00} + P_{01} + P_{10} + P_{11}$$

Substituting in (3), (5), and (6)

$$P = P_{00} + \frac{R_f P_{00}}{k_2} + \frac{R_f P_{00}}{k_1} + \frac{R_f^2 P_{00}}{k_1 k_2}$$

simplifying, $$k_1 k_2 P = P_{00}(k_1 k_2 + R_f k_1 + R_f k_2 + R_f^2) = P_{00}(k_1 + R_f)(k_2 + R_f)$$

or $$P_{00} = \frac{k_1 k_2 P}{(k_1 + R_f)(k_2 + R_f)} = k_1 k_2 PA$$

further, $$P_{01} = \frac{R_f}{k_2} \frac{k_1 k_2 P}{(k_1 + R_f)(k_2 + R_f)} = \frac{k_1 R_f P}{(k_1 + R_f)(k_2 + R_f)} = k_1 R_f PA$$

$$P_{10} = \frac{R_f}{k_1} \frac{k_1 k_2 P}{(k_1 + R_f)(k_2 + R_f)} = \frac{k_2 R_f P}{(k_1 + R_f)(k_2 + R_f)} = k_2 R_f PA$$

$$P_{11} = \frac{{R_f}^2}{k_1 k_2} \frac{k_1 k_2 P}{(k_1 + R_f)(k_2 + R_f)} = \frac{{R_f}^2 P}{(k_1 + R_f)(k_2 + R_f)} = {R_f}^2 PA$$

where $$A = \frac{1}{(k_1 + R_f)(k_2 + R_f)}$$

Thus, the relative concentrations of the 4 states (or, the relative promoter occupancies):

$$P_{00} : P_{01} : P_{10} : P_{11} = k_1 k_2 : k_1 R_f : k_2 R_f : {R_f}^2$$

If R_f is approximated by R, i.e., when there are many more regulator than promoter molecules, so that the amount of R that is bound to the promoters ($P_{01} + P_{10} + 2P_{11}$) can be ignored. $Rf \sim R = 0.9$ μM at the peak of the PhoP-P surge, assuming 10% of PhoP is phosphorylated. Occupancy describes the fractional occupancies of the individual binding sites with respect to P.

Supplemental Figure Legends

Figure S1. Correlations between the order and level of promoter occupancy by PhoP-P and the product of PhoP-activated mRNAs

(A-D) Scatter plot between the rank orders of genes derived from clustering the mRNA levels and time at which they are produced, and the promoter occupancy of the corresponding genes by PhoP-P (Fig. 1). The dashed green lines describe the linear correlation among genes. Green and red boxes designate good and bad correlations. (A) Linear correlation between the levels and the time of promoter binding by the PhoP-P protein. (B) Linear correlation between promoter binding time by the PhoP-P protein and transcription time of the corresponding genes. (C) Absence of a linear correlation between the time and the levels with which PhoP-activated mRNAs are produced. (D) Absence of a linear correlation between promoter binding levels and mRNA levels for PhoP-activated genes.

Figure S2. *In vitro* binding of PhoP-P to PhoP boxes.

EMSA assays were carried out as described in Experimental procedures with purified PhoP-His6-P protein and DNA fragments harboring the 19 nucleotides corresponding to the PhoP box flanked by the same sequences for all PhoP boxes. Sequences corresponding to each PhoP box, and the affinity constants are described in Table S2. X

axis constitutes the amount of PhoP-P (pmoles). The affinity at 50% of occupancy (K_d) was derived from polynomial fit (sixth order).

Figure S3. Transcription time in early PhoP-activated genes is determined by the affinity of the PhoP protein for its target promoters

Normalized mRNA levels of the *phoP* gene (blue line) or *gfp* gene (red line) in strain EG19625 (harboring the *phoP4* derivative of the early *phoP* promoter driving transcription of a promoterless *gfp* gene for the *att*Tn*7*). Normalized mRNA levels of the *phoP* gene (green line) or *gfp* gene (cyan line) in strain EG19627 (harboring a *phoP4* promoter derivative where the PhoP box was replaced by a PhoP box from the late *pagK* promoter). The mRNA timing for *gfp* in strain EG19625 is the same as that of the *phoP* gene. The timing for the mRNA for *gfp* in strain EG19627 is delayed with respect to the timing of the *phoP* mRNA.

Figure S4. Correlations between the promoter occupancy based on *in vitro* affinities and the *in vivo* times and levels of binding and mRNAs of PhoP-activated promoters

(A) Linear correlation (central dashed line) between the occupancy of the maximum affinity PhoP box by the PhoP-P protein in a promoter and its temporal rank order considered in Fig. S1. Square colors reflect the time range for achieving the 50% of binding occupancy. Promoters with lower correlation diverge from the central

correlation line (*e.g.*, the *virK* promoter). (B) Linear correlation (central dashed line) between the total binding occupancy of the PhoP boxes by the PhoP-P protein in a promoter and its ranked levels considered in Fig. S1. Square colors reflect the range of binding levels. Promoters with lower correlation diverge from the central correlation line (*e.g.*, the *pcgL* promoter).

Figure S5. H-NS and SlyA regulation of PhoP-activated promoters.

(A) mRNA levels of PhoP-activated genes harvested from isogenic *rpoS* (EG14749), *hns rpoS* (EG17828), and *hns rpoS phoP* (EG18482) strains grown in LB broth at OD600 = 0.6. mRNA levels were determined by quantitative real time PCR and normalized to those corresponding to the PhoP-independent *rrs* gene. (B) mRNA levels of PhoP-activated genes harvested from isogenic PhoP-HA (EG13918), EG13918 *slyA*+ (EG14078) and EG13918 *slyA* (EG17254) strains grown in N-minimal medium containing 10, 50, and 200μM$MgCl_2$. mRNA levels were determined by quantitative real time PCR and normalized to those corresponding to the PhoP-independent *rrs* gene.

SUPPLEMENTAL TABLES

Table S1. Functional and kinetic characterization of 20 PhoP regulated genes. (Binding and mRNA rise time correspond to the time at the 50% of binding and mRNA promoter behavior, respectively (Figure 1)).

