Supplemental Materials

Supplemental Materials and Methods

Strain Construction

For construction of the Δ*mogR*Δ*degU*/p*Hy* strain, the primer pair #366 and #780 were used with *Lm* EGDe genomic DNA to amplify ~0.8 kb of the region upstream of *mogR*. The 0.8 kb PCR product was purified, digested with *KpnI* and *EagI*, and ligated to plasmid pHPL3 (Gründling *et al*., 2004) digested with the same restriction enzymes, and transformed into XL1-Blue to generate strain DH-E1878 carrying plasmid pHPL3-5'Δ*mogR*. Primer #366 and #707 were used to amplify the 5'Δ*mogR* DNA fragment and the p_{Hy} promoter from plasmid pHPL3-5'Δ*mogR*. The p_{Hy} promoter is located at the 3' end of the 5'Δ*mogR* fragment with transcription initiating at nucleotide 36 of *mogR* proceeding divergently towards the 5' start of *mogR* and *fliN*. Primers #706 and #368 were used to amplify ~1 kb of DNA downstream of *mogR*. The 5' and 3' PCR products were gel purified using the QIAquick gel extraction kit (Qiagen) and used as templates for a splicing by overlap extension (SOE) PCR reaction (Horton *et al*., 1989). The flanking primers, #366 and #368, were used to amplify an ~1.8 kb PCR product containing an in-frame deletion of sequences encoding amino acids 13 to 295 of MogR and the p_{Hy} promoter sequence inserted between sequences encoding amino acid 12 and 296 oriented to initiate transcription towards amino acid 12 of *mogR*. The SOE PCR product was gel-purified, digested with *KpnI* and *BamHI* and ligated to plasmid pCON1 digested with the same restriction enzymes to generate plasmid pCON1-Δ*mogR*/$p_{Hy.}$ The resulting plasmid was transformed into XL1-Blue to generate strain DH-E1819. Plasmid isolated from DH-E1819 was used to transform the conjugal mating strain SM10 to generate strain DH-E1821. pCON1-Δ*mogR*/p_{Hy} was transformed into the Δ*degU* strain (DH-L1273) through conjugative transfer from strain DH-E182. Allelic exchange was then performed to generate strain Δ*mogR*Δ*degU*/p_{Hy} (DH-L1818).

To construct an in-frame deletion of *lmo1021*, primer pair #504 and #506 were used to amplify ~1 kb of the upstream sequence of *lmo1021*. Primer pair #505 and #507 were used to amplify ~1 kb of the downstream sequence of *lmo1021*. The resulting PCR products were mixed together with flanking primers #504 and #507 to amplify an ~2 kb DNA product by SOE PCR. The SOE PCR product was digested with *SpeI* and *KpnI*, ligated to pCON1 digested with *XbaI* and *KpnI*, and transformed into XLI-Blue to generate strain DH-E1352. The resulting plasmid, pCON1-Δ*lmo1021* was electroporated into EGDe, and allelic exchange was performed to generate strain DH-L1344 in which codons 13 to 334 of *lmo1021* have been deleted.

The *Lm*DegU $D_{55}N$ strain was constructed by allelic exchange in wild-type EGDe. Primer pair #464 and #484 were used with *Lm* EGDe genomic DNA to amplify ~1 kb of the region upstream of *Lm*DegU encoding amino acid 55 and primer pair #466 and #483 were used with *Lm* EGDe genomic DNA to amplify ~1 kb of DNA downstream of sequences encoding amino acid 55. Primers #483 and #484 contain the point mutant required for the amino acid change of $D_{55}N$. The 5' and 3' PCR products were gel purified and used as templates for PCR SOE. The flanking primers, #464 and #466, were used to amplify a 2 kb PCR product containing the point mutant required for the amino acid change $D_{55}N$ of *Lm*DegU. The PCR SOE product was gel-purified and digested with *XbaI* and *KpnI*, ligated into plasmid pCON1 digested with the same restriction enzymes and transformed into CLG190 to generate strain DH-E1342. The resulting plasmid, pCON1-*Lm*DegU $D_{55}N$, was transformed into the conjugal

mating strain SM10 and then conjugated into EGDe where allelic exchange was performed to generate the *Lm*DegU $D_{55}N$ strain (DH-L1339).

