SUPPLEMENTARY MATERIAL

Multiple Flagellin Proteins Have Distinct and Synergistic Roles in *Agrobacterium tumefaciens* Motility

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Running title: Multiple flagellins of *A. tumefaciens*

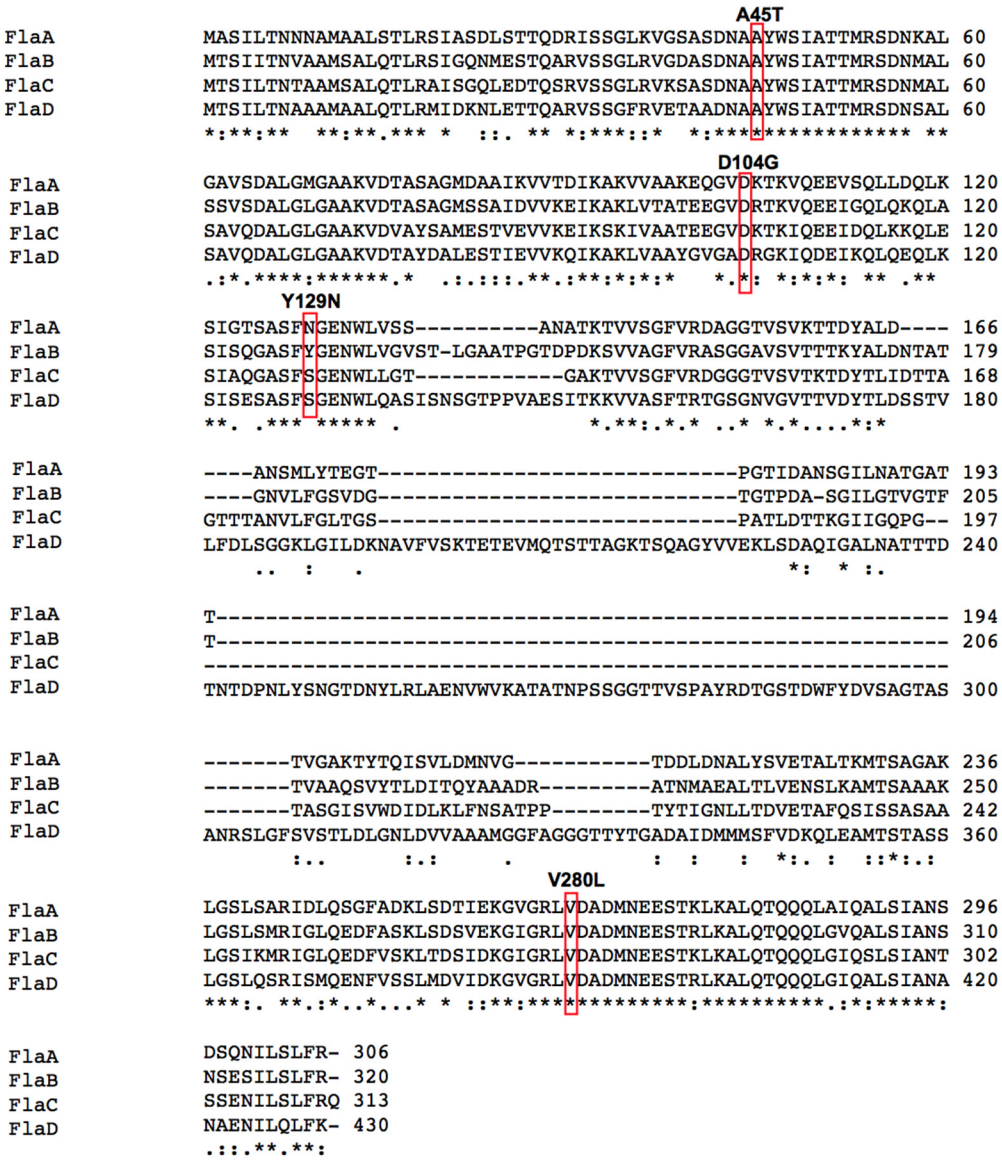


Figure S1. Amino acid sequence alignments of flagellins.

Pairwise alignments were performed with ClustalW2. Asterisks, colons and semicolons mark residues that are conserved, highly similar, and weakly similar, respectively. The alignment of *A.tumefaciens* FlaA, FlaB, FlaC and FlaD is shown. Red boxes indicate residues found to be mutated in a particular flagellin in suppressor mutants and the mutations are indicated on top of the red box: FlaBA45T, FlaDD104G, FlaBY129N and FlaBV280L.

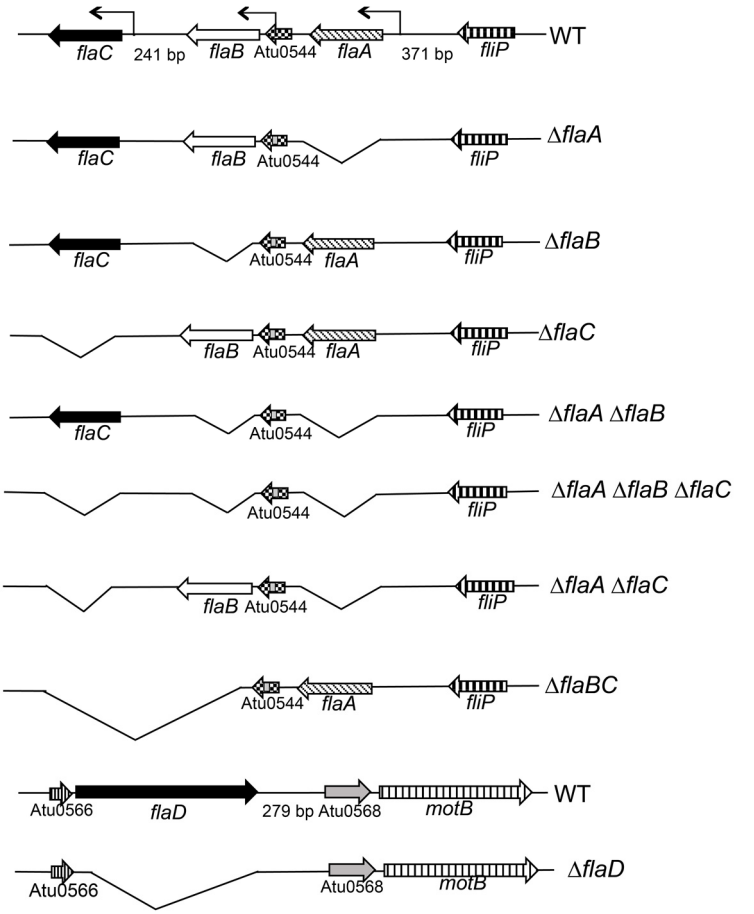
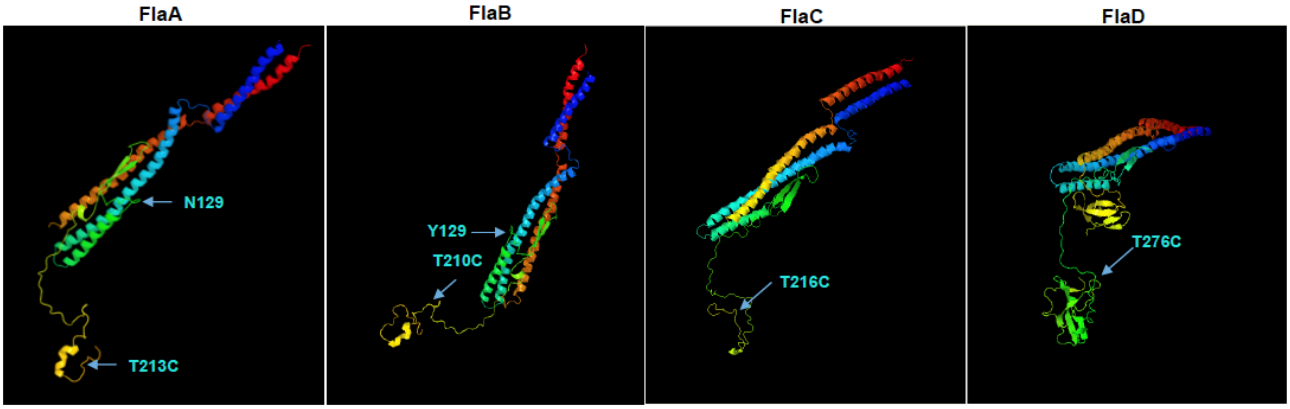