Gene Name	Function of Product	Binding Occupancy				mRNA			
		Rise Time	Label	Level	Label	Rise Time	Label	Level	Label
yrbL	Putative cytoplasmic protein	-4	early	37.63	high	-5	early	0.76	low
phoP	Response regulator	0	early	26.05	high	7	early	31.38	high
mgtA	Mg2+ transporter	-1.5	early	49.08	high	1.5	early	4.51	low
slyB	Outer membrane lipoprotein	-1.5	early	17.70	high	6	early	39.99	high
pmrD	Connector of two-component systems	3	early	26.88	high	9	early	3.87	low
rstA	Response regulator	-2	early	47.84	high	7.5	early	5.88	low
ompX	Outer membrane protein	-0.5	early	32.53	high	9	early	24.45	high
ybjX		-3	early	87.89	high	-0.5	early	16.97	high
pdgL	D-ala-D-ala dipeptidase	-1.5	early	2.78	low	10	early	4.6	low
yobG	Putative inner membrane protein	0.5	early	9.75	low	6	early	2.62	low
pagP		0.5	early	18.62	low	11.5	early	5.44	low
pagD		8.5	late	8.80	low	18.5	early	2.4	low
virK		2	late	62.72	high	14	late	32.56	high
mig-14	Putative transcription activator	5	late	51.74	high	19	late	24.57	high
ugtL	Inner membrane protein that modifies the LPS	3	late	12.51	low	10	late	6.47	low
pagK		2	late	10.25	low	15.5	late	5.14	low
pgtE	Outer membrane protease	-5	late	1.11	low	12	late	1.07	low
mgtC	Inner membrane virulence protein that aids grouth in low Mg2+	1	late	9.48	low	13.5	late	0.68	low
pipD		1	late	6.27	low	17.5	late	0.79	low
pagC	Outer membrane protein	8.5	late	8.80	low	14.5	late	4.75	low

Table S2. Promoter dataset corresponding to 20 PhoP regulated promoters. ("Occupancy" describes the fractional occupancies of the PhoP box(es))

Gene Name	Sequence_PhoP-box	Affinity (Molar)	Occupancy				
			P00	P10	P01	P11	Total
yrbL	TCGTTTAGGTTTTGTTTAA	7.33E-06					0.109
phoP	TGGTTTATTAACTGTTTAT	8.67E-06					0.094
mgtA	TGGTTTATCGTTGGTTTAA	1.27E-05					0.066
slyB	TCGTTTAAGATTGGTTAAT	1.27E-05					0.066
pmrD	CTATTGCCGTTTTGTTTAT	1.40E-05					0.060
rstA	TCGTTTAGAAAAGATTTAT	1.20E-05					0.070
ompX	CGGTTGAGGGTTCGTTGAA	6.67E-06					0.119
ybjX	GTATTGACGATTGGTTAAT	4.00E-05	0.913	0.065	0.021	0.001	0.087
	TTGTTTAGATACGGTTTAC	1.27E-05					
pdgL	ATTTTAACCATCTGTTTAA	1.47E-05	0.907	0.035	0.056	0.002	0.093
	GAGTTTATATTTTGCTTAT	2.33E-05					
yobG	ACAGTTACTCCTGGTTTAA	2.20E-05	0.945	0.016	0.039	0.001	0.055
	GTTTTTAGGAATGATTCAC	5.40E-05					
pagP	CTGTTTATAGTTTGTTAAG	1.87E-05	0.937	0.017	0.045	0.001	0.063
	TTTGTGAAAGCTTATTAAG	4.87E-05					
pagD	TGGTTAACTCTTCGTTGAA	5.47E-05	0.953	0.031	0.016	0.001	0.047
	GTGTTTAGAGAGAATTTAC	2.80E-05					
virK	TCGTTGCCTTTACGTTTAA	1.33E-05	0.825	0.111	0.056	0.008	0.175
	CCATTGATAAACTGTTTAA	6.67E-06					
mig-14	ACATTTTTATTTGGTTAAG	1.67E-05	0.919	0.030	0.050	0.002	0.081
	ATGTTTAGCTTGTATTTAA	2.73E-05					
ugtL	CGGTTGAGCAACTATTTAC	1.67E-05	0.924	0.024	0.050	0.001	0.076
	AATAATACTTTTAGTTTAA	3.40E-05					
pagK	CCATTTATAAAATATTTAA	4.40E-05	0.930	0.050	0.019	0.001	0.060
	ACGTTTAATATCTATAGTA	1.67E-05					
pgtE	ATTTTTACCTTATATTGAA	1.27E-05	0.91	0.018	0.058	0.001	0.075
	ATGATTATAGATTGCTTAT	3.07E-05					
mgtC	CTGTTTAAGTTTGTTTGAT	1.33E-05	0.917	0.019	0.061	0.001	0.060
	ATGTTTAAACACGCTTTAT	4.20E-05					
	ATGTTTCCTTATATTTTAA	3.53E-05					
pipD	TTATTGAGGTTGTATTGAT	2.00E-05	0.943	0.014	0.042	0.001	0.043
	CCGTTACGCTGCTGGGTAT	5.93E-05					
pagC	GTGTTTAGAGAGAATTTAC	2.60E-05	0.911	0.056	0.032	0.002	0.054
	TTATTTACGGTGTGTTTAA	1.47E-05					

Table S4. Bacterial strains used in this study.

Strain	Description	Reference
S. enterica		
14028s	Wild-type	ATCC
EG13918	*phoP*-HA	(*1*)
TJH18	Wild-type Δ*rpoS* Δ*slyA*::Cm	This work
TJH19	Wild-type Δ*rpoS* Δ*hns* Δ*slyA*::Cm	This work
EG17828	Wild-type Δ*rpoS*::Cm Δ*hns*::Kn	(*2*)
EG14749	Δ*rpoS*::Cm	This work
EG18482	Δ*rpoS phoP*::Tn10 Δ*hns*::Km	This work
EG14078	Δ*slyA*::Cm	This work
EG17254	PhoP-HA Δ*slyA*::Cm	This work
EG19625	PhoP-HA attTn7::*phoP4*	This work
EG19627	PhoP-HA attTn7::*phoP4-pagK*	This work
MS7953s	*phoP*7953::Tn10	This work

