Supplemental information

ExsA and LcrF recognize a similar consensus-binding site but differences in their oligomeric state influence interactions with promoter DNA

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Table S1. Bacterial strains used in this study.

Bacterial strains	Relevant characteristics	Reference
P. aeruginosa strains		
PA103 *exsA*::Ω	chromosomal interposon insertion in *exsA*	(1)
Y. pestis strains[a,b]		
KIM5-3001 (parent)	Sm^R, pCD1, pPCP1, pMT1	(2, 3)
KIM5-3001-F1(*ΔlcrF*)	Sm^R, pCD1 (*ΔlcrF::dhfr*), pPCP1, pMT1	This study
KIM5-3233 (*yopM::lacZYA*)	Sm^R, pCD1 (*yopM::lacZYA*), pPCP1, pMT1	(4)
KIM5-3233-F2 (*ΔlcrF yopM::lacZYA*)	Sm^R, pCD1 (*ΔlcrF; yopM::lacZYA*), pPCP1, pMT1	This study

[a]All *Y. pestis* strains used in this study are avirulent due to a deletion of the *pgm* locus (5).

[b]Plasmids native to *Y. pestis* include pCD1(6), pPCP1 (7) and pMT1 (8).

Table S2. Primers used in this study.

Plasmid construction

Primer ID	Name	Primer
NA	LcrF-KpnI	5'-TTTGGTACCTTTAGATTTTTAGGACAGTAT
NA	LcrF-HindIII	5'-TTTAAGCTTACTTTATAGTCCAAAAGTGTC
81126789	lcrF3'sacI	5'-GTCAGAGCTCATCCCGTATTTTACTTTATAGTC
81126790	lcrF5'xbaI	5'-GTCATCTAGAAGGACAGTATAACATTTATGGC
81559809	lcrF5'Nde	5'-GTCACATATGGCATCACTAGAGATTATTAAATTAG
82001905	lcrF3'BamHI	5'-GTCAGGATCCATCCCGTATTTTACTTTATAGTC
85928866	VxsA5'Nde	5'-GTGACATATGGATGTGTCAGGCCAACTAAACAC
86519886	VxsASac-2	5'-GTATGAGCTCTCAATTAGCGATGGCGACTTGCTCA
88733522	lcrF148A	5'-GCGTATTGAGGAATTACTGCTTGCGCTTGCCTTTAGCTCGCAAGGGAC
89928045	lcrF140Ashort	5'-GATTTTCCGTCAGTATTAACGCAAGCGCGTATTGAGGAATTAC

Site-directed mutagenesis

Primer ID	Name	Primer
81126787	lcrF(A126T)bot	5'-CCCCTCATCTAAATCATAAGTCTGACCGCTGCTCTGCC
81126788	lcrF(A126T)top	5'-GGCAGAGCAGCGGTCAGACTTATGATTTAGATGAGGGG
88022425	minictxJK511	5'-CTAAAACTCGCGGCTCCCGCTACATTTTTTTAGCGTT
88022426	minictxJK508	5'-CTAAAAACTCGCGGCTCCCGCTCCAATTTTTTAGCGTT
88022427	minictxJK510	5'-CTAAAAACTCGCGGCTCCCGCTACGATTTTTTAGCGTT
88022428	minictxJK507	5'-CTAAAAACTCGCGGCTCCCGCAACAATTTTTTAGCGTT
88022429	minictxJK506	5'-CTAAAAACTCGCGGCTCCCGATACAATTTTTTAGCGTT
88022430	minictxJK505	5'-CTAAAAACTCGCGGATCCCGCTACAATTTTTTAGCGTT
88022431	minictxJK509	5'-CTAAAAACTCGCGGCTCCCGCTAAAATTTTTTAGCGTT
88022432	minictxJK504	5'-CTAAAAACTCGCAGCTCCCGCTACAATTTTTTAGCGTT
88022433	minictxJK503	5'-CTAAAAAGTCGCGGCTCCCGCTACAATTTTTTAGCGTT

Promoter probes

Primer ID	Name	Primer
85973161	PyopN3'Eco	5'-CGACGAATTCGTATTGCCATAAGATAGGTTATG
85973164	PyscN3'Eco	5'-CGACGAATTCTACAATGCCATGACGAATATGATGAG
85973165	PlcrG3'Eco	5'-CGACGAATTCGTCGGCTATTGCCAGTTCTGCCTG
88203765	PlcrG5'Hind	5'-CGACAAGCTTAAAGTATCACAAGTCATCACCTTATC
88203767	PyscN5'Hind	5'-CGACAAGCTTAAATCAGCCGAGAAATTTTAGTGCTG

Table S2 (continued). Primers used in this study.