To generate the complementing constructs of DegU (*Lm*DegU, *Bs*DegU and *Lm*DegU Cterm), the pLOV site-specific integration vector was used (Shen *et al*., 2006). DegU from *Lm* carrying an optimized *ermC* ribosome binding site (RBS) was amplified from EGDe genomic DNA using primers #485 and #486. *Bs*DegU carrying an optimized *ermC* RBS was amplified from *Bs* strain PY79 genomic DNA using primers #708 and #709. The C-terminal DNA binding domain of *Lm*DegU was amplified from *Lm* EGDe genomic DNA using the 5' primer #710 that contains an optimized *ermC* RBS followed by a start codon sequence and the 3' primer #486, together this primer pair amplifies *Lm*DegU sequences encoding from amino acid 133 to the stop codon. The resulting PCR products were gel purified and the *Lm*-derived constructs were digested with *PstI* and *SalI* and the *Bs* construct was digested with *SalI* and *KpnI*. All fragments were ligated into pLOV digested with the same restriction enzymes and transformed into CLG190 to generate strains DH-E1822, DH-E1823, and DH-E1824. The resulting plasmids (pLOV-*Lm*DegU, pLOV-*Bs*DegU, and pLOV-*Lm*DegU-Cterm) were sequenced and transformed into the *E. coli* conjugation strain SM10 to generate strains DH-E1825, DH-E1826, and DH-E1827. The SM10 strains were mated with *Lm* Δ*degU* to integrate the plasmids in single copy into the $tRNA^{Arg}$ locus and generate strains DH-L1798, DH-L1865, and DH-L1799.

To produce the transcriptional reporter fusions, a reporter construct was generated using the pPL3 site integration vector (Gründling *et al*., 2004). Primers #166 and #713 were used to amplify *gusA* from pNF470 plasmid DNA (Shetron-Rama *et al*., 2003). The *gusA* PCR product was purified using QIAquick PCR purification kit (Qiagen), digested with *PstI* and *SalI* restriction enzymes, ligated into pPL3 digested with the same restriction enzymes, and transformed into XL1-Blue to generate strain DH-E1828. The resulting plasmid pPL3-*gusA* is a reporter construct that can be used to generate transcriptional fusions to β-glucoronidase. To generate the p*fliN-gmaR* reporter constructs, truncations of the p*fliN-gmaR* region DNA were amplified from *Lm* EGDe genomic DNA using 5' primers #715, #717 or #718 and 3' primer #716. The resulting PCR products (-278, -209, or -173 to +274 relative to the transcriptional start site) were gel purified, digested with *EagI* and *PstI*, ligated into pPL3-*gusA* digested with the same restriction enzymes and transformed into XL1-Blue to generate strains DH-E1829, DH-E1831, and DH-E1832. The resulting plasmids (pPL3-p_{fliN}*gusA*-278, pPL3-p_{fliN}*gusA*-209, pPL3-p_{fliN}*gusA*-173) were sequenced and transformed into the conjugative mating strain SM10 to generate strains DH-E1833, DH-E1834, and DH-E1835. The *Lm* wild-type and Δ*mogR* strains were transformed by electroporation, and the Δ*mogR*Δ*degU* strain was transformed through conjugation with SM10 to generate the following strains: p_{fliN}*gusA*-278 (DH-L1866), Δ*mogR*/p_{fliN}*gusA*-278 (DH-L1867), Δ*mogR*Δ*degU*/p_{fliN}*gusA*-278 (DH-L1868), p_{fliN}*gusA*-209 (DH-L1869), Δ*mogR*/p_{fliN}*gusA*-209 (DH-L1871), Δ*mogR*Δ*degU*/p_{fliN}*gusA*-209 (DH-L1872), p_{fliN}*gusA*-173 (DH-L1873), Δ*mogR*/p_{fliN}*gusA*-173 (DH-L1874), Δ*mogR*Δ*degU*/p_{fliN}*gusA*-173 (DH-L1811). Additional transcriptional fusions to *gusA* were generated as described above using genomic DNA from EGDe and primer pair #719 and #720 to produce a PCR product (*fliNgmaRgusA*-2) spanning ~2 kb from nucleotide 178 of *fliN* to nucleotide 127 of *flhB*; primer pair #721 and #722 to produced a PCR product (*fliNgmaRgusA*-3) spanning ~3 kb from nucleotide 127 of *flhB* to nucleotide 127 of *flhF*; primer pair #723 and #724 to produce a PCR product (*fliNgmaRgusA*-4) spanning ~3 kb from nucleotide -26 of *flhF* to nucleotide 154 of *lmo0684*; primer pair #725 and #726 to produce a PCR product (*fliNgmaRgusA*-5) spanning ~2.5 kb from nucleotide 170 of *lmo684* to nucleotide 4 of *gmaR*; and primer pair #729 and #730 to produce a PCR product (p_{cheY}*gusA*) spanning –175 nucleotides upstream of *cheY* to nucleotide 48 of *cheY*. The resulting PCR products were purified, digested with *EagI* and *PstI* restriction enzymes, ligated into pPL3-*gusA* that was previously digested with the same restriction enzymes, and transformed into XL1-Blue to generate strains DH-