Table S5. Promoter-specific primers used in Gel Shift assay

Number	Name	Sequence
9319	mgtA_s33_1	5' TGGTTTATCGTTGGTTTAAggatcctctagatttaagaa 3'
9320	mgtA_s33_2	5' TTAAACCAACGATAAACCAcctcgaggtgaagacgaaag 3'
9321	mgtC_s34_3	5' ATGTTTAAACACGCTTTATggatcctctagatttaagaa 3'
9322	mgtC_s34_4	5' ATAAAGCGTGTTTAAACATcctcgaggtgaagacgaaag 3'
9323	mgtC_s35_5	5' ATCAAACAAACTTAAACAGggatcctctagatttaagaa 3'
9324	mgtC_s35_6	5' CTGTTTAAGTTTGTTTGATcctcgaggtgaagacgaaag 3'
9325	mgtC_s36_7	5' ATGTTTCCTTATATTTTAAggatcctctagatttaagaa 3'
9326	mgtC_s36_8	5' TTAAAATATAAGGAAACATcctcgaggtgaagacgaaag 3'
9327	mgtC_s37_9	5' TTAAACAGAACGTCACTAAggatcctctagatttaagaa 3'
9328	mgtC_s37_10	5' TTAGTGACGTTCTGTTTAAcctcgaggtgaagacgaaag 3'
9329	mig-14_s38_11	5' TTAAATACAAGCTAAACATggatcctctagatttaagaa 3'
9330	mig-14_s38_12	5' ATGTTTAGCTTGTATTTAAcctcgaggtgaagacgaaag 3'
9331	ompX_s2_13	5' CGGTTGAGGGTTCGTTGAAggatcctctagatttaagaa 3'
9332	ompX_s2_14	5' TTCAACGAACCCTCAACCGcctcgaggtgaagacgaaag 3'
9333	pagC_s3_15	5' TTAAACACACCGTAAATAAggatcctctagatttaagaa 3'
9334	pagC_s3_16	5' TTATTTACGGTGTGTTTAAcctcgaggtgaagacgaaag 3'
9335	pagC_s4_17	5' GTAAATTCTCTCTAAACACggatcctctagatttaagaa 3'
9336	pagC_s4_18	5' GTGTTTAGAGAGAATTTACcctcgaggtgaagacgaaag 3'
9337	pagD_s5_19	5' GTGTTTAGAGAGAATTTACggatcctctagatttaagaa 3'
9338	pagD_s5_20	5' GTAAATTCTCTCTAAACACcctcgaggtgaagacgaaag 3'
9339	pagP_s6_21	5' CTGTTTATAGTTTGTTAAGggatcctctagatttaagaa 3'
9340	pagP_s6_22	5' CTTAACAAACTATAAACAGcctcgaggtgaagacgaaag 3'
9341	pgtE_s7_23	5' ATGATTATAGATTGCTTATggatcctctagatttaagaa 3'
9342	pgtE_s7_24	5' ATAAGCAATCTATAATCATcctcgaggtgaagacgaaag 3'
9343	phoP_s8_25	5' TGGTTTATTAACTGTTTATggatcctctagatttaagaa 3'
9344	phoP_s8_26	5' ATAAACAGTTAATAAACCAcctcgaggtgaagacgaaag 3'
9345	pipD_s9_27	5' ATCAATACAACCTCAATAAggatcctctagatttaagaa 3'
9346	pipD_s9_28	5' TTATTGAGGTTGTATTGATcctcgaggtgaagacgaaag 3'
9347	pmrD_s10_29	5' CTATTGCCGTTTTGTTTATggatcctctagatttaagaa 3'
9348	pmrD_s10_30	5' ATAAACAAAACGGCAATAGcctcgaggtgaagacgaaag 3'
9349	rstA_s11_31	5' TCGTTTAGAAAAGATTTATggatcctctagatttaagaa 3'
9350	rstA_s11_32	5' ATAAATCTTTTCTAAACGAcctcgaggtgaagacgaaag 3'
9351	slyB_s12_33	5' TCGTTTAAGATTGGTTAATggatcctctagatttaagaa 3'
9352	slyB_s12_34	5' ATTAACCAATCTTAAACGAcctcgaggtgaagacgaaag 3'
9353	ugtL_s14_35	5' GTAAATAGTTGCTCAACCGggatcctctagatttaagaa 3'
9354	ugtL_s14_36	5' CGGTTGAGCAACTATTTACcctcgaggtgaagacgaaag 3'
9355	virK_s15_37	5' CCATTGATAAACTGTTTAAggatcctctagatttaagaa 3'
9356	virK_s15_38	5' TTAAACAGTTTATCAATGGcctcgaggtgaagacgaaag 3'
9357	virK_s16_39	5' ATAAACTGTTTAACAACATggatcctctagatttaagaa 3'
9358	virK_s16_40	5' ATGTTGTTAAACAGTTTATcctcgaggtgaagacgaaag 3'
9359	virK_s17_41	5' TCGTTGCCTTTACGTTTAAggatcctctagatttaagaa 3'
9360	virK_s17_42	5' TTAAACGTAAAGGCAACGAcctcgaggtgaagacgaaag 3'
9361	ybjX_s18_43	5' GTAAACCGTATCTAAACAAggatcctctagatttaagaa 3'
9362	ybjX_s18_44	5' TTGTTTAGATACGGTTTACcctcgaggtgaagacgaaag 3'
9363	ybjX_s19_45	5' TTAACCAGAAAGTAAACCGggatcctctagatttaagaa 3'

Number	Name	Sequence
9364	ybjX_s19_46	5' CGGTTTACTTTCTGGTTAAcctcgaggtgaagacgaaag 3'
9365	ybjX_s20_47	5' GTATTGACGATTGGTTAATggatcctctagatttaagaa 3'
9366	ybjX_s20_48	5' ATTAACCAATCGTCAATACcctcgaggtgaagacgaaag 3'
9367	yobG_s21_49	5' ACAGTTACTCCTGGTTTAAggatcctctagatttaagaa 3'
9368	yobG_s21_50	5' TTAAACCAGGAGTAACTGTcctcgaggtgaagacgaaag 3'
9369	yrbL_s22_51	5' TCGTTTAGGTTTTGTTTAAggatcctctagatttaagaa 3'
9370	yrbL_s22_52	5' TTAAACAAAACCTAAACGAcctcgaggtgaagacgaaag 3'
9371	pagK_s26_53	5' TTAAATATTTTATAAATGGggatcctctagatttaagaa 3'
9372	pagK_s26_54	5' CCATTTATAAAATATTTAAcctcgaggtgaagacgaaag 3'
9373	pdgL_s27_55	5' ATTTTAACCATCTGTTTAAggatcctctagatttaagaa 3'
9374	pdgL_s27_56	5' TTAAACAGATGGTTAAAATcctcgaggtgaagacgaaag 3'
9375	ugtl_50_s28_57	5' TTAAACTAAAAGTATTATTggatcctctagatttaagaa 3'
9376	ugtl_50_s28_58	5' AATAATACTTTTAGTTTAAcctcgaggtgaagacgaaag 3'
9377	izwI_1_30_59	5' GCTTAATATTAACTTAATAggatcctctagatttaagaa 3'
9378	izwI_1_30_60	5' TATTAAGTTAATATTAAGCcctcgaggtgaagacgaaag 3'