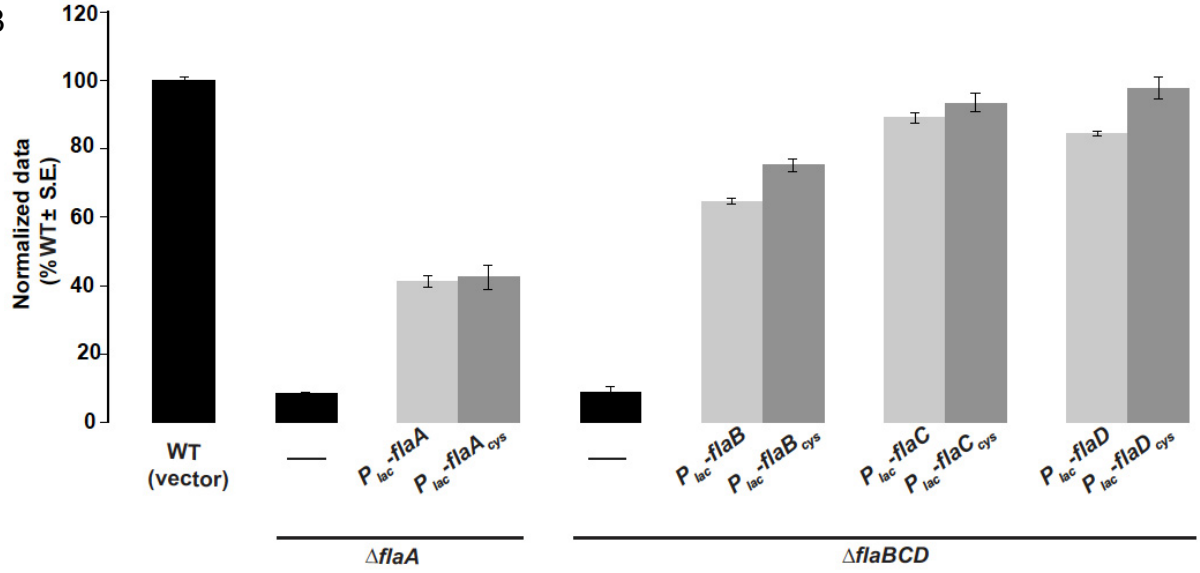
Figure S2. Construction of flagellin deletion mutants.

(A) Mutants were generated via allelic replacement (detailed in Materials and Methods) keeping in consideration that the existing genes are intact. The wild type shows the arrangement of three flagellin genes with their transcriptional start sites marked by arrows that has been mapped by Deakin et al. 1999. The bottom panels show the sequential deletion of genes. (B) *flaD* is located 15 kb away from *flaABC* in opposite orientation and its transcription start site has not been mapped. *flaD* deletion mutant was then combined with other flagellin mutants using the same strategy, in order to generate all combinations of flagellin mutants. FlaA is 306 aa, FlaB is 320 aa, FlaC is 313 aa and FlaD is 430 aa long.

A


FlaA
FlaB
FlaC
FlaD
N129
T213C
Y129
T210C
T216C
T276C

B


Normalized data
(% WT± S.E)
120
100
80
60
40
20
0
WT
(vector)
—
P lac -flaA
P lac -flaA cys
—
P lac -flaB
P lac -flaB cys
P lac -flaC
P lac -flaC cys
P lac -flaD
P lac -flaD cys
ΔflaA
ΔflaBCD

C

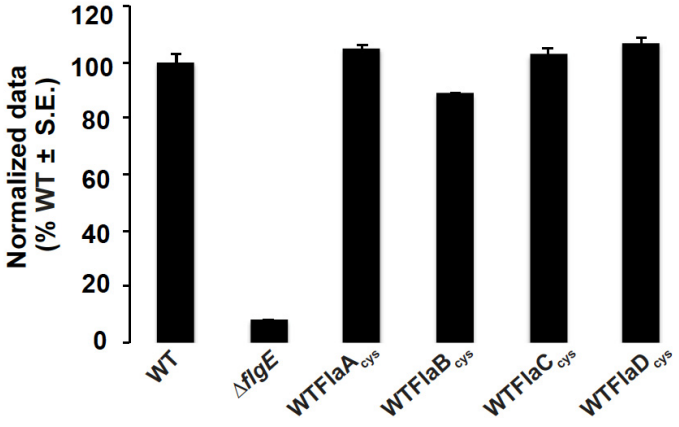

Normalized data
(% WT ± S.E.)
120
100
80
60
40
20
0
WT
ΔflgE
WTFlaA cys
WTFlaB cys
WTFlaC cys
WTFlaD cys

Figure S3. Flagellin mutants with introduced cysteine residues

(A) Phyre structural analysis of FlaA, FlaB, FlaC and FlaD. A structural model for all flagellins based on the *Salmonella* FliC flagellin (PDB 3A5ZX) structure using Phyre2. Images are rainbow colored N →C terminus. Threonine residues mutated to cysteine via site-specific mutagenesis are mapped on the flagellin structures (indicated by arrow). FlaAN129 and FlaBY129 residues are also indicated. (B) Complementation phenotype of all Δfla*BCD* mutants with plasmid borne cysteine knock-in constructs of flagellins. Swim ring formation after 7 days in the Δ*flaA* or Δ*flaBCD* mutants with plasmid borne expression of either $flaA_{Cys}$ or $flaB_{Cys}$ or $flaC_{Cys}$ expressed from P_{lac}. These constructs were all induced with 400 µm IPTG (dark grey bars). The complementation by either *flaA* or *flaB* or *flaC* are shown for comparison (light grey bars). Wild type and other controls*: ΔflaA and ΔflaBCD* are shown in black bars. $flaA_{Cys}$ refers to FlaAT213C, $flaB_{Cys}$ refers to FlaBT190C, $flaC_{Cys}$ refers to FlaCT216C and $flaD_{Cys}$ refers to FlaDT276C. Data is normalized to wild type. (C) Swim phenotype for cells having chromosomal copies of cysteine knock in flagellins. Swim ring diameter data after 7 days showing the swimming proficiency of strains that are otherwise wild type but have cysteine knock in flagellins: $FlaA_{Cys}$ or $FlaB_{Cys}$ or $FlaC_{Cys}$ or $FlaD_{Cys}$ as indicated. Values are the averages for three swim plates per strain, with error bars in SEM.