88203768	PyopN5'Hind	5'-CGACAAGCTTGCGGGAGCCGCGAGTTTTTAGAA
88859457	probe4top	5'-AATTCCGGGGAATACCGGTCAAAACCACGGCCAATCCTGATAGTACCGTGGCCAACA
88859458	probe2top	5'-AATTCCGGGGAATACCTAAAAAAACCACGGCCAATCCTGATAGTACCGTGGCCAACA
88859460	probe4bottom	5'-AGCTTGTTGGCCACGGTACTATCAGGATTGGCCGTGGTTTTGACCGGTATTCCCCGG
88859461	probe6bottom	5'-AGCTTGTTGGCCACGGTAAGATCAGGATTGGCCGTGGTTTTTTTAGGTATTCCCCGG
88859462	probe7top	5'-AATTCCGGGGAATACCGGTCAAAACCACGGCCAATCCTGATCTTACCGTGGCCAACA
88859464	probe7bottom	5'-AGCTTGTTGGCCACGGTAAGATCAGGATTGGCCGTGGTTTTGACCGGTATTCCCCGG
88859465	probe2bottom	5'-AGCTTGTTGGCCACGGTACTATCAGGATTGGCCGTGGTTTTTTTAGGTATTCCCCGG
88859466	probe6top	5'-AATTCCGGGGAATACCTAAAAAAACCACGGCCAATCCTGATCTTACCGTGGCCAACA

***lcrF* deletion mutant**

Primer ID	Name	Primer sequence
NA	LcrF-dhfr-P1	5'- TTTACAAGATGGCCTATATATTCTTTTGCAAGGTCAGATATAGACGGCATGCACGATTTG
NA	LcrF-dhfr-P2	5'- GAAGCGACGTCGATAACTTTGAGTGAAATAAGACTGACTCGCATCCAATGTTTCCGCCAC
NA	LcrF-kan-P1	5'- GGCATCACTAGAGATTATTAAATTAGAATGGGTCACACCTGTGTAGGCTGGAGCTGCTTC
NA	LcrF-kan-P2	5'- CGACGTCGATAACTTTGAGTGAAATAAGACTGACTCGAGCATATGAATATCCTCCTTAGT
NA	LcrF-F1	5'- CCTCATTAGATAAATATATACAAG
NA	LcrF-R1	5'- ATCCCGTATTTTACTTTATAGTCC

Table S3. Transcriptional reporters used in this study.

Reporter fusions	Integration Plasmid	Experiment	Primer Pair	Reference
$P_{exoT\text{-}lacZ}$	mini-CTX-$P_{exoT\text{-}lacZ}$ (-179 to + 17)	Fig. 1A, 4B, 10, S3C	NA	(9)
$P_{exsC\text{-}lacZ}$	mini-CTX-$P_{exsC\text{-}lacZ}$ (-186 to + 17)	Fig. 1B	NA	(10)
$P_{exsD\text{-}lacZ}$	mini-CTX-$P_{exsD\text{-}lacZ}$ (-152 to + 16)	Fig. 1C	NA	(10)
EB126	mini-CTX-$P_{exoT\text{-}lacZ}$ (C-50G)	Fig. 4B	NA	(11)
EB127	mini-CTX-$P_{exoT\text{-}lacZ}$ (C-45A)	Fig. 4B	NA	(11)
EB134	mini-CTX-$P_{exoT\text{-}lacZ}$ (G-47A)	Fig. 4B	NA	(11)
EB135	mini-CTX-$P_{exoT\text{-}lacZ}$ (T-38A)	Fig. 4B	NA	(11)
EB136	mini-CTX-$P_{exoT\text{-}lacZ}$ (G-37C)	Fig. 4B	NA	(11)
JK121	mini-CTX-$P_{exoT\text{-}lacZ}$ (C-39A)	Fig. 4B	NA	(11)
JK122	mini-CTX-$P_{exoT\text{-}lacZ}$ (A-36C)	Fig. 4B	NA	(11)
JK123	mini-CTX-$P_{exoT\text{-}lacZ}$ (T-35G)	Fig. 4B	NA	(11)
$P_{yopN\text{-}lacZ}$	mini-CTX-$P_{yopN\text{-}lacZ}$	Fig. 7A	88203768-85973161	This study
$P_{yscN\text{-}lacZ}$	mini-CTX-$P_{yscN\text{-}lacZ}$	Fig. 7B, 8B	88203767-85973164	This study
$P_{lcrG\text{-}lacZ}$	mini-CTX-$P_{lcrG\text{-}lacZ}$	Fig. 7C	88203765-85973165	This study
JK503	mini-CTX-$P_{yscN\text{-}lacZ}$ (C-50G)	Fig. 8B	88022433-85973164	This study
JK504	mini-CTX-$P_{yscN\text{-}lacZ}$ (G-47A)	Fig. 8B	88022432-85973164	This study
JK505	mini-CTX-$P_{yscN\text{-}lacZ}$ (C-45A)	Fig. 8B	88022430-85973164	This study
JK506	mini-CTX-$P_{yscN\text{-}lacZ}$ (C-39A)	Fig. 8B	88022429-85973164	This study
JK507	mini-CTX-$P_{yscN\text{-}lacZ}$ (T-38A)	Fig. 8B	88022428-85973164	This study
JK508	mini-CTX-$P_{yscN\text{-}lacZ}$ (A-37C)	Fig. 8B	88022426-85973164	This study
JK509	mini-CTX-$P_{yscN\text{-}lacZ}$ (C-36A)	Fig. 8B	88022431-85973164	This study
JK510	mini-CTX-$P_{yscN\text{-}lacZ}$ (A-35G)	Fig. 8B	88022427-85973164	This study
JK511	mini-CTX-$P_{yscN\text{-}lacZ}$ (A-34T)	Fig. 8B	88022425-85973164	This study