Table S6. Promoter-specific primers used in ChIP assay

Number	Name	Sequence
5852	mgtA	forward 5′ GTGAGCCGGTTTTGCATC 3'
5853	mgtA	reverse 5′ CTCCGGTAAGTAAATAATTTGCG 3'
3564	mgtC	forward 5′ CAAACAAACTTAAACAGAACGTCACTAA 3'
3565	mgtC	reverse 5′ TTGCGCTTATTATAGTCAGCCAAT 3'
6805	mig-14	forward 5′ CCTTCACCATAACGTCATCGATTA 3'
6806	mig-14	reverse 5′ GCTATTTTGTATTATTTTTTTGCTTAACCAA 3'
7142	ompX	forward 5′ GTAAAGGTGGCAGTGTAATGGAAA 3'
7143	ompX	reverse 5′ TTTATGGAATCCTGGAAATTTTTTTT 3'
5865	pagC-D	forward 5′ CCACTCTTAATAATAATGGGTTTTA 3'
5866	pagC-D	reverse 5′ TATAAGAATAGAATCAACACCACA 3'
7197	pagK	forward 5′ TTGTGGCACACTATCCTTACGG 3'
7198	pagK	reverse 5′ TACTCACCTGATGGTAATGAATAACGT 3'
3046	pagP	forward 5′ GTTGTGCTTTGTTTTGTGTTTTTTG 3'
3047	pagP	reverse 5′ GGCGTTTGATTTAGCACTCAGA 3'
5867	pcgL	forward 5′ ATATAAACTCTCCGTTTGTGATAAG 3'
5868	pcgL	reverse 5′ GCATTTTACCATATGTTACACCTC 3'
7141	pgtE	forward 5′ CGCATCACCGTAATGGTAAATC 3'
7144	pgtE	reverse 5′ AAATAGAATTTGATGCTCAGGTGAATAAT 3'
3039	phoP	forward 5′ ATCGGTCGCGCTGTGACT 3'
3040	phoP	reverse 5′ AGAGGGTGAGGCAGGCATT 3'
5988	pipD	forward 5′ TAGCTAAGGTCAGGACAGGTT 3'
5989	pipD	reverse 5′ GGATGAATTGACCCTGGAT 3'
6584	pmrD	forward 5′ AATATACAACCATTCCATCGCTATTG 3'
6585	pmrD	reverse 5′ CCAACCATTCCATAGCGCC 3'
6588	rstA	forward 5′ GCCTTAACACGCTAATAACAACAGC 3'
6589	rstA	reverse 5′ AAACGCCAACATACACCGC 3'
6586	slyB	forward 5′ TGTCGTCCACGGATATCAATGA 3'
6587	slyB	reverse 5′ TCTGCCGAAAGAATGTGAAAATC 3'
5990	somA	forward 5′ TAATGACCAGATAACGCTTTTTAA 3'
5991	somA	reverse 5′ GCTGTGTCATGATCGTAATCC 3'
6398	ugtL	forward 5′ CATGTACTATGAGTAATGATTAATTACGCACTAT 3'
6399	ugtL	reverse 5′ CATCTCATTGTTGTTAGCCTAATAATACTTT 3'
3158	virK	forward 5′ TTCTGGACTCATCCCACTCATTAG 3'
3159	virK	reverse 5′ AACAGTTTATCAATGGCGGTAATAAA 3'
7199	yobG	forward 5′ TGCCGCTACAGTTACTCCTGG 3'
7200	yobG	reverse 5′ CGAGAACGACCCATCGAAATT 3'
4149	rpoD	forward 5′ ACCGTGGCACAAATGATGCT 3′
4150	rpoD	reverse 5′ TCGGCAATCGCCTTATCTG 3′

Table S7. Primers used in transcription assay

Number	Name	Sequence
4489	phoP	forward 5′ GATGAAGACGGCCTTTCCTTAA 3′
4490	phoP	reverse 5′ GAACCGGCAGTGAAACATCA 3′
4443	mgtA	forward 5′ TAATTGCCACAAAACTTATG 3′
4446	mgtA	reverse 5′ TCGCGGGAGAGGGGTGGGTT 3′
4310	mig-14	forward 5′ CGCAATACGGCGGTAGTATCA 3′
4311	mig-14	reverse 5′ ATGCCAGTTATAGCGCTTCATG 3′
6964	pagC	forward 5′ AAAAGATTAAATCGGAGCGGGA 3′
6965	pagC	reverse 5′ TGACGCTCCATCCGCAATA 3′
7016	pagD	forward 5′ ACATCATGCTTTTATGCTTTGGTC 3′
7017	pagD	reverse 5′ AAACCAGAACAATGGCCTGAA 3′
4491	pmrD	forward 5′ GGTTAAGAAATCGCATTATGTCAAAA 3′
4492	pmrD	reverse 5′ CGAACCGCCGCTATCG 3′
7116	yobG	forward 5' TGAAAAAATTTCGATGGGTCG 3'
7118	yobG	reverse 5' TGATATTAAACACCTGCGCCC 3'
4485	mgtC	forward 5' GCGGGATTACGCACTAATGC 3'
4486	mgtC	reverse 5' GTCATGGAGCTCAGAATAAAAACG 3'
4487	pagP	forward 5' CAGTCTCTGCGGCGGATAAA 3'
4488	pagP	reverse 5' AGGCTGTCGCCACGTTTCT 3'
4493	slyB	forward 5' CAAGTTCAGAATGTAACGTACGGTACT 3'
4494	slyB	reverse 5' GAATCATCACCGCCCTGAAT 3'
7018	pagK	forward 5' ACGTCAAGAGCGTATTTTTAGCAA 3'
7019	pagK	reverse 5' TCTGCCGCTATTGTAAGAGCAG 3'
7022	ybjX	forward 5' TCGCGGATTACGATCATGAC 3'
7023	ybjX	reverse 5' AATTGCCCGCGCGTC 3'
7107	ompX	forward 5' CTGTTCTGGCTTTTTCCGCA 3'
7105	ompX	reverse 5' GCGTCGCTCTGAGCGTAAC 3'
7112	virK	forward 5' TAGTATGACGATGCAGCAAAGTGA 3'
7113	virK	reverse 5' CGTCTGCGCCATGACTTCTT 3'
9544	yjeH	forward 5' CGGCCAGGCGCTGTT 3'
9547	yjeH	reverse 5' GTACCCAGCTCCCCAAATAAGA 3'
9540	orgB	forward 5' GGCGGAAGAAGAGGCAAAG 3'
9546	orgB	reverse 5' TTCCCTGCTCGTAACCTTCCT 3'
9537	yhaO	forward 5' TGGCGCAGGGATCGTT 3'
9539	yhaO	reverse 5' AAAAAGACCCAAAGTCCCATCA 3'
9541	pipB2	forward 5' ATGGAGCGTTCACTCGATAGTCT 3'
9542	pipB2	reverse 5' TCCCCGCGCCAAAAG 3'
9545	phoN	forward 5' TCTGACAATGGGCGGCTACT 3'
9549	phoN	reverse 5' GGGCGGGTACGCATATAATATTT 3'
6627	pcgL	forward 5' GGCGACCGTAGTAATATCGACAA 3'
6628	pcgL	reverse 5' CTTTCCTCCTGTTCAGCCTGTT 3'
7108	ugtL	forward 5' CGATTAGCTGACGGCTTTGTTT 3'