E1836, DH-E1837, DH-E1838, DH-E1839, and DH-E1842. The resulting plasmids (pPL3-*fliNgmaRgusA*-2, pPL3-*fliNgmaRgusA*-3, pPL3-*fliNgmaRgusA*-4, pPL3-*fliNgmaRgusA*-5, and pPL3-p_{cheY}*gusA*) were sequenced, transformed into *Lm* EGDe by electroporation and integrated into the $tRNA^{Arg}$ locus to generate strains DH-L1812, DH-L1813, DH-L1814, DH-L1815, and DH-L1817.

To clone N-terminally His_6-tagged DegU derivatives, primer pair #535 and #486 was used with either EGDe or *Lm*DegU $D_{55}N$ genomic DNA to amplify the *degU* coding sequence or a *degU* coding sequence harboring a point mutation in codon 55 (resulting in Asp55 to Asn). The resulting PCR products were digested with *NdeI* and *SalI* and ligated to pET28a vector digested with the same enzymes. The ligations were transformed into XLI-Blue, generating strains DH-E1445 and DH-E1449. The resulting plasmids pET28a-*Lm*DegU and pET28a-*Lm*DegU $D_{55}N$ were transformed into JM109 (DE3) pLysS, yielding strains DH-E1446 and DH-E1451, respectively. For purification of *Bs*DegU, primer pair #559 and #560 were used with *Bs* PY79 genomic DNA to amplify the *Bs*DegU coding sequence. The resulting PCR product was digested with *NdeI* and *SalI* and ligated to pET28a digested with the same enzymes. Transformation of the ligation into XLI-Blue yielded strain DH-E1519 harboring pET28a-*Bs*DegU. Transformation of the plasmid into JM109 (DE3) pLysS generated strain DH-E1521, permitting IPTG-inducible expression of an N-terminally His-tagged *Bs*DegU in *E. coli*. To purify the *Bs* histidine kinase DegS, primer pair #561 and #562 was used to amplify the DegS coding sequence using *Bs* PY79 genomic DNA as the template. The resulting PCR product was digested with *NdeI* and *SalI*, ligated to pET28a digested with the same enzymes, and transformed into XLI-Blue to generate strain DH-E1522. The resulting plasmid, pET28-*Bs*DegS was transformed into JM109 (DE3) pLysS to generate strain DH-E1523, which permits tightly controlled IPTG-inducible expression of N-terminally His-tagged *Bs*DegS in *E. coli*.

Gel mobility shift analysis

Gel mobility shift analysis was performed as previously described (Shen & Higgins, 2006). Primer pairs #715 , #717, or #718 with primer #711 were used to generate the -278, -209, or -173 to +100 p*fliN-gmaR* DNA probes used for the p*fliN-gmaR* gel shift analysis. Identical DNA probes were used for analysis of p*cheY* and p*flaA* as previously described (Shen & Higgins, 2006).