Table S4. Expression plasmids used in this study.

Expression Plasmids	Experiment	Primer Pair	Reference
pJN105	Fig. 1, 7A-C, 9A-B, S3C	NA	(12)
pEB124 (referred to as pExsA in the text)	Fig. 1, 4B, 7A-C, 8B, 9A-B, 10	NA	(9)
pBAD30-LcrF	NA	NA	Plano
pJK29	NA	81126788-81126787	This study
pJK32 (referred to as pLcrF in the text)	Fig. 1, 4B, 7A-C, 8B, 9A-B, 10, S3C	81126790-81126789	This study
pLcrF$_m$	Fig. S3C	NA	This study
pET16b ExsA	ExsA purification	NA	(9)
pET16b LcrF	LcrF purification	81559809-82001905	This study
pET16b LcrF$_m$	LcrF$_m$ purification	89928045/88733522-82001905	This study
pAxsA	Fig. 10	NA	(9)
pPxsA	Fig. 10	NA	(9)
pVxsA	Fig. 10	85928866-86519886	This study

Table S5. EMSA/DNaseI footprinting probes used in this study.

Probe	Experiment	Primer Pair	Reference
EMSA Probes (PCR products)			
$P_{exsC\text{-}lacZ}$	Fig. 2A-B	NA	(10)
$P_{exoT\text{-}lacZ}$	Fig. 2C-D, 4C-D	NA	(9)
$P_{exsD\text{-}lacZ}$	Fig. 2E-F	NA	(10)
EB126 (C-50G)	Fig. 4C-D	NA	(11)
EB134 (G-47A)	Fig. 4C-D	NA	(11)
EB127 (C-45A)	Fig. 4C-D	NA	(11)
JK121 (C-39A)	Fig. 4C-D	NA	(11)
EB135 (T-38A)	Fig. 4C-D	NA	(11)
EB136 (G-37A)	Fig. 4C-D	NA	(11)
JK122 (A-36C)	Fig. 4C-D	NA	(11)
JK123 (T-35G)	Fig. 4C-D	NA	(11)
EB137 (A-34T)	Fig. 4C-D	NA	(3)
$P_{yopN\text{-}lacZ}$	Fig. 7D	88203768-85973161	This study
$P_{yscN\text{-}lacZ}$	Fig. 7E, 8B	88203767-85973164	This study
$P_{lcrG\text{-}lacZ}$	Fig. 7F	88203765-85973165	This study
JK503 (C-50G)	Fig. 8B	88203767-85973164	This study
JK504 (G-47A)	Fig. 8B	88203767-85973164	This study
JK505 (C-45A)	Fig. 8B	88203767-85973164	This study
JK506 (C-39A)	Fig. 8B	88203767-85973164	This study
JK507 (T-38A)	Fig. 8B	88203767-85973164	This study
JK508 (A-37C)	Fig. 8B	88203767-85973164	This study
JK509 (C-36A)	Fig. 8B	88203767-85973164	This study
JK510 (A-35G)	Fig. 8B	88203767-85973164	This study
JK511 (A-34T)	Fig. 8B	88203767-85973164	This study
EMSA Probes (annealed oligonucleotides)			
P_{exsC} (50 bp)	Fig. 3A	NA	(9)
P_{exsD} (50 bp)	Fig. 3B	NA	(9)
P_{exoT} (50 bp)	Fig. 3C, 5A	NA	(9)
P_{exoT} probe 1	Fig. 3E	88859458-88859465	This study
P_{exoT} probe 2	Fig. 3E	88859457-88859460	This study
P_{exoT} probe 3	Fig. 3E	88859466-88859461	This study
P_{exoT} probe 4	Fig. 3E	88859462-88859464	This study