Number	Name	Sequence
7114	ugtL	reverse 5' GATTTCTTCATTTTGAGCCTCCTC 3'
7109	pgtE	forward 5' TGCAGTAATGATGATCGCCG 3'
7111	pgtE	reverse 5' TCAGGAGAGACGTCCGGAAT 3'
7020	pipD	forward 5' AAAAGTATCTTGCATTCGCCG 3'
7021	pipD	reverse 5' GAAGGAGCCGTCAGCCG 3'
4236	rstA	forward 5' ACGCTGAAGTGGGTTCTCTCA 3'
4237	rstA	reverse 5' CACGCGGCTCAACAATGA 3'
7110	yrbL	forward 5' TCTTATCGAAACAGACTCCGCTG 3'
7117	yrbL	reverse 5' GCAACGCCGGGCATT 3'
3023	rrs	forward 5’ CCAGCAGCCGCGGTAAT 3’
3024	rrs	reverse 5’ TTTACGCCCAGTAATTCCGATT 3’

References

1. D. Shin, E. A. Groisman, *J Biol Chem* **280**, 4089 (Feb 11, 2005).

2. J. C. Perez, T. Latifi, E. A. Groisman, *J Biol Chem* **283**, 10773 (Apr 18, 2008).

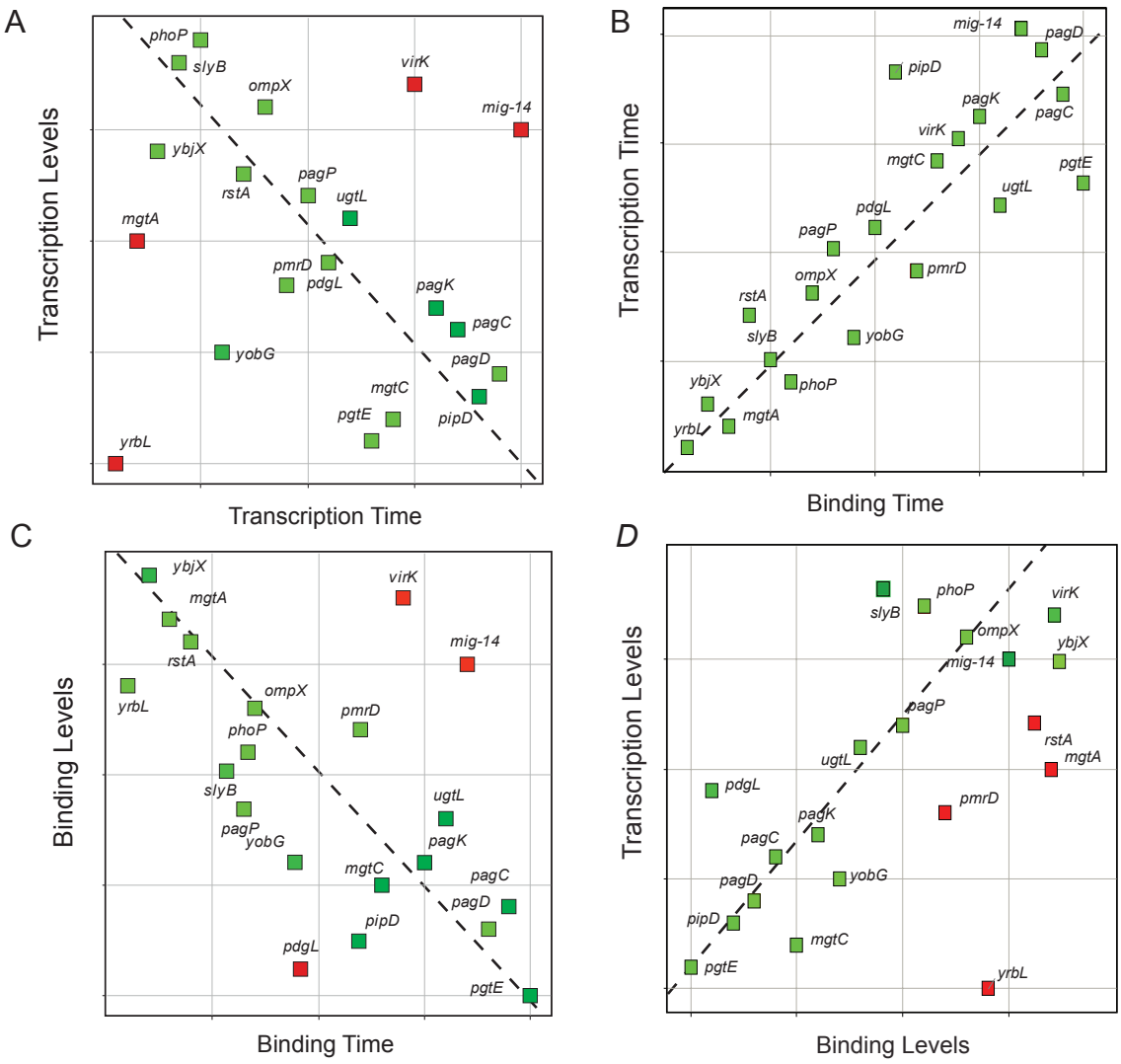
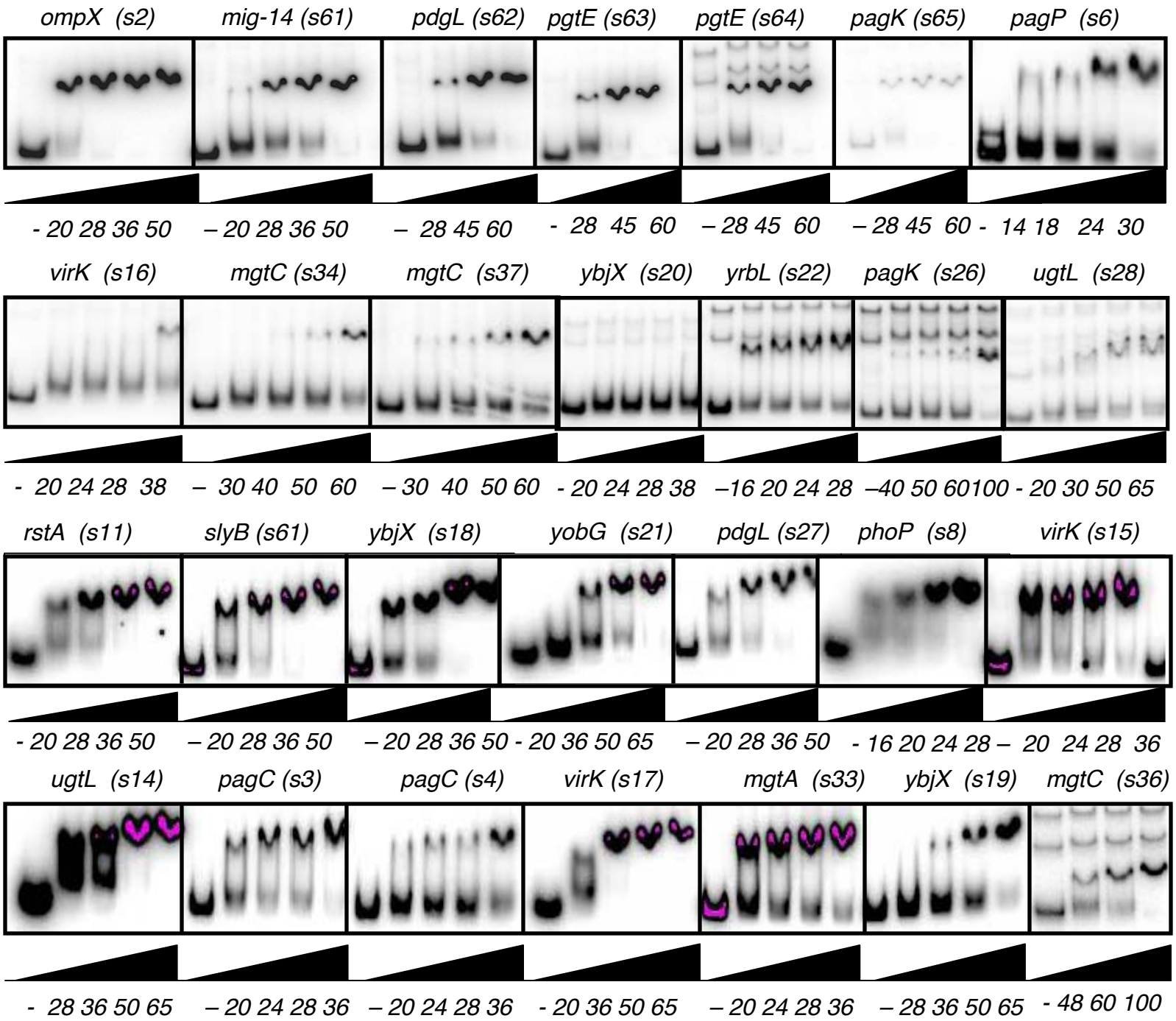