Supplemental Table 1. ***Listeria monocytogenes*** **strains used in this study.**

Strain	**Genotype and relevant features**	**Strain Designation**	**Reference**
DH-L478	Wild-type *L. monocytogenes* strain EGDe	wild-type	M. Loessner
DH-L1156	*mogR* in-frame deletion in EGDe	Δ*mogR*	(Gründling *et al*., 2004)
DH-L1273	*degU* in-frame deletion in EGDe	Δ*degU*	(Shen & Higgins, 2006)
DH-L1274	*degU* in-frame deletion in DH-L1156	Δ*mogR* Δ*degU*	(Shen & Higgins, 2006)
DH-L1339	D_{55}N point mutation in *degU* in DH-L478	*Lm*DegU D_{55}N	This study
DH-L1344	*lmo1021* in-frame deletion in EGDe	Δ*lmo1021*	This study
DH-L1798	pLOV-*Lm*DegU in DH-L1273	Δ*degU*/*Lm*DegU	This study
DH-L1799	pLOV-Cterm in DH-L1273	Δ*degU*/Cterm	This study
DH-L1865	pLOV-*Bs*DegU in DH-L1273	Δ*degU*/*Bs*DegU	This study
DH-L1866	pPL3-p_{fliN}*gusA*-278 in EGDe	p_{fliN}*gusA*-278	This study
DH-L1867	pPL3-p_{fliN}*gusA*-278 in DH-L1156	Δ*mogR*/p_{fliN}*gusA*-278	This study
DH-L1868	pPL3-p_{fliN}*gusA*-278 in DH-L1274	Δ*mogR*Δ*degU*/p_{fliN}*gusA*-278	This study
DH-L1869	pPL3-p_{fliN}*gusA*-209 in EGDe	p_{fliN}*gusA*-209	This study
DH-L1871	pPL3-p_{fliN}*gusA*-209 in DH-L1156	Δ*mogR*/p_{fliN}*gusA*-209	This study
DH-L1872	pPL3-p_{fliN}*gusA*-209 in DH-L1274	Δ*mogR*Δ*degU*/p_{fliN}*gusA*-209	This study
DH-L1873	pPL3-p_{fliN}*gusA*-173 in EGDe	p_{fliN}*gusA*-173	This study
DH-L1874	pPL3-p_{fliN}*gusA*-173 in DH-L1156	Δ*mogR*/p_{fliN}*gusA*-173	This study
DH-L1811	pPL3-p_{fliN}*gusA*-173 in DH-L1274	Δ*mogR*Δ*degU*/p_{fliN}*gusA*-173	This study
DH-L1812	pPL3-*fliNgmaRgusA*-2 in EGDe	*fliNgmaRgusA*-2	This study
DH-L1813	pPL3-*fliNgmaRgusA*-3 in EGDe	*fliNgmaRgusA*-3	This study
DH-L1814	pPL3-*fliNgmaRgusA*-4 in EGDe	*fliNgmaRgusA*-4	This study
DH-L1815	pPL3-*fliNgmaRgusA*-5 in EGDe	*fliNgmaRgusA*-5	This study
DH-L1817	pPL3-p_{cheY}*gusA* in EGDe	p_{cheY}*gusA*	This study
DH-L1818	pCON1-Δ*mogR*/p_{Hy} allelic exchange in DH-L1273	Δ*mogR*Δ*degU*/p_{Hy}	This study