Table S5 (continued). EMSA/DNaseI footprinting probes used in this study

pBEND fragments			
P_{exsD} probe 1	Fig. 3E	NA	(3)
P_{exsD} probe 2	Fig. 3E	NA	(3)
P_{exsD} probe 3	Fig. 3E	NA	(3)
P_{exsD} probe 4	Fig. 3E	NA	(3)
DNaseI Footprinting Probes (PCR product)			
P_{exsC} (200bp)	Fig. S5A	N/A	(9)

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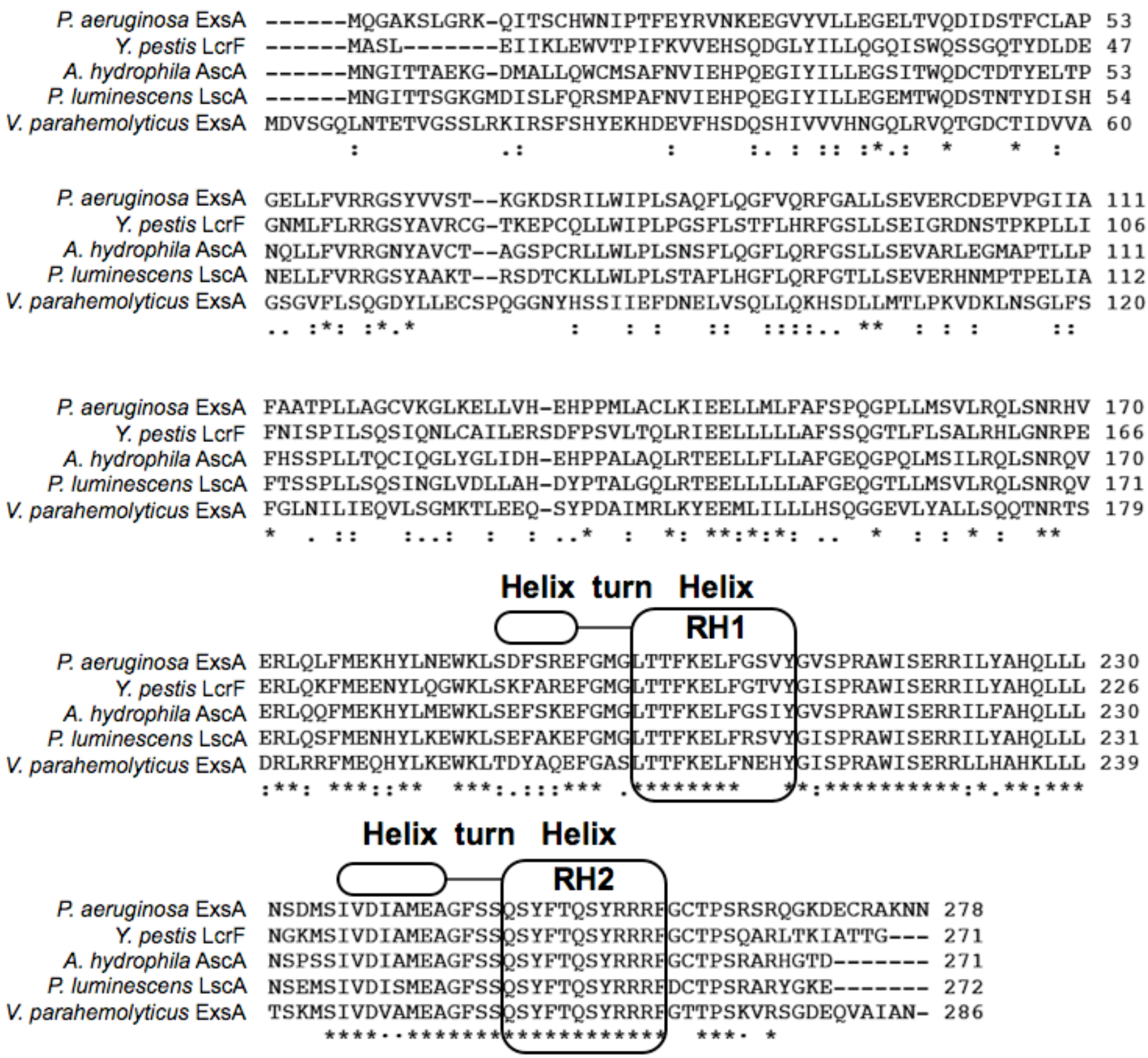

Figure S1. Amino acid sequence alignment of ExsA (*P. aeruginosa*), LcrF (*Y. pestis*), AxsA (*A. hydrophila*), LxsA (*P. luminescens*), and ExsA (*V. parahaemolyticus*), respectively. The recognition helices (RH1 and RH2) of the two helix-turn-helix DNA binding motifs are indicated with rounded boxes. Residues that are identical (*), conserved (:), or similar (.) are indicated.