A
Transcription Levels
Transcription Time
phoP
slyB
ompX
virK
mig-14
ybjX
pagP
rstA
ugtL
mgtA
pmrD
pdgL
pagK
pagC
yobG
pagD
mgtC
pgtE
pipD
yrbL
B
Transcription Time
Binding Time
mig-14
pagD
pipD
pagK
pagC
virK
mgtC
pgtE
ugtL
pdgL
pagP
pmrD
ompX
rstA
slyB
yobG
ybjX
phoP
yrbL
mgtA
C
Binding Levels
Binding Time
ybjX
mgtA
virK
rstA
mig-14
yrbL
ompX
pmrD
phoP
slyB
ugtL
pagP
pagK
yobG
mgtC
pagC
pagD
pipD
pdgL
pgtE
D
Transcription Levels
Binding Levels
slyB
phoP
virK
ompX
ybjX
mig-14
pagP
rstA
mgtA
ugtL
pdgL
pmrD
pagK
pagC
pagD
yobG
pipD
mgtC
pgtE
yrbL

Fig. S1


ompX (s2)
mig-14 (s61)
pdgL (s62)
pgtE (s63)
pgtE (s64)
pagK (s65)
pagP (s6)
- 20 28 36 50
– 20 28 36 50
– 28 45 60
- 28 45 60
– 28 45 60
– 28 45 60 - 14 18 24 30
virK (s16)
mgtC (s34)
mgtC (s37)
ybjX (s20)
yrbL (s22)
pagK (s26)
ugtL (s28)
- 20 24 28 38
– 30 40 50 60
– 30 40 50 60
- 20 24 28 38
–16 20 24 28
–40 50 60100 - 20 30 50 65
rstA (s11)
slyB (s61)
ybjX (s18)
yobG (s21)
pdgL (s27)
phoP (s8)
virK (s15)
- 20 28 36 50
– 20 28 36 50
– 20 28 36 50 - 20 36 50 65
– 20 28 36 50
- 16 20 24 28 – 20 24 28 36
ugtL (s14)
pagC (s3)
pagC (s4)
virK (s17)
mgtA (s33)
ybjX (s19)
mgtC (s36)
- 28 36 50 65
– 20 24 28 36
– 20 24 28 36
- 20 36 50 65
– 20 24 28 36
– 28 36 50 65
- 48 60 100

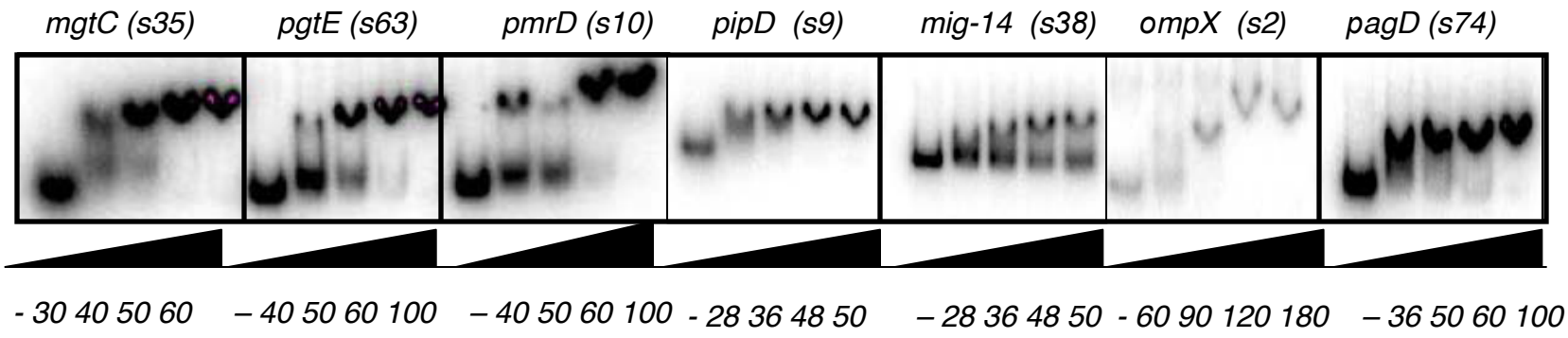

mgtC (s35)
pgtE (s63)
pmrD (s10)
pipD (s9)
mig-14 (s38)
ompX (s2)
pagD (s74)
- 30 40 50 60
– 40 50 60 100
– 40 50 60 100
- 28 36 48 50
– 28 36 48 50
- 60 90 120 180
– 36 50 60 100

