The polar flagellar transcriptional regulatory network in *Vibrio campbellii* deviates from canonical *Vibrio* species

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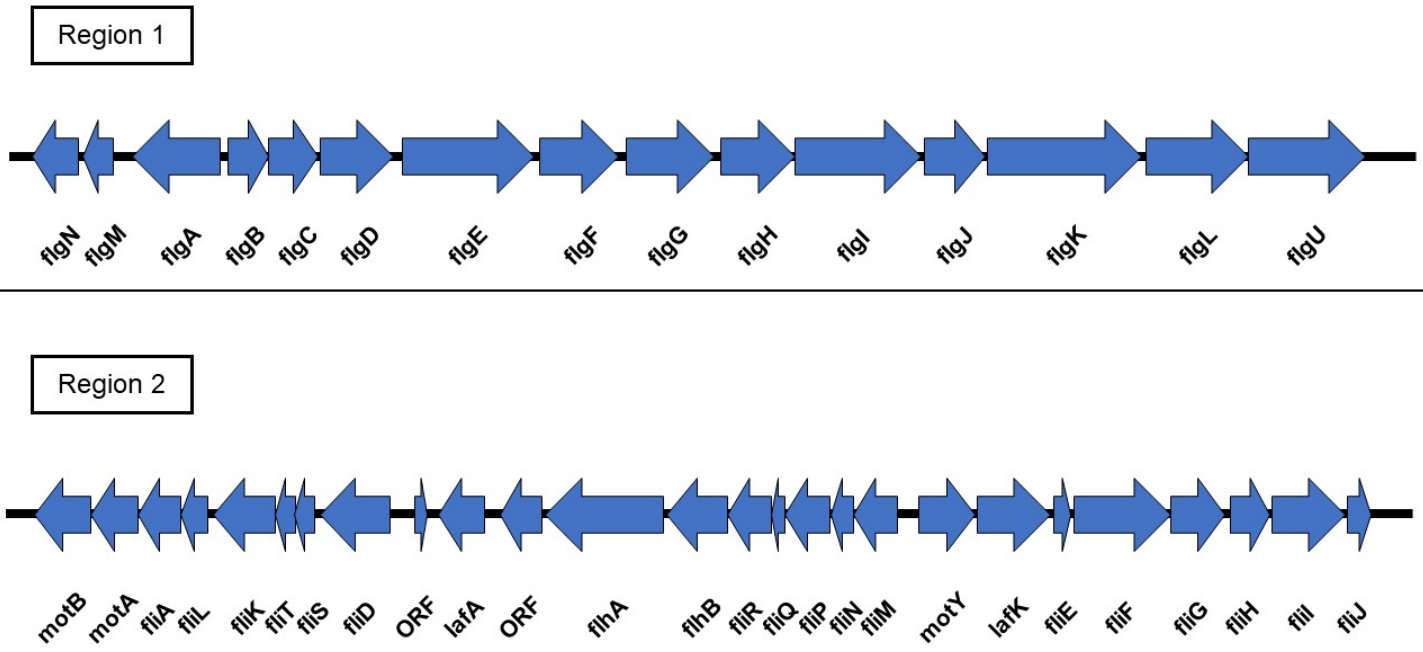
Supplemental Figures S1-S3
Supplemental Tables S1-S3
Supplemental Datasets S1-S3

Dataset S1:
RNA-seq differential expression analysis from experiments comparing *V. campbellii* DS40M4 strains wild-type, Δ*rpoN*, Δ*flrA*, Δ*flrC*, and Δ*fliA* strains. NCBI GEO accession: GSE167483

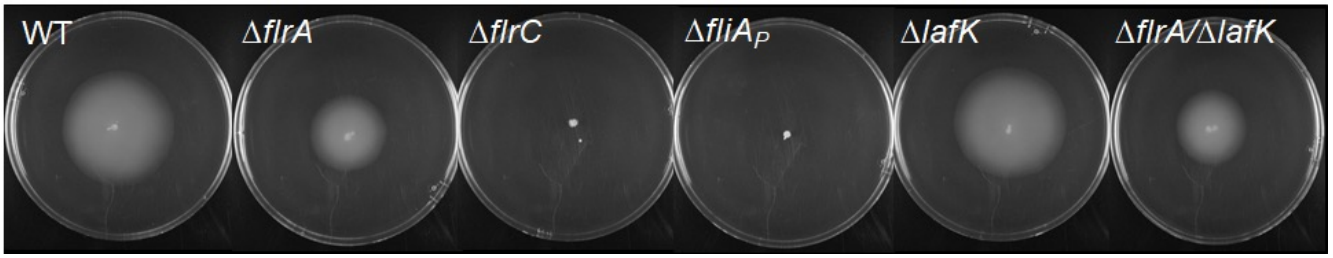
Dataset S2:
Transcriptional start site analysis of data from differential RNA-seq experiments performed with *V. campbellii* DS40M4 wild-type and Δ*luxR* strains. NCBI GEO accession: GSE147616