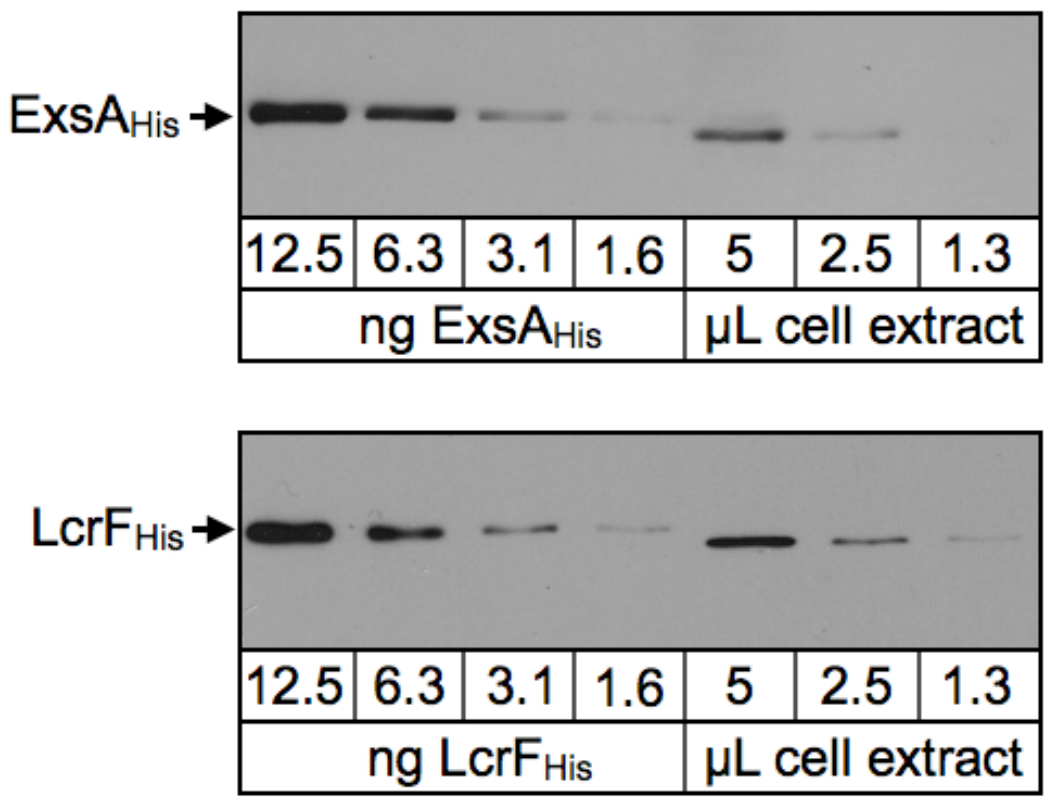

Figure S2. Intracellular expression levels of ExsA and LcrF are comparable. PA103 *exsA*::Ω carrying the pExsA (top panel) or pLcrF (bottom panel) expression plasmid was cultured under inducing (+ EGTA) conditions for T3SS gene expression. Cellular extracts and purified ExsA (top panel) and LcrF (bottom panel) standards were analyzed by SDS-PAGE and immunoblot analyses. ExsA and LcrF intracellular protein expression levels were estimated by comparing the amount of ExsA or LcrF in cellular extracts to the purified ExsA or LcrF standards.