Fig. S2

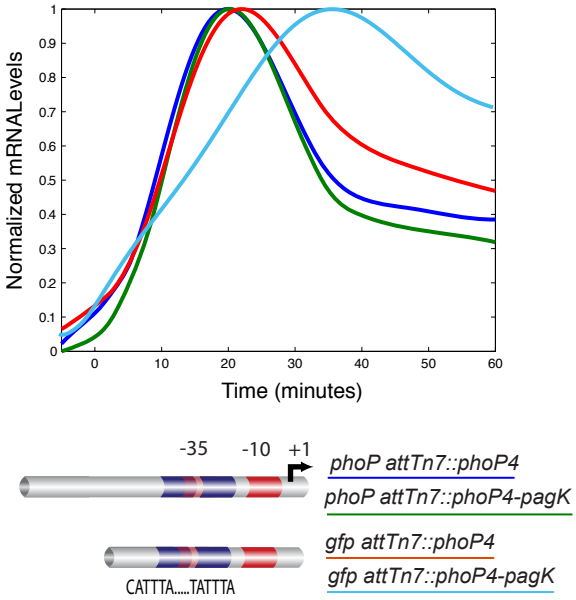

Normalized mRNALevels
Time (minutes)
-35
-10
+1
phoP attTn7::phoP4
phoP attTn7::phoP4-pagK
gfp attTn7::phoP4
gfp attTn7::phoP4-pagK
CATTTA.....TATTTA