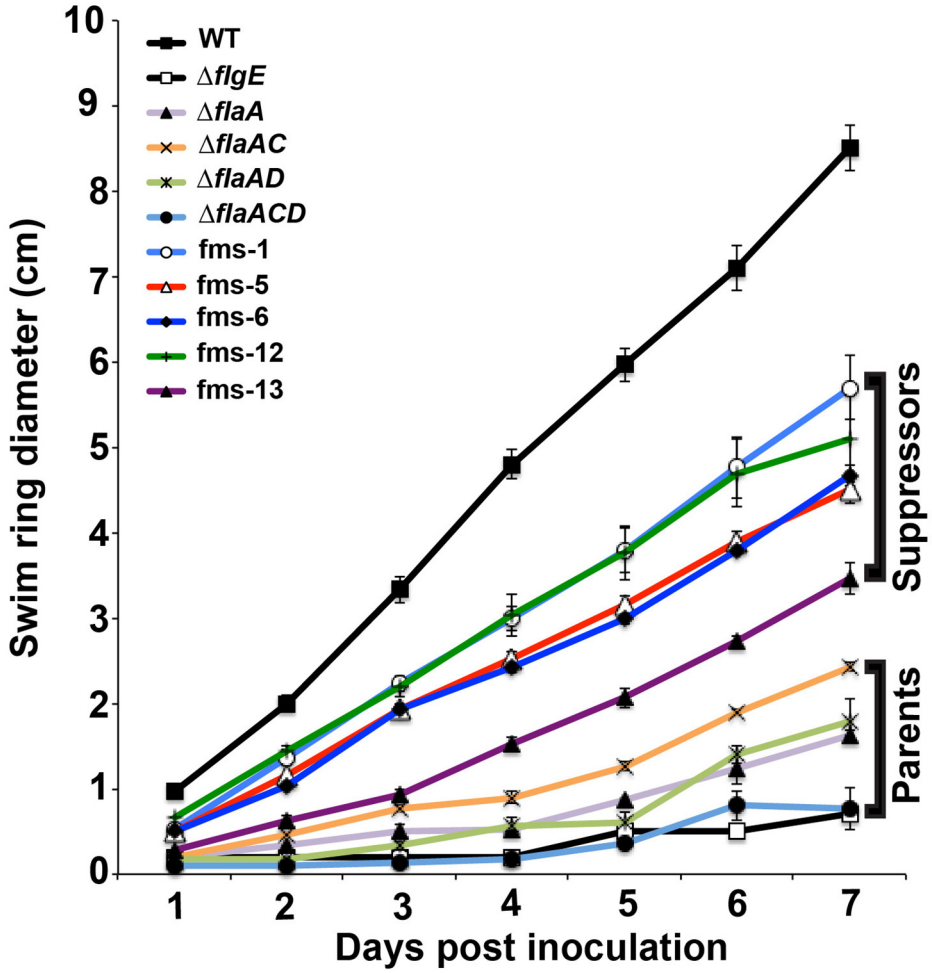


Figure S4. Motility agar migration of fms mutants.

Quantified swim ring diameters of WT and Δ*flgE* (black lines), the parent mutants: Δ*flaA* (light purple), Δ*flaAC* (orange), Δ*flaAD* (light green), Δ*flaACD* (light blue) and the suppressor mutants: fms-1 (dark blue1), fms-5 (red), fms-6 (dark blue2), fms-12 (dark green) and fms-13 (dark purple). Open marker styles are for parent mutants and filled marker styles are for suppressors. Values are the averages for three swim plates per strain. Error bars show SEM. Parentheses indicate parent and suppressor groups.

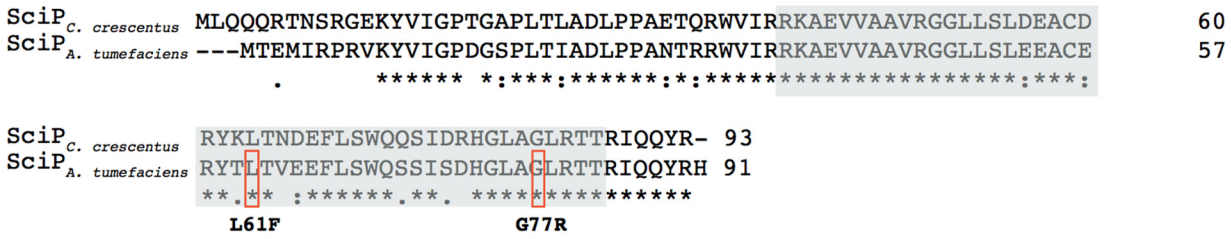


Figure S5. Amino acid sequence alignments of SciP.

Pairwise alignments were performed with Clustal Omega. Asterisks, colons and semicolons mark residues that are conserved, highly similar, and weakly similar, respectively. The alignment of *A.tumefaciens* Atu2430 and *C. crescentus* SciP is shown. Gray shaded region indicates the conserved helix turn helix motif and the red boxes indicate conserved residues found to be mutated in suppressor mutants.

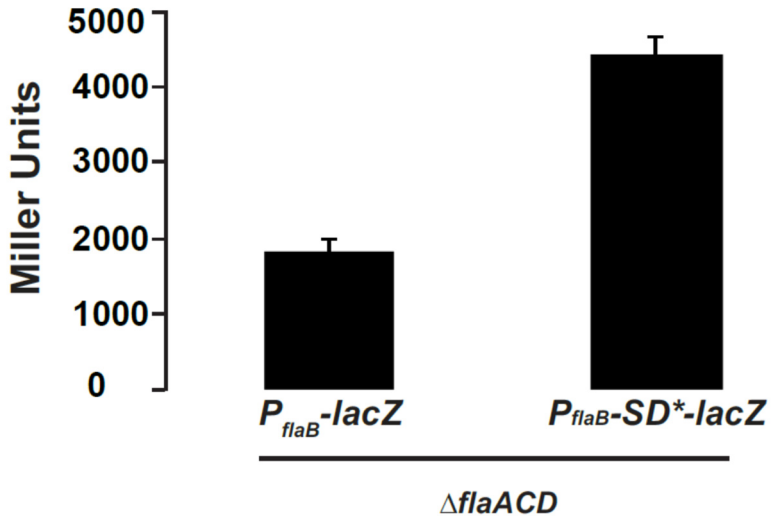


Figure S6. Beta galactosidase assay for wild type SD-*flaB* and fms-6 SD*-*flaB* in Δ*flaACD* mutants.