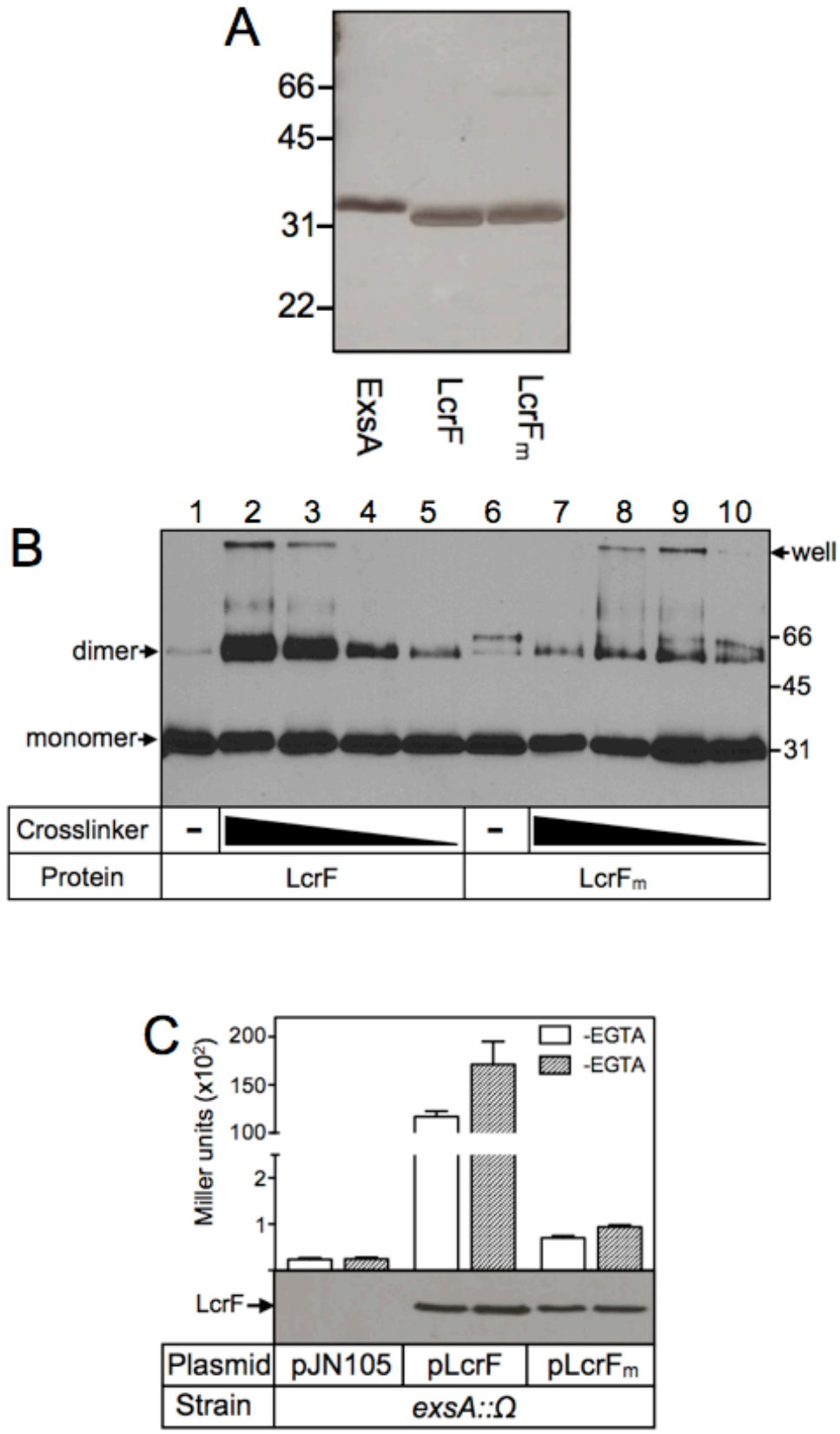

Figure S3. Characterization of LcrF and LcrF$_m$. (A) Silver-stained gel of purified ExsA, LcrF, and LcrF$_m$ (1 µg each). (B) Crosslinking experiments demonstrate that purified LcrF is dimeric in solution and that LcrF$_m$ is primarily monomeric. Reactions containing 125 ng of purified LcrF (lanes 1-5) or LcrF$_m$ (lanes 6-10) were incubated in the absence (lanes 1 and 6) or presence of sulfo-EGS crosslinker (1 mg/ml [lanes 2 and 7], 0.33 mg/ml [lanes 3 and 8], 0.11 mg/ml [lanes 4 and 9], 0.033 mg/ml [lanes 5 and 10]) for 60 min at 25˚C. Reactions were immediately subjected to electrophoresis on 12% SDS-polyacrylamide gels and immunoblotted for LcrF. Monomeric and dimeric species are indicated on the left side of the blot and molecular weight standards are indicated on the right side. (C) PA103 *exsA*:: carrying the P$_{exoT\text{-}lacZ}$ transcriptional reporter was transformed with a vector control (pJN105), pLcrF, or pLcrF$_m$. The resulting strains were cultured under non-inducing (- EGTA) and inducing (+ EGTA) conditions for T3SS gene expression and assayed for -galactosidase activity (reported in Miller units). Whole cell extracts were immunoblotted for LcrF.