Fig. S3

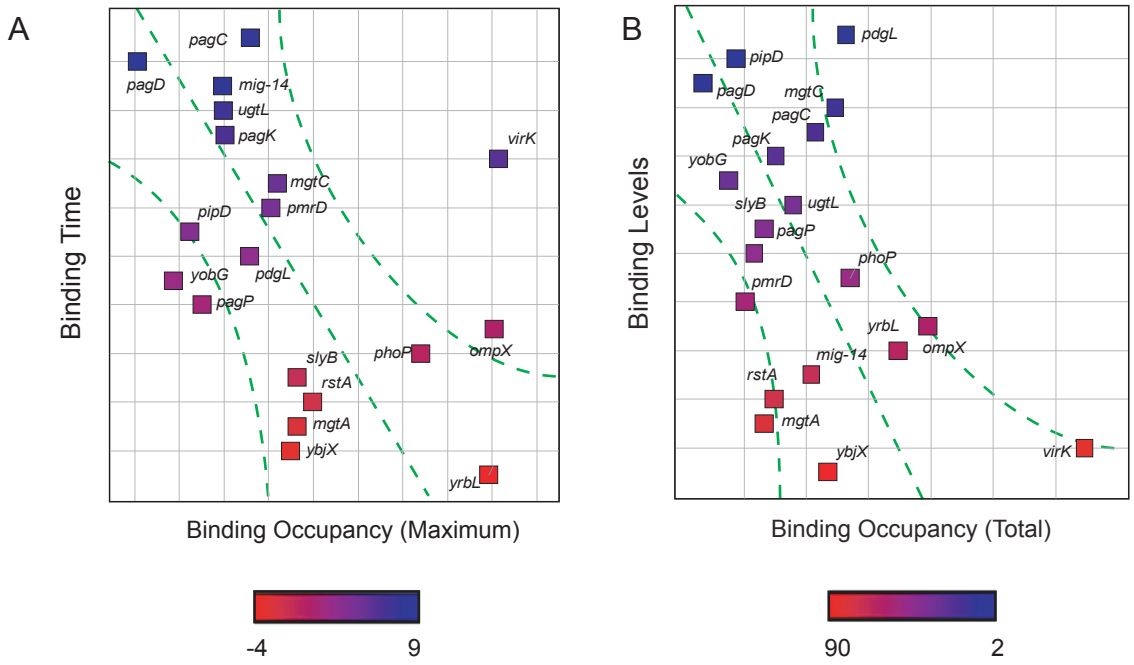
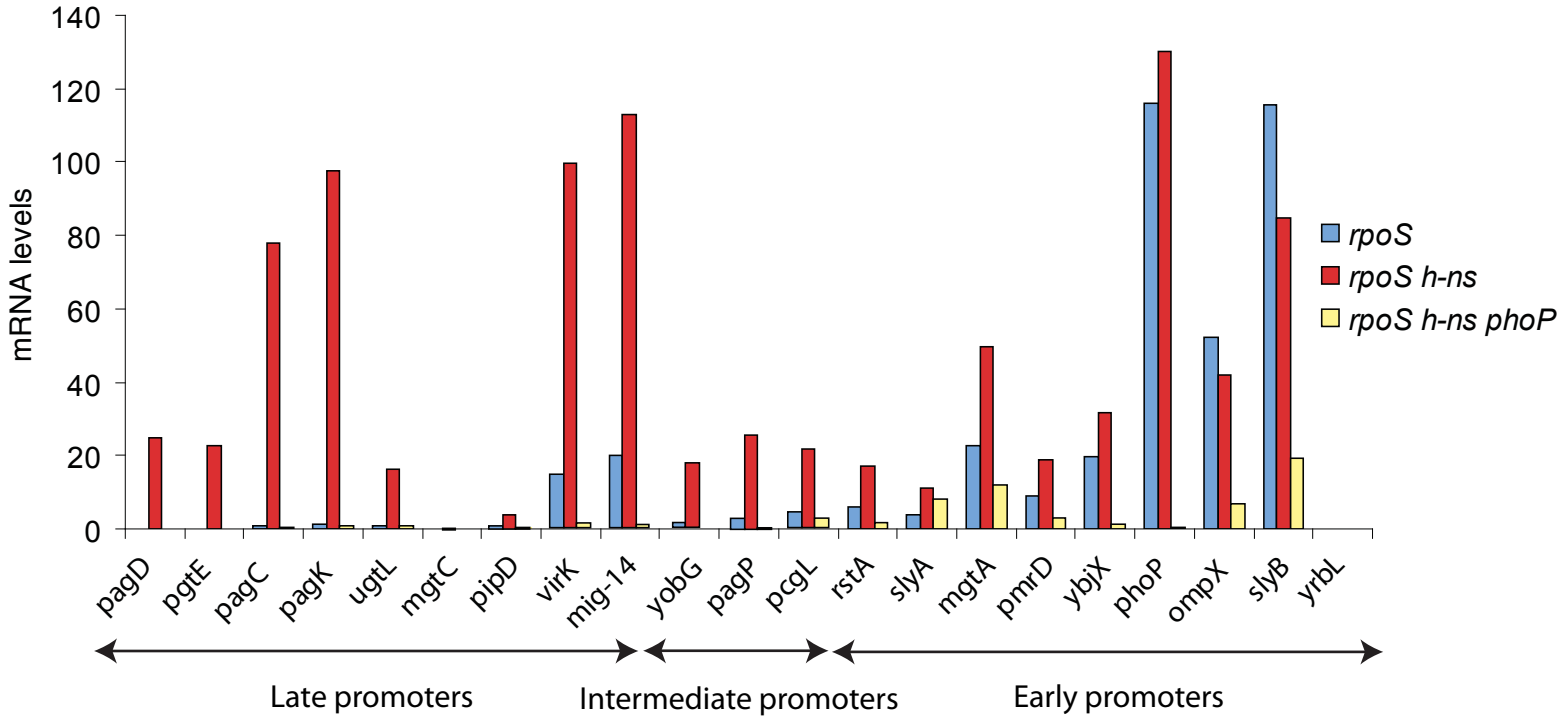
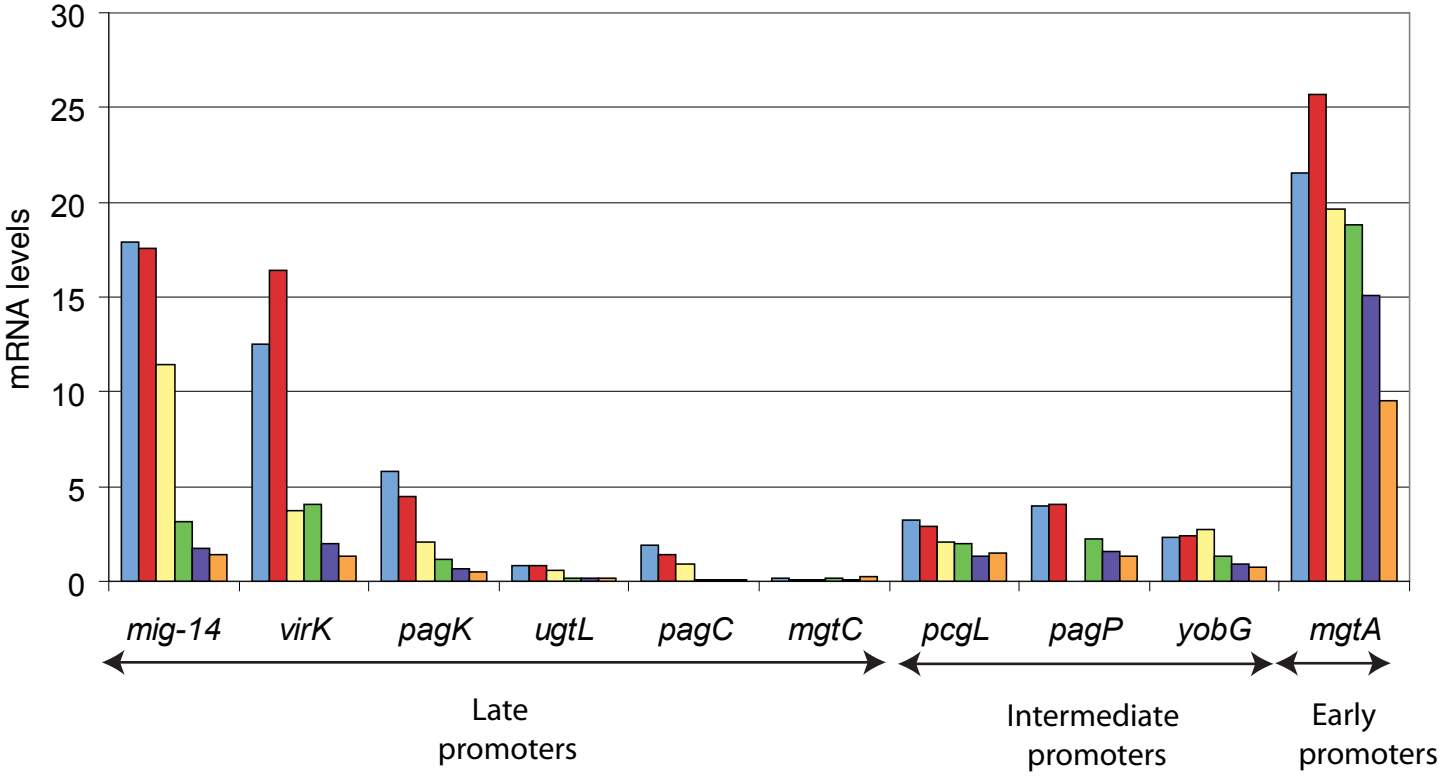

A
Binding Time
Binding Occupancy (Maximum)
pagC
pagD
mig-14
ugtL
pagK
virK
mgtC
pmrD
pipD
yobG
pdgL
pagP
slyB
phoP
ompX
rstA
mgtA
ybjX
yrbL
-4
9
B
Binding Levels
Binding Occupancy (Total)
pdgL
pipD
pagD
mgtC
pagC
pagK
yobG
slyB
ugtL
pagP
phoP
pmrD
yrbL
ompX
mig-14
rstA
mgtA
ybjX
virK
90
2

Fig. S4

A


140
120
100
80
60
40
20
0
mRNA levels
rpoS
rpoS h-ns
rpoS h-ns phoP
pagD
pgtE
pagC
pagK
ugtL
mgtC
pipD
virK
mig-14
yobG
pagP
pcgL
rstA
slyA
mgtA
pmrD
ybjX
phoP
ompX
slyB
yrbL
Late promoters
Intermediate promoters
Early promoters

B


30
25
20
15
10
5
0
mRNA levels
mig-14
virK
pagK
ugtL
pagC
mgtC
pcgL
pagP
yobG
mgtA
Late promoters
Intermediate promoters
Early promoters


slyA+ 10uM
slyA+ 50uM
slyA+ 200uM
slyA 10uM
slyA 50uM
slyA 200uM

Fig. S5