Expression analysis via b-galactosidase assay for P_{flaB}-*lacZ* that either includes the wild type Shine Dalgarno or the fms-6 mutation fused to *lacZ*. Expression analysis was done in the Δ*flaACD* parent mutant background from which the fms-6 suppressor had been isolated. Miller units are the averages for three replicates per strain. Error bars show SEM.

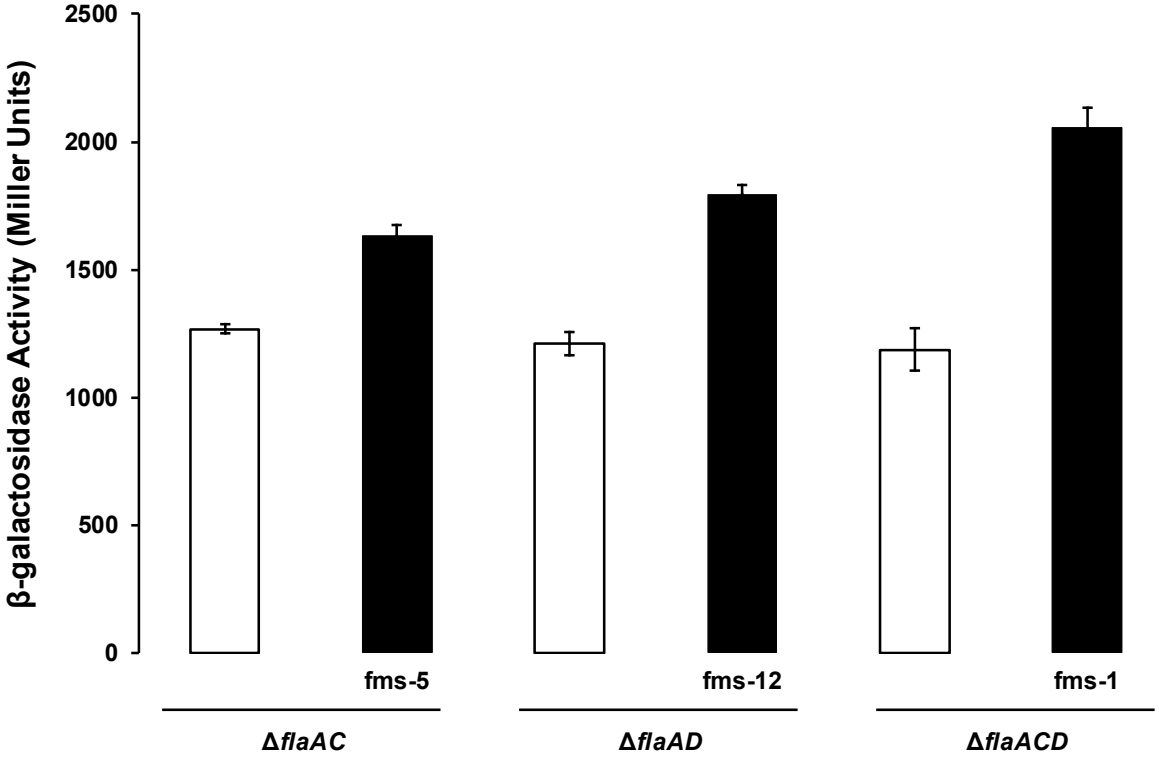


Figure S7. Expression of P_{flaB} in *sciP* fms suppressor mutants.

Expression analysis via β-galactosidase assay for plasmid-borne P_{flaB}-*lacZ* (pBM196) introduced into the suppressor mutant backgrounds where *sciP* mutations were isolated (solid bars) and their isogenic parents (open bars), as indicated under each data set. The reported Miller units are the averages for five replicates per strain, and error bars are S.E.M.

Table S1. Comparison of *A. tumefaciens* flagellin genes

Protein	Gene[a]	Length (aa)	% Sequence Identity (Conservation) with:			
			FlaA	FlaB	FlaC	FlaD
FlaA	Atu0545	306		62 (76)	60 (74)	54 (69)
FlaB	Atu0543	320			62 (77)	55 (70)
FlaC	Atu0542	313				57 (74)
FlaD	Atu0567	430				

[a] Atu numbers as available through BioCyc database (https://biocyc.org/)

Table S2. Extracted flagellin peptide signals in relative proportions to flagellin FlaB: Mass Spectrometry was conducted for three samples.

Comparison of FlaA with FlaB

FlaA peptide	Start	End	Extracted	Retention	Sample 1	Sample 2	Sample 3
Sequence	Residue	Residue	m/z range	Time	m/z Area	m/z Area	m/z Area
SIASDLSTTQDR	19	30	647.22-647.42	17.54	1.18E+09	2.45E+08	3.93E+08
VGSASDNAAYWSIATTMR	37	54	950.84-951.04	24.82	1.92E+08	1.71E+07	3.19E+07
VDTASAGMDAAIK	74	86	625.21-625.41	17.16	9.31E+08	9.29E+07	2.20E+08
IDLQSGFADK	244	253	547.18-547.38	19.73	1.29E+09	8.73E+07	2.12E+08

FlaB peptide	Start	End	Extracted	Retention	Sample 1	Sample 2	Sample 3	FlaA:FlaB	FlaA:FlaB	FlaA:FlaB
Sequence	Residue	Residue	m/z range	Time	m/z Area	m/z Area	m/z Area	Sample 1	Sample 2	Sample 3
SIGQNMESTQAR	19	30	669.21-669.41	14.28	7.39E+07	2.88E+07	6.04E+07	15.968	8.507	6.507
VGDASDNAAYWSIATTMR	37	54	964.84-965.04	25.1	1.03E+08	2.50E+07	4.88E+07	1.864	0.684	0.654
VDTASAGMSSAIDVVK	74	89	775.79-775.99	20.86	6.82E+08	6.98E+07	1.21E+08	1.365	1.331	1.819
IGLQEDFASK	258	267	554.19-554.39	19.6	9.41E+08	1.79E+08	3.39E+08	1.371	0.488	0.625

Comparison of FlaB with FlaC

FlaB peptide	Start	End	Extracted	Retention	Sample 1	Sample 2	Sample 3
Sequence	Residue	Residue	m/z range	Time	m/z Area	m/z Area	m/z Area
SIGQNMESTQAR	19	30	669.21-669.41	14.28	7.39E+07	2.88E+07	6.04E+07
SDNMA-combo	55	73	903.85-904.05	26.43	4.36E+08	2.14E+08	1.39E+08
VDTASAGMSSAIDVVK	74	89	775.79-775.99	20.86	4.60E+08	2.52E+07	2.67E+07
LVTATEEGVDR	95	105	595.21-595.41	15.09	4.97E+08	1.49E+08	2.13E+08
IGLQEDFASK	258	267	554.19-554.39	19.6	9.41E+08	1.79E+08	3.39E+08
SVVAGFVR	152	159	417.64-417.84	19.2	4.50E+08	5.50E+07	6.39E+07

FlaC peptide	Start	End	Extracted	Retention	Sample 1	Sample 2	Sample 3	FlaC:FlaB	FlaC:FlaB	FlaC:FlaB
Sequence	Residue	Residue	m/z range	Time	m/z Area	m/z Area	m/z Area	Sample 1	Sample 2	Sample 3
AISGQLEDTQSR	19	30	652.73-652.93	15.04	7.29E+07	2.47E+07	4.56E+07	0.985	0.856	0.754
SDNMA-combo	55	73	916.36-916.56	28.55	4.43E+07	3.84E+07	3.00E+07	0.101	0.180	0.216
VDVAYSAMESTVEVVK	74	89	863.83-864.03	36.38	1.40E+07	5.10E+06	1.20E+06	0.030	0.202	0.045
IVAATEEGVDK	95	105	680.77-680.97	13.46	1.13E+07	1.16E+07	1.26E+07	0.023	0.078	0.059
IGLQEDFVSK	250	259	568.2-568.4	20.55	9.45E+07	2.76E+07	3.42E+07	0.100	0.154	0.101
TVVSGFVR	141	148	432.65-432.85	18.7	9.00E+08	6.71E+07	2.00E+08	2.000	1.219	3.129

Comparison of FlaB with FlaD

FlaB	Start	End	Extracted	Retention	Sample 1	Sample 2	Sample 3
Sequence	Residue	Residue	m/z range	Time	m/z Area	m/z Area	m/z Area
VDTASAGMSSAIDVVK	74	89	775.79-775.99	20.86	4.60E+08	2.52E+07	2.67E+07
LVTATEEGVDR	95	105	595.21-595.41	15.09	4.97E+08	1.49E+08	2.13E+08

FlaD	Start	End	Extracted	Retention	Sample 1	Sample 2	Sample 3	FlaD:FlaB	FlaD:FlaB	FlaD:FlaB
Sequence	Residue	Residue	m/z range	Time	m/z Area	m/z Area	m/z Area	Sample 1	Sample 2	Sample 3
VDTAYDALESTIEVVK	74.00	89.00	876.85-877.05	27.01	1.14E+07	1.50E+07	1.80E+07	0.025	0.595	0.674
LVAAYGVGADR	95.00	105.00	546.2-546.4	17.56	2.13E+07	5.20E+06	8.54E+06	0.043	0.035	0.040

Table S3. Strains and plasmids used in this study

Strain or Plasmid	Relevant feature(s)	Source or Reference
***A. tumefaciens* strains**		
C58	Nopaline type strain, pTiC58, pAtC58	(1)
BM110	Δ*flaB* C58 derivative	This study
BM111	Δ*flaD* C58 derivative	This study
BM114	Δ*flaD,* Δ*flaB* derivative	This study
BM117	Δ*flaA* C58 derivative	This study
BM118	Δ*flaC* C58 derivative	This study
BM120	Δ*flaA,* Δ*flaC* derivative	This study
BM121	Δ*flaA,* Δ*flaD* derivative	This study
BM122	Δ*flaC,* Δ*flaD* derivative	This study
BM124	Δ*flaD,* Δ*flaA* Δ*flaC* derivative	This study
BM126	Δ*flaBC,* C58 derivative	This study
BM128	Δ*flaBC,* Δ*flaD* derivative	This study
BM129	C58 (FlaA T213C), C58 derivative	This study
BM135	C58 (FlaD T276C), C58 derivative	This study
BM136	Δ*flaA* Δ*flaB* C58 derivative	This study
BM139	Δ*flaA* Δ*flaB* Δ*flaC,* C58 derivative	This study
BM140	Δ*flaD,* Δ*flaA* Δ*flaB* Δ*flaC* derivative	This study
BM141	Δ*flaD*, Δ*flaA* Δ*flaB* derivative	This study
BM143	C58 (FlaC T216C), C58 derivative	This study
BM149	C58 (FlaB T190C), C58 derivative	This study
BM150	Δ*flaA*, C58 derivative without the base change	This study
BM151	fms-5 *flaD* mutation replaced in native Δ*flaAC* background	This study
BM152	fms-12 *flaB* mutation replaced in native Δ*flaAD* background	This study
BM153	fms-13 *flaB* mutation replaced in native Δ*flaA* background	This study
BM154	fms-6 SD* +*flaB* mutation replaced in native Δ*flaACD* background	This study

BM157	fms-5 *flgE* mutation replaced in native Δ*flaAC* background	This study
BM158	fms-5 *flgE and flaD* mutation replaced in native Δ*flaAC* background	This study
BM159	FlaBT190C replaced in fms-6 suppressor	This study
BM160	FlaBT190C replaced in fms-2 suppressor	This study
BM161	FlaBT190C replaced in fms-13 suppressor	This study
BM162	fms-1 *flaB* mutation replaced in native Δ*flaACD* background	This study
BM163	fms-12 *sciP* mutation replaced in BM152	This study
BM164	fms-1 *sciP* mutation replaced in BM162	This study
Δ*flaA* Δ*flaC* Δ*flaD,* fms-1	Spontaneous suppressor of Δ*flaA* Δ*flaC* Δ*flaD*	This study
Δ*flaA* Δ*flaC,* fms-2	Spontaneous suppressor of Δ*flaA* Δ*flaC*	This study
Δ*flaA* Δ*flaC,* fms-5	Spontaneous suppressor of Δ*flaA* Δ*flaC*	This study
Δ*flaA* Δ*flaC* Δ*flaD,* fms-6	Spontaneous suppressor of Δ*flaA* Δ*flaC* Δ*flaD*	This study[i]
Δ*flaA* Δ*flaD,* fms-12	Spontaneous suppressor of Δ*flaA* Δ*flaD*	This study
Δ*flaA,* fms-13	Spontaneous suppressor of Δ*flaA*	This study
***E.coli* strains**		
DH5α λ*pir*	Cloning host	(2)
S17-1 λ*pir*	Cloning host	(3)
TOP10 F’	Cloning host	Invitrogen
Plasmids		
pGEM-T Easy	PCR cloning vector; Amp^r	Promega
pNPTS138	colE1 origin; *sacB*; Km^r	gift of M. Alley
pRK2013	Self transmissible tra+, helper	(4)
pRK2073	Self transmissible tra+, helper	(5)
pSRKKm	Broad host range P_{lac} expression vector; $lacI^q$; $lacZ\alpha^+$; Km^r	(6)
pRA301	Broad host range; Promoterless *lacZ* ; Sp^r	(7)

pBM120	pGEM-T Easy carrying *flaB* deletion construct	This study
pBM121	pGEM-T Easy carrying *flaD* deletion construct	This study
pBM122	pNPTS138 carrying *flaB* deletion construct	This study
pBM123	pNPTS138 carrying *flaD* deletion construct	This study
pBM126	pNPTS138 carrying *flaA* deletion construct	This study
pBM127	pGEM-T Easy carrying *flaC* deletion construct	This study
pBM128	pGEM-T Easy carrying *flaD* for complementation	This study
pBM129	pNPTS138 carrying *flaC* deletion construct	This study
pBM136	pGEM-T Easy carrying *flaBC* deletion construct	This study
pBM138	pNPTS138 carrying *flaBC* deletion construct	This study
pBM140	pGEM-T Easy carrying *flaA* T213C for allelic replacement and complementation	This study
pBM143	pSRKKm P_{lac}- *flaA* T213C	This study
pBM145	pNPTS138 carrying *flaA* T213C for allelic replacement	This study
pBM147	pGEM-T Easy carrying *flaC* for complementation	This study
pBM149	pNPTS138 carrying *flaD* T276C for allelic replacement	This study
pBM150	pGEM-T Easy carrying *flaAB* deletion construct	This study
pBM152	pNPTS138 carrying *flaAB* deletion construct	This study
pBM154	pSRKKm P_{lac}-*flaD*	This study
pBM157	pGEM-T Easy carrying *flaABC* deletion construct	This study
pBM158	pNPTS138 carrying *flaABC* deletion construct	This study
pBM161	pSRKKm P_{lac}–*flaC*	This study
pBM165	pNPTS138 carrying *flaC* T216C for allelic replacement	This study
pBM173	pSRKKm P_{lac}–*flaA*	This study
pBM181	pGEM-T Easy carrying P_{flaA} + *flaA*	This study

pBM183	pSRKKm P_{lac} - P_{flaA} - *flaA*	
pBM185	pGEM-T Easy carrying *flaC*T216C for allelic replacement and complementation	This study
pBM186	pGEM-T Easy carrying *flaD*T276C for allelic replacement and complementation	This study
pBM187	pGEM-T Easy carrying *flaB*T190C for allelic replacement and complementation	This study
pBM188	pSRKKm - P_{lac} -*flaC*T216C	This study
pBM189	pSRKKm - P_{lac} -*flaD*T276C	This study
pBM190	pSRKKm - P_{lac} -*flaB*T190C	This study
pBM192	pNPTS138 carrying *flaBT190C* for allelic replacement	This study
pBM193	pGEM-T Easy carrying P_{flaB}	This study
pBM195	pGEM-T Easy carrying fms-6 P_{flaB}	This study
pBM196	pRA301- P_{flaB} - *lacZ*	This study
pBM198	pRA301- fms-6 P_{flaB} - *lacZ*	This study
pBM199	pGEM-T Easy carrying P_{flaA} - *flaAT213C* for complementation	This study
pBM205	pSRKKm P_{lac} - P_{flaA} *flaA*T213C	This study
pBM207	pGEM-T Easy carrying fms-6 (US of *flaB* + *flaB*) fragment for allelic replacement	This study
pBM208	pGEM-T Easy carrying fms-5 (*flaD*) fragment for allelic replacement	This study
pBM209	pGEM-T Easy carrying fms-5 *(flgE)* fragment for allelic replacement	This study
pBM210	pGEM-T Easy carrying fms-12 (*flaB*) fragment for allelic replacement	This study
pBM211	pGEM-T Easy carrying fms-13 (*flaB*) fragment for allelic replacement	This study

pBM214	pNPTS138 carrying fms-5 *(flaD)* fragment for allelic replacement	This study
pBM215	pNPTS138 carrying fms-5 *(flgE)* fragment for allelic replacement	This study
pBM216	pNPTS138 carrying fms-12 (*flaB*) fragment for allelic replacement	This study
pBM217	pNPTS138 carrying fms-13 (*flaB*) fragment for allelic replacement	This study
pBM219	pGEM-T Easy carrying *flaA* deletion fragment	This study
pBM221	pNPTS138 carrying *flaA* deletion construct	This study
pBM222	pSRKKm P_{lac} - *flaB*	This study
pBM223	pNPTS138 carrying fms-6 (US of *flaB* + *flaB*) fragment for allelic replacement	This study
pBM225	pGEM-T Easy carrying *flaB*T190C for allelic replacement in fms-13	This study
pBM227	pNPTS138 - *flaB*T190C for allelic replacement in fms-13	This study
pBM228	pGEMT-Easy carrying P_{flaA} - *flaB*T190C for complementation	This study
pBM230	pSRKKm - P_{lac}- P_{flaA}- *flaB*T190C	This study
pBM232	pGEM-T Easy carrying fms-1 Y129N for allelic replacement	This study
pBM233	pNPTS138 carrying fms-1 Y129N for allelic replacement	This study
pBM234	pGEM-T Easy carrying P_{flaA}- *flaB*Y129N+T190C for complementation	This study
pBM236	pSRKKm - P_{lac}- P_{flaA}- *flaB*Y129N+T190C for complementation	This study
pBM237	pGEM-T Easy carrying Atu2430 C181T fragment for allelic replacement	This study

pBM238	pGEM-T Easy carrying Atu2430 G229C fragment for allelic replacement	This study
pBM239	pNPTS138 carrying Atu2430 C181T fragment for allelic replacement	This study
pBM240	pNPTS138 carrying Atu2430 G229C fragment for allelic replacement	This study
pBM243	pGEM-T Easy carrying P_{flaB}-SD*-*flaB*Y129N+T190C for complementation	This study
pBM244	pGEM-T Easy carrying P_{flaB}-SD*-*flaB*T190C for complementation	This study
pBM246	pSRKKm - P_{lac} - P_{flaB} - SD*-*flaB*T190C for complementation	This study
pBM251	pGEM-T Easy carrying P_{flaB} - *flaB*Y129N+T190C for complementation	This study
pBM253	pSRKKm - P_{lac} - P_{flaB} - *flaB*Y129N+T190C for complementation	This study
pBM254	pSRKKm - P_{lac} - P_{flaB} - SD_2-*flaB*Y129N+T190C for complementation	This study
pBM256	pGEM-T Easy carrying P_{flaA} – *flaA*N129Y+T213C for complementation	This study
pBM257	pGEM-T Easy carrying P_{flaB}-*flaB*T190C for complementation	This study
pBM258	pSRKKm - P_{lac} - P_{flaA} – *flaA*N129Y+T213C for complementation	This study
pBM259	pSRKKm - P_{lac} - P_{flaB}-*flaB*T190C for complementation	This study

TABLE S4. Primers used in this study

Primer	Sequence	Use
flaA P1	actagt GCATGATGATGCTGCCGC	P1 for *flaA* SOE deletion fragment
flaA P2	*AAGCTTGGTACCGAATTC* GTT GTT GGT CAG AAT GCT TGC CAT	P2 for *flaA* SOE deletion fragment
flaA P3	*GAA TTC GGT ACC AAG CTT* CTCTTCCGCTAAGAGCCGAGC	P3 for *flaA* SOE deletion fragment
flaA P4	gcatgc CCT GGG TGG ATT CCA TGT TCT GGC	P4 for *flaA* SOE deletion fragment
flaB P1	actagt GCT CTG ACG AAG ATG ACC AGC	P1 for *flaB* SOE deletion fragment
flaB P2	*AAGCTTGGTACCGAATTC* GAT AAT GCT CGT CAT AGT AGT GTG CCC	P2 for *flaB* SOE deletion fragment
flaB P3	*GAATTCGGTACCAAGCTT* TCG CTC TTC CGT TAA TCG AAA GCT	P3 for *flaB* SOE deletion fragment
flaB P4	gcatgc GTC GAC ACC CTC TTC GGT TGC	P4 for *flaB* SOE deletion fragment
flaC P1	actagt CTTCCAAGCTGTCCGACTCCG	P1 for *flaC* SOE deletion fragment
flaC P2	*AAGCTTGGTACCGAATTC* ACT TGT CAT AAT TGC CCC TCT GAG CTG	P2 for *flaC* SOE deletion fragment
flaC P3	*GAA TTC GGT ACC AAG CTT* TC GCT ATT CCG CCA GTA AGC G	P3 for *flaC* SOE deletion fragment
flaC P4	gcatgc GCC CGA ACC TGC TGT TTG C	P4 for *flaC* SOE deletion fragment
flaD P1	actagt CTC GAT CTC GAA CTC AAG CCG	P1 for *flaD* SOE deletion fragment
flaD P2	*AAGCTTGGTACCGAATTC* GCT TGT CAT GGG GTC CAC	P2 for *flaD* SOE deletion fragment
flaD P3	*GAATTCGGTACCAAGCTT* CAG CTC TTC AAG TAA CGG GTT TCG	P3 for *flaD* SOE deletion fragment
flaD P4	gggccc ATC GAT ACC TTC ACG CAG ACG	P4 for *flaD* SOE deletion fragment

flaB P2-2	AGC TTT CGA TTA ACG GAA GAG CGA *GAT AAT GCT CGT CAT AGT AGT GTG CCC*	P2 for *flaB* SOE deletion fragment for creating Δ*flaAB* and Δ*flaABC*
flaB P3-2	*GGG CAC ACT ACT ATG ACG AGC ATT ATC* TCG CTC TTC CGT TAA TCG AAA GCT	P3 for *flaB* SOE deletion fragment for creating Δ*flaAB* and Δ*flaABC*
flaC P2-2	CGC TTA CTG GCG GAA TAG CGA *ACT TGT CAT AAT TGC CCC TCT GAG CTG*	P2 for *flaC* SOE deletion fragment for creating Δ*flaAB* and Δ*flaABC*
flaC P3-2	*CAG CTC AGA GGG GCA ATT ATG ACA AGT* TCG CTA TTC CGC CAG TAA GCG	P3 for *flaC* SOE deletion fragment for creating Δ*flaAB* and Δ*flaABC*
fms-2/fms-6 AR P1	actagt CGG GTT TGG TTT CTG GAA CTA AGC	P1 for allelic replacement of fms-2/ fms-6 mutated allele
fms-2/fms-6 AR P2	gcatgc GCG TAT TTC GTG GTC GTA ACG	P2 for allelic replacement of fms-2/ fms-6 mutated allele
fms-5 (flaD) AR P1	actagt TGC CGG TTT ATG CGA AAC CCG	P1 for allelic replacement of fms-5 (*flaD*) mutated allele
fms-5 (flaD) AR P2	gcatgc GAG GGA ACG ATT GGC AGA AGC	P2 for allelic replacement of fms-5 (*flaD*) mutated allele
fms-12 AR P1	actagt AAG GTT CAG GAA GAA ATC GGC	P1 for allelic replacement of fms-12 mutated allele
fms-12 AR P2	gcatgc GCG GAG AGA GCC ATG TTG TCG	P2 for allelic replacement of fms-12 mutated allele

fms-13 AR P1	actagt GATCCATTTCTTCCCACAAGC	P1 for allelic replacement of fms-13 mutated allele
fms-13 AR P2	gcatgc GCC TTA AGC GAG TTT TCA ACC AGC	P2 for allelic replacement of fms-13 mutated allele
fms-1 AR P1	actagt CCTAATGCTCATCGAAACGGC	P1 for allelic replacement of fms-1 mutated allele
fms-1 AR P2	gcatgc ACA GGA TGC TTT CGG AGT TGC	P2 for allelic replacement of fms-1 mutated allele
Atu2430 AR P1	actagt TCTCGTCATGCTGTCACTGGC	P1 for allelic replacement of Atu2430 mutated allele
Atu2430 AR P2	gcatgc TCT ACT CTC AGA CAG AGG ATA GCG	P2 for allelic replacement of Atu2430 mutated allele
fms-5 (flgE) AR P1	actagt ATT TCT ACT ACA CCA AGA CCG GCG	P1 for allelic replacement of fms-5 (*flgE*) mutated allele
fms-5 (flgE) AR P2	gcatgc CTT GAG CAG TTC TTT CAT ATA GTT GGC	P2 for allelic replacement of fms-5 (*flgE*) mutated allele
flaBT190C AR P1	actagt CTT CCG TTT CCG ACG CTC TCG	For allelic replacement of FlaBT190C mutated allele in fms-13
flaBT190C AR P2	gcatgc GGA AAC GAC TGA GCG GCT TGA TGC	For allelic replacement of FlaBT190C

		mutated allele in fms-13
PflaB +flaB - P1	*ggcagc* actagt TGA GGC CTT ATC TTT TTT TTA AAC TTT TGC	For cloning *flaB* along with its native promoter
flaA N129Y mutagenic - P2	CGA TCG GCA CGA GCG CGT CTT TCT ACG GTG AAA ACT GGC TCG TTT CCA	Forward mutagenic primer
flaA N129Y mutagenic - P3	TGG AAA CGA GCC AGT TTT CAC CGT AGA AAG ACG CGC TCG TGC CGA TCG	Reverse mutagenic primer
(PflaB+flaB) wt - P2	GAC ATT CGT GAT AAT GCT CGT CAT AGT AGT GTG CCC CTT GAA TGG CTG ATT	Reverse primer for amplifying across the Shine Dalgarno of *flaB* – FlaB coding sequence junction
(PflaB+flaB) wt - P3	AAT CAG CCA TTC AAG GGG CAC ACT ACT ATG ACG AGC ATT ATC ACG AAT GTC	Forward primer for amplifying across the Shine Dalgarno of *flaB* – FlaB coding sequence junction
(PflaB+flaB) fms-6 - P2	GAC ATT CGT GAT AAT GCT CGT CAT AGT AGT GTG CCT CTT GAA TGG CTG ATT	Reverse primer for amplifying across the Shine Dalgarno of *flaB* – FlaB coding sequence junction
(PflaB+flaB) fms-6 - P3	AAT CAG CCA TTC AAG AGG CAC ACT ACT ATG ACG AGC ATT ATC ACG AAT GTC	Forward primer for amplifying across the Shine Dalgarno of *flaB* – FlaB coding sequence junction
flaA T213C mutagenic F	CGACATGAACGTCGGCTGTGACGAC CTCGACAACGC	Reverse mutagenic primer

flaA T213C mutagenic R	GCG TTG TCG AGG TCG TCA CAG CCG ACG TTC ATG TCG	Forward mutagenic primer
flaB T190C mutagenic F	CGG TTC GGT CGA CGG TTG TGG CAC TCC CGA CGC	Reverse mutagenic primer
flaB T190C mutagenic R	GCG TCG GGA GTG CCA CAA CCG TCG ACC GAA CCG	Forward mutagenic primer
flaC T216C mutagenic F	GCTGTTCAATTCTGCATGTCCGCCGA CCTACACCATCG	Reverse mutagenic primer
flaC T216C mutagenic R	CGA TGG TGT AGG TCG GCG GAC ATG CAG AAT TGA ACA GC	Forward mutagenic primer
flaD T276C mutagenic F	CGT CTA GCG GCG GCT GTA CCG TTT CCC CTG C	Reverse mutagenic primer
flaD T276C mutagenic R	GCA GGG GAA ACG GTA CAG CCG CCG CTA GAC G	Forward mutagenic primer
flaA comp P1	*ggcagc* catatg GCA AGC ATT CTG ACC AAC AAC	*flaA* cloning
flaA comp P2	*tctaga* ggtacc TTA GCG GAA GAG CGA CAG GAT	*flaA* cloning
flaB comp P1	*ggcagc* catatg ACG AGC ATT ATC ACG AAT GTC	*flaB* cloning
flaB comp P2	t*ctaga* ccgcgg TTA ACG GAA GAG CGA CAG GAT	*flaB* cloning
flaB comp P2- 2	*tctaga* gctagc TTA ACG GAA GAG CGA CAG GAT	*flaB* cloning along with its native promoter
flaC comp P1	*ggcagc* catatg ACA AGT ATT CTG ACG AAC ACC	*flaC* cloning
flaC comp P2	*tctaga* ggtacc TTA CTG GCG GAA TAG CGA CAG	*flaC* cloning
flaD comp P1	*ggcagc* catatg ACA AGC ATT TTG ACC AAT GCG	*flaD* cloning
flaD comp P2	*tctaga* ggtacc TTA CTT GAA GAG CTG GAG GAT ATT	*flaD* cloning
PflaA+flaA P1	*ggcagc* actagt TGA CGGCCCTTATACCCCGCT	

		flaA cloning along with its native promoter
PflaB-lacZ P1	*gagctc* ggtacc CTC TTC CGC TAA GAG CCG AGC	Forward cloning promoter of *flaB* fused to *lacZ*
PflaB-lacZ P2	*ctgcag* gcatgc CAT GTT CTG GCC GAT AGA GCG	Reverse cloning promoter of *flaB* fused to *lacZ*
flaA seq F1	TGC GAA ATT CTT CCC ACA CGC AGC	Sequencing primer for *flaA* locus
flaA seq F2	CCGCCATCAAGGTCGTGACCG	Sequencing primer for *flaA* locus
flaA seq R1	CGC TGG TCA TCT TCG TCA GAG CG	Sequencing primer for *flaA* locus
flaA seq R2	ATA AGG CCG CAT CCG TTT CCG	Sequencing primer for *flaA* locus
flaB seq F1	CCGACAACATGGCTCTCTCTTCCG	Sequencing primer for *flaB* locus
flaB seq R1	AAC GGA AGA GCG ACA GGA TGC	Sequencing primer for *flaB* locus
flaB seq F2	CGGAAACGGATGCGGCCTTAT	Sequencing primer for *flaB* locus
flaB seq F3	CAG AAG CAG CTC GCA TCG ATC TCG	Sequencing primer for *flaB* locus
flaB seq R3	CGA GAT CGA TGC GAG CTG CTT CTG	Sequencing primer for *flaB* locus
flaC seq F1	GAA GTC GTC AAG GAA ATC AAG TCG	Sequencing primer for *flaC* locus
flaC seq R1	ATA TTC TGT TGC TGC CAG AGC	Sequencing primer for *flaC* locus
flaC seq F2	AGA GTT CAT TAA CCA AAT CAC GGC	Sequencing primer for *flaC* locus
flaC seq R2	CCC ACT GGA TGA AGA ATA TCG	Sequencing primer for *flaC* locus
flaC seq R3	GCC GTG ATT TGG TTA ATG AAC TCT	Sequencing primer for *flaC* locus

flaD seq F1	GCA TCC TTC ACC AGG ACC GGC	Sequencing primer for *flaD* locus
flaD seq R1	CCT TGT CGA TCA CAT CCA TCA AGC TCG	Sequencing primer for *flaD* locus
flaD seq F2	TGT CAT CGG GCT TTC GTG TCG	Sequencing primer for *flaD* locus
flaD seq R2	CTG ACA AAA ACG GCG TTC TTA TCG	Sequencing primer for *flaD* locus
flgE seq F1	CGA GCC AGA TCA AGG ATG TCG	Sequencing primer for *flgE* locus
flgE seq R2	GGT TCG CTT GAC GTA GTT CGC	Sequencing primer for *flgE* locus
flgE seq F2	GAACGCTGCTGGCTTCCAGC	Sequencing primer for *flgE* locus
flgE seq reverse	GTCGTGAGCTTGGTGGC	Sequencing primer for *flgE* locus
flaF seq F1	GTCGCCATGAAGTCGGTGACG	Sequencing primer for *flaF* locus
flaF seq R1	CGC TTC AGT TCC GCC AGA ATC TCG	Sequencing primer for *flaF* locus
flbT seq F1	GAGGCAAGGGACTCCTCTTCG	Sequencing primer for *flbT* locus
flbT seq R1	GGT GGG TGT TGG TCT TGA TCG	Sequencing primer for *flbT* locus
flbT seq F2	GAA TGT ACC AGT TTT CCT ACG CCG	Sequencing primer for *flbT* locus
flbT seq R2	TGT AGC AGC ACT TTA TCA CCG	Sequencing primer for *flbT* locus
Atu2430 seq F1	CGACCTGCTTAGGCAATTTTTAAAGG TACG	Sequencing primer for Atu2430 locus
Atu2430 seq R1	GCT ATT CGT TTT TTC CAA TGT AAC GGC	Sequencing primer for Atu2430 locus
Atu2430 seq R2	AGG TCA TCT GGG CCT TGT GGC	Sequencing primer for Atu2430 locus
Atu0544 seq F1	AGA GTC CAC CAA GCT GAA GGC	Sequencing primer for Atu0544 locus

Atu0544 seq R1	CGA GCT CAT ACC GGC GGA AGC	Sequencing primer for Atu0544 locus
Atu0544 seq R2	CGT TAG CTC GCC GTT TCG ATG AGC	Sequencing primer for Atu0544 locus
pUC/M13F	CGCCAGGGTTTTCCCAGTCACGAC	pGEM-T Easy and pNPTS138 sequencing
pUC/M13R	TCACACAGGAAACAGCTATGAC	pGEM-T Easy and pNPTS138 sequencing
JEH89	GCG TTG GCC GAT TCA TTA ATG CA	pSRKKm sequencing
JEH90	GTC AAT TAT TAC CTC CAC GGG GA	pSRKKm sequencing

Lowercase indicates engineered restriction sites.

Italics indicate overlapping sequences for respective P2/P3 primers used to generate SOE deletion fragments.

Lowercase italics indicate protector sequences added for ease of direct cloning to pSRKKm.

seq refers to sequencing primers.

Underline indicates the base mutagenized via site-directed mutagenesis.

Artificial stop codons highlighted in yellow.

P1 = forward primer, P2 = reverse primer, P3 = forward primer, P4 = reverse primer, F = forward primer and R = reverse primer, unless mentioned otherwise.

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