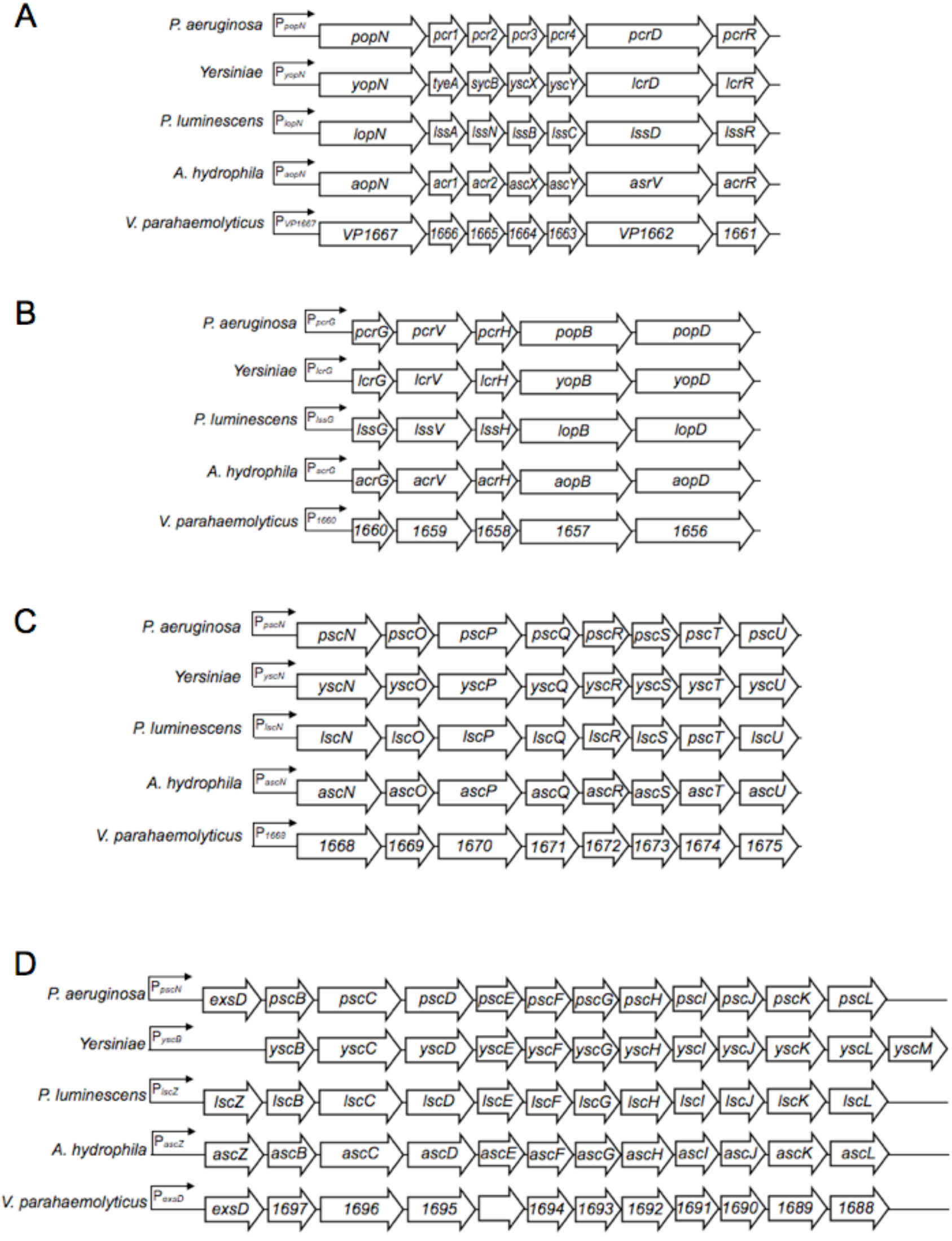

Figure S4. Conserved T3SS promoter regions and gene clusters in *P. aeruginosa*, yersiniae, *P. luminescens*, *A. hydrophila*, and *V. parahaemolyticus*.