Supplemental Table 2. ***Escherichia coli*** **strains used in this study.**

Strain	Genotype and relevant features	Reference
DH-E121	pET28a in BL21(DE3)	Novagen
DH-E278	JM109(DE3) pLysS	Novagen
DH-E474	SM10	(Simon *et al*., 1983)
DH-E123	pCON1 in JM109	(Higgins, 2006)
DH-E182	XL1-Blue {F' *proAB lacI*^{q} *Δ(lacZ)*M15 Tn10} *RecA1 endA1 gyrA96 thi-1 hsdR17 supE relA1 lac*	Stratagene
DH-E375	CLG190 (F' *lac*, *pro*, *lacI*^{q}) Δ*malF3*, Δ*phoA*, *phoR*, Δ*lac*X74, Δ*ara*Δ*leu*7697, *araD139*, *galE, galK*, Str^R, *pcnB*, *zad*::Tn10, *recA*	D. Boyd
DH-E898	pPL3 in XL1-Blue	(Gründling *et al*., 2004)
DH-E1225	pLOV in XL1-Blue	(Higgins, 2006)
DH-E1819	pCON1-Δ*mogR*/p$_{Hy}$ in XL1-Blue	This study
DH-E1821	pCON1-Δ*mogR*/p$_{Hy}$ in SM10	This study
DH-E1352	pCON1-Δ*lmo1021* in XL1-Blue	This study
DH-E1342	pCON1- DegU-D$_{55}$N in CLG190	This study
DH-E1822	pLOV-*Lm*DegU in CLG190	This study
DH-E1823	pLOV-*Bs*DegU in CLG190	This study
DH-E1824	pLOV-*Lm*DegU-Cterm in CLG190	This study
DH-E1825	pLOV-*Lm*DegU in SM10	This study
DH-E1826	pLOV-*Bs*DegU in SM10	This study
DH-E1827	pLOV-*Lm*DegU-Cterm in SM10	This study
DH-E1445	pET28a-*Lm*DegU in XL1-Blue	This study
DH-E1446	pET28a-*Lm*DegU in JM109(DE3) pLysS	This study
DH-E1449	pET28a-*Lm*DegU D$_{55}$N in XL1-Blue	This study
DH-E1451	pET28a-*Lm*DegU D$_{55}$N in JM109(DE3) pLysS	This study
DH-E1519	pET28a-*Bs*DegU in XL1-Blue	This study
DH-E1521	pET28a-*Bs*DegU in JM109(DE3) pLysS	This study
DH-E1522	pET28a-*Bs*DegS in XL1-Blue	This study
DH-E1523	pET28a-*Bs*DegS in JM109(DE3) pLysS	This study
DH-E1828	pPL3-*gusA* in XL1-Blue	This study
DH-E1829	pPL3-p$_{fliN}$*gusA*-278 in XL1-Blue	This study
DH-E1831	pPL3-p$_{fliN}$*gusA*-209 in XL1-Blue	This study
DH-E1832	pPL3-p$_{fliN}$*gusA*-173 in XL1-Blue	This study
DH-E1833	pPL3-p$_{fliN}$*gusA*-278 in SM10	This study
DH-E1834	pPL3-p$_{fliN}$*gusA*-209 in SM10	This study
DH-E1835	pPL3-p$_{fliN}$*gusA*-173 in SM10	This study
DH-E1836	pPL3-*fliNgmaRgusA*-2 in XL1-Blue	This study
DH-E1837	pPL3-*fliNgmaRgusA*-3 in XL1-Blue	This study
DH-E1838	pPL3-*fliNgmaRgusA*-4 in XL1-Blue	This study
DH-E1839	pPL3-*fliNgmaRgusA*-5 in XL1-Blue	This study
DH-E1842	pPL3-p$_{cheY}$*gusA* in XL1-Blue	This study
DH-E1878	pHPL3-5'Δ*mogR* in XL1-Blue	This study

Supplemental Table 3. Oligonucleotides used in this study.

Number	Sequence	Site[a]
133	CGCAGCAAATGCTGTTACCGCAATCCCAGCTGTAGCCGCG	
166	AAACTGCAGAGGAGGAAAAATATGTTACGTCCTGTAGAAA	*PstI*
326	CTTGCGCTTGAGTCATGCCTTCGTTATTTTTACGAAG	
366	GGGGTACCGTCCAACAAGCACTTGGTTGGGCGGCAAATTCG	*KpnI*
368	CGGGATCCGTAGAGTTTAACTAAAATACCTCAATAACATTTTG	*BamHI*
375	ACTAAAAAATAAGAAATTCCCGCAAGTACTGCCAAAAGTAC	
464	GCTCTAGAATTCAACAATAGTGGCTAG	*XbaI*
466	GGGGTACCCTTAATTCAAATGTTACTTCTGG	*KpnI*
483[b]	AAACCAGATATTGTTTTAATG**A**ATATTAATATGCCAACTGTG	
484[b]	CACAGTTGGCATATTAATAT**T**CATTAAAACAATATCTGGTTT	
485	AACTGCAGGATAAGTGAAGGAGGAGTAG	*PstI*
486	ACGCGTCGACGAGATTTCTTTAGCGAATG	*SalI*
504	GGACTAGTAATCAGATCCAGATGCCATTC	*SpeI*
505	CTGATGATGGCTGTTTGCTCAAGTGTAGAAATAAAAATCCCA	
506	TGGGATTTTTATTTCTACACTTGAGCAAACAGCCATCATCAG	
507	GGGGTACCCGGTAACGTATTTCACGCCCA	*KpnI*
535	GGAATTCCATATGGCACTCAAAATCATGAT	*NdeI*
559	GGAATTCCATATGGTGACTAAAGTAAACATTGTTATTA	*NdeI*
560	CGGGATCCTTATCTCATTTCTACCCAGCCAT	*BamHI*
561	GGAATTCCATATGAATAAAACAAAGATGGATTCC	*NdeI*
562	CGGGATCCTTATTAAAGAGATAACGGAACCTTA	*BamHI*
706	GATAAAGTCAACAACTTTTTGGTATTCAAAGAAAATTATAAAC	
707	GTTTATAATTTTCTTTGAATACCAAAAAGTTGTTGACTTTATC	
708	ACGCGTCGACAGGAGGAAAAATGTGACTAAAGTAAACATTG	*SalI*
709	GGGGTACCATTATACTATCTCATTTCTAC	*KpnI*
710	AAAACTGCAGAGGAGGAAAAATATGAAATTAATTCGTGAATACC	*PstI*
711	CCATTCAAGTAAGGTAAATTCACCTTTCACTCCCTTC	
713	ACGCGTCGACTCATTGTTTGCCTCCCTGC	*SalI*
715	AGATACCGGCCGGCATACAATCACATACCTCTC	*EagI*
716	AAACTGCAGCCAGAAGAACTGCACGTAACTGATG	*PstI*
717	AGATACCGGCCGGTGTTAGTTGTGTAAAGGGAACGCGAG	*EagI*
718	AGATACCGGCCGGGATTGGGTATGAAGAACC	*EagI*
719	AGATACCGGCCGCATCAGTTACGTGCAGTTCTTCTGG	*EagI*
720	AAACTGCAGGAAGCCCAGCTACAATTAGCAGTG	*PstI*
721	AGATACCGGCCGCACTGCTAATTGTAGCTGGGCTTC	*EagI*
722	AAACTGCAGCAAGCTTTGTGACACGTTCGTC	*PstI*
723	AGATACCGGCCGCTTGATGAGGGAGGGAATCCGTAGCAATG	*EagI*
724	AAACTGCAGCTTTATCCCCGATTTCATAGCCAACTG	*PstI*
725	AGATACCGGCCGGGACAAACCTGGACTCGCAGAACAAATTC	*EagI*
726	AAACTGCAGGCATGAATCTTCCCTCTTTC	*PstI*
729	AGATACCGGCCGGTAAATGGGGCACAGAGCCAATG	*EagI*
730	AAACTGCAGCATCGTACGCATGAACATCTGCATG	*PstI*
731	**Biotin**-CCATTCAAGTAAGGTAAATTCACCTTTCACTCCCTTC	

732	GCGAGTTTGCTGATTACTTATTG	
733	CCTTGTTTGGTCACTTCGTC	
734	GCAGAAGTGAAAACGGAAGC	
735	CTGTTTGTTGTTGCGTTGCT	
736	AGATACCGGCCGCCATTCAAGTAAGGTAAATTCACCTTTCACTCCCTTC	*EagI*
780	AGATACCGGCCGAAGTAATTTTCTTATTTCTGATTTAGGCAT	*EagI*

a. The indicated restriction endonuclease site is underlined within the oligonucleotide sequence.
b. Deviations from the wild-type sequence are in bold and underlined within the oligonucleotide sequence.

Supplemental References

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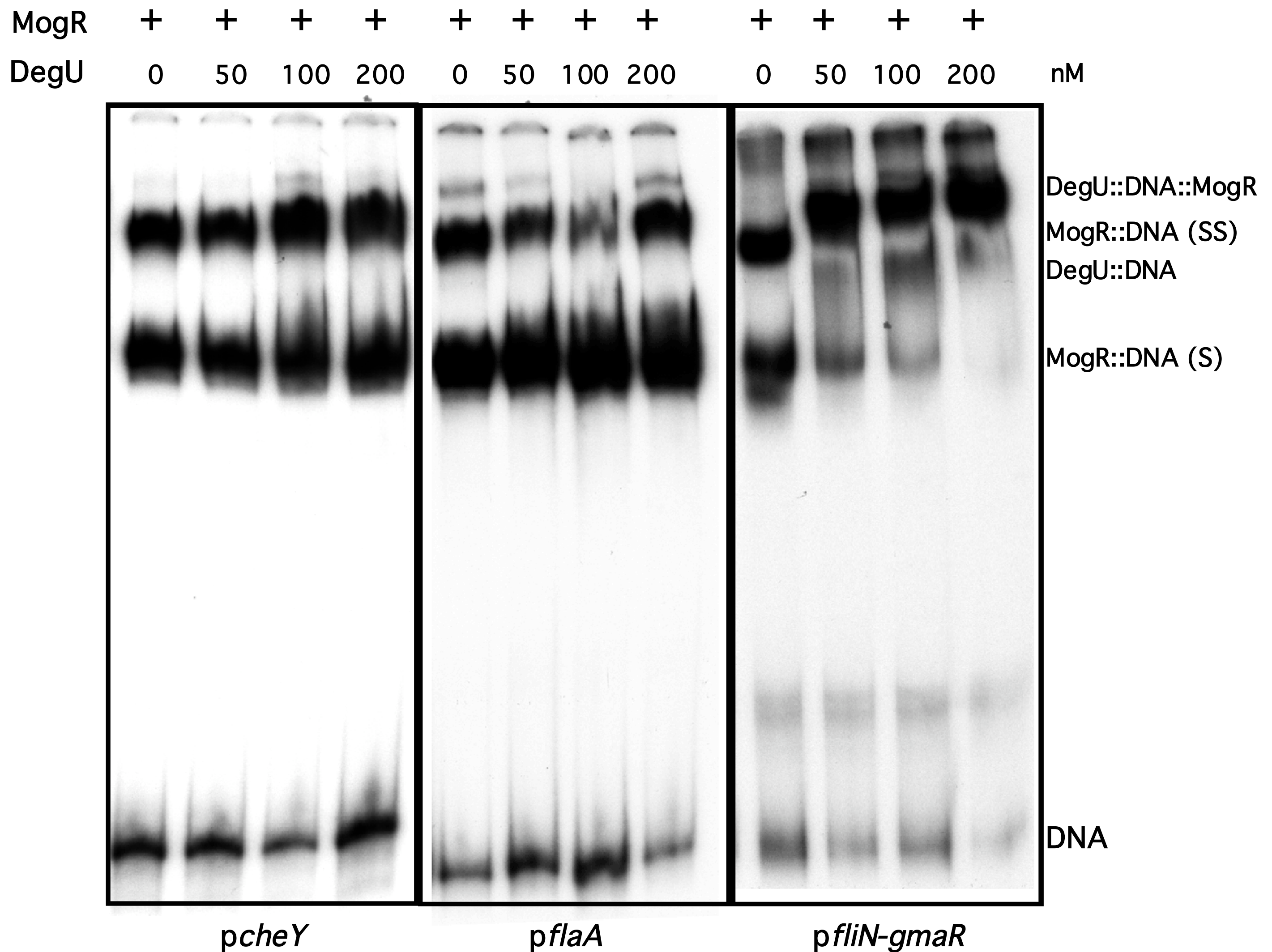

Figure S1. Gel shift analysis of MogR and DegU binding to *fliN-gmaR*, *cheY*, and *flaA* promoter region DNA. Radiolabeled promoter region DNA fragments spanning –278 to +100 for *fliN-gmaR*, -108 to +74 for *cheY*, and –162 to +8 for *flaA* (relative to the transcriptional start sites) were incubated at room temperature (RT, 18-25°C) with 20 nM MogR-His_6 for 30 min. Increasing amounts of purified His_6-tagged DegU were added for an additional 30 min at RT for a total incubation of 1 h. Binding reactions were separated by non-denaturing PAGE and detected by autoradiography. Shifted (S) and Super-shifted (SS) complexes of MogR::DNA were detected for p*fliN-gmaR*, p*cheY*, and p*flaA*. A DegU::DNA band and a DegU::DNA::MogR band were only detected for p*fliN-gmaR*.