Dataset S3:
qRT-PCR raw data analyzing expression of flagellar genes (*flhF*, *flgO*, *fliK*, *flaA, hfq*). Calculations for standard curves and normalization to *hfq* (endogenous control gene) are included. Experiments were performed with five biological replicates and two technical replicates.



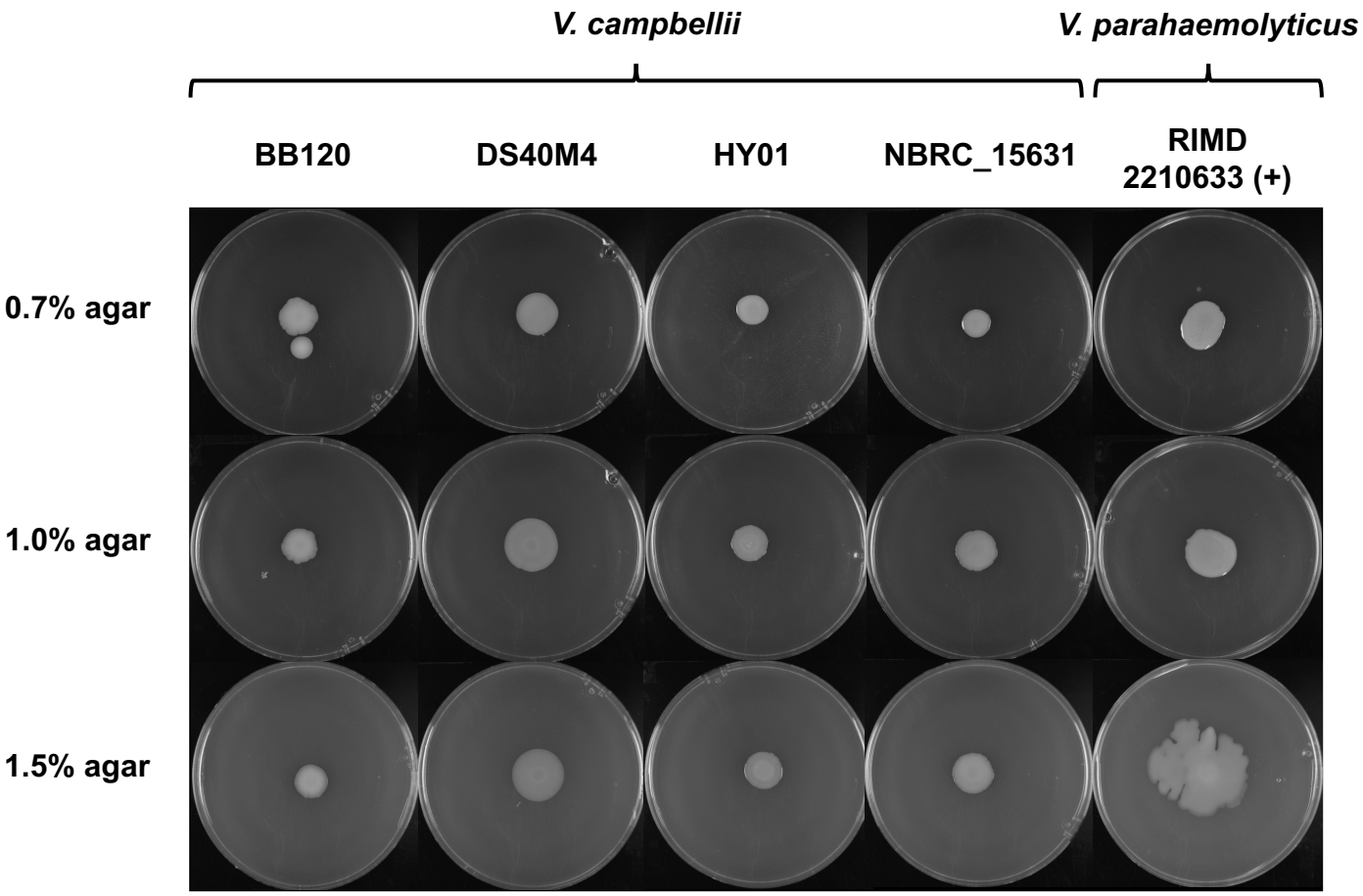
Supplemental Fig. 1: Organization of the lateral flagellar gene system in *V. campbellii* DS40M4

A) Diagram showing the two regions of lateral flagellar genes in encoded within Chromosome II (GenBank accession: CP030789.1) in *V. campbellii*.



Supplemental Fig. 2. **Swimming phenotypes of *V. campbellii* BB120 flagellar regulator mutants**

A) Soft agar (0.3%) swim plates showing swim halo phenotypes for wild-type and mutant BB120 strains.



Supplemental Fig. 3. **Swarming motility in *V. campbellii***
Hard agar (0.7, 1.0, or 1.5%) swarm plates showing swarming phenotypes for each indicated *Vibrio* strain: *V. campbellii* BB120, DS40M4, HY01, and NBRC_15631 or *V. parahaemolyticus* RIMD 2210633. All plates were incubated at 30°C for 72h.

Table S1. Strains used in this study.

Name	Description	Reference
BB120	*V. campbellii* type strain, derivative of BB7 (wild-type)	(1)
DS40M4	Wild-type *V. campbellii*	(2)
cas0034	DS40M4, pMMB67EH-tfox-kanR	(3)
cas0107	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}	This study
BDP107	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*pomA*	This study
BDP033	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*pomB*	This study
BDP114	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgT*	This study
BDP135	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgO*	This study
BDP116	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgP*	This study
BDP117	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgN*	This study
BDP045	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgM*	This study
BDP117	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgA*	This study
BDP134	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*cheV*	This study
BDP113	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*cheR*	This study
BDP144	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgB*	This study
BDP108	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgC*	This study
BDP109	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgD*	This study
BDP110	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgE*	This study
BDP111	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgF*	This study
BDP112	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgG*	This study
BDP125	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgH*	This study
BDP146	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgI*	This study
BDP123	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgJ*	This study
BDP098	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgK*	This study

BDP105	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flgL*	This study
CAS0130	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flaC*	This study
CAS0149	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flaB*	This study
CAS0142	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flaE*	This study
BDP104	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, ΔmotY	This study
BDP118	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flrD*	This study
BDP136	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*cheW*	This study
BDP119	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*ORF2*	This study
BDP120	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*ORF1*	This study
BDP145	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*cheB*	This study
BDP137	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*cheA*	This study
BDP121	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*cheZ*	This study
BDP101	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*cheY*	This study
BDP046	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliA*	This study
ML006	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flhG*	This study
BDP100	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flhF*	This study
BDP126	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flhA*	This study
BDP106	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flhB*	This study
BDP122	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliR*	This study
BDP127	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliQ*	This study
BDP138	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliP*	This study
BDP128	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliO*	This study
BDP129	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliN*	This study
BDP133	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliM*	This study
BDP141	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliL*	This study

BDP124	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliK*	This study
BDP142	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliJ*	This study
BDP130	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliI*	This study
BDP131	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliH*	This study
BDP132	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliG*	This study
BDP143	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliF*	This study
BDP102	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliE*	This study
BDP031	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flrC*	This study
BDP099	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flrB*	This study
BDP030	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flrA*	This study
BDP150	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliS*	This study
BDP151	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliS*	This study
BDP139	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*fliT*	This study
BDP140	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flaG*	This study
CAS0128	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flaA*	This study
CAS0150	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flaD*	This study
CAS0131	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flaF*	This study
BDP029	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, ΔrpoN	This study
BDP103	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*motX*	This study
ML05	DS40M4, pMMB67EH-tfox-kanR, Δ*luxB*::*spec*^{R}, Δ*flrA, ΔflrC*	This study
BDP152	DS40M4, pMMB67EH-tfox-kanR, ΔflrA, ΔflrC, Δ*luxB*::Ptac-flrA-*Tm*^{R},	This study
BDP153	DS40M4, pMMB67EH-tfox-kanR, ΔflrA, ΔflrC, Δ*luxB*::Ptac-flrC-*Tm*^{R},	This study
BDP037	DS40M4, pMMB67EH-tfox-kanR, ΔrpoN, Δ*luxB*::