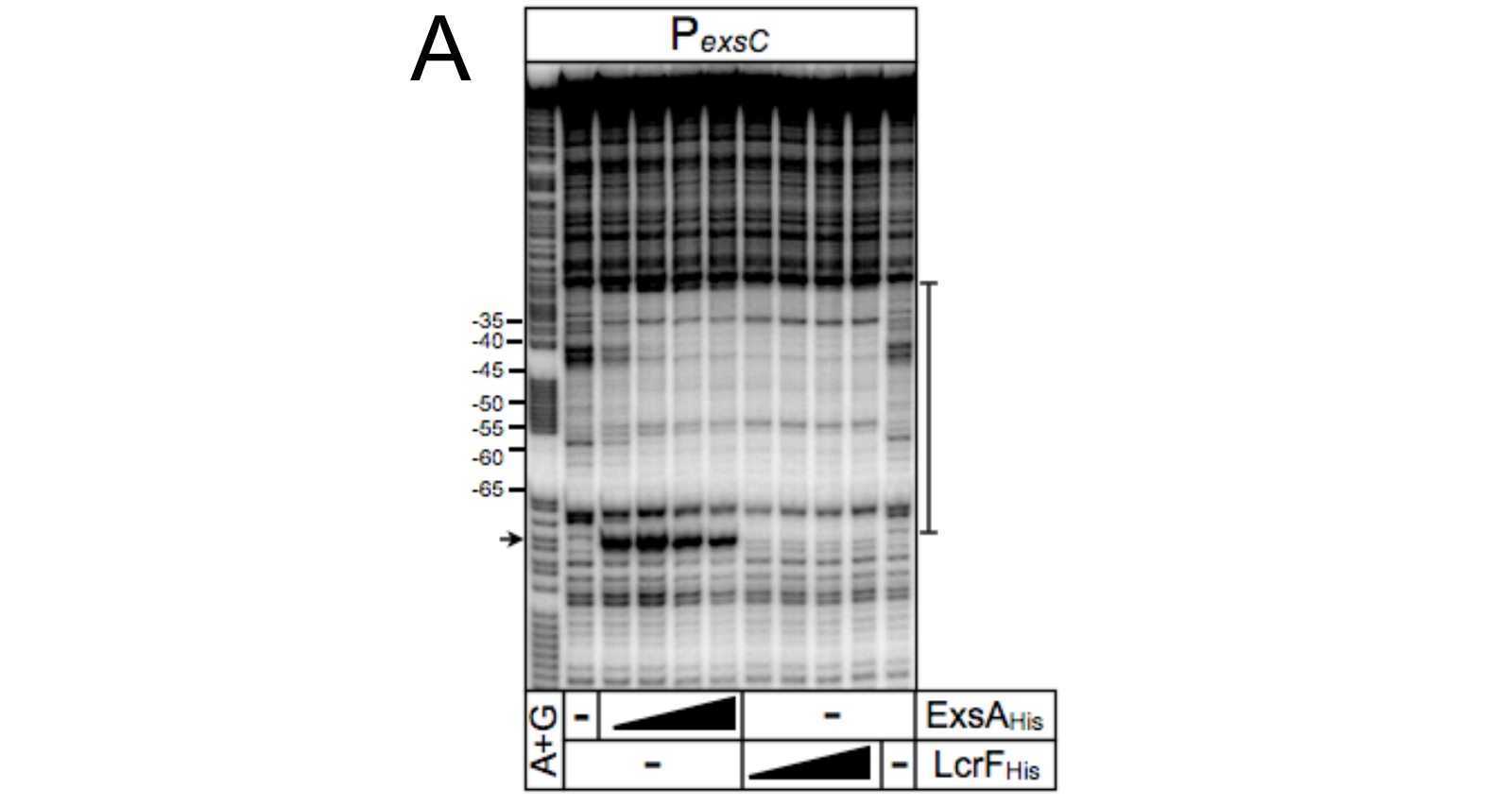

Figure. S5. DNase I footprinting assay. (A). Radiolabelled promoter fragments (5 nM) were incubated alone (-) or in the presence of 62.5, 125, 250, or 500 nM ExsA or LcrF for 15 min. Reactions were treated with DNase I and subjected to electrophoresis and phosphorimaging. Promoter regions protected by ExsA and LcrF from DNase I cleavage are indicated by the solid line on the right of the gel. The hypersensitive DNase I cleavage site is indicated by an arrow on the left side of the gel. Maxam–Gilbert A+G sequencing reaction, and nucleotide positions are included on the left of the gel. (B). Diagram of the P_{exsC}, P_{exsD} and P_{exoT} promoter regions protected by ExsA and LcrF from DNase I cleavage. The solid line above the nucleotide sequence represents the ExsA- and LcrF-protected regions of the promoter DNA and the dotted line below indicates binding sites 1 and 2. Arrowheads indicate nucleotides that are hypersensitive to DNase I cleavage. The highly conserved GnC and TGnnA nucleotides, and adenine-rich region are shown in bold. The -10 promoter element is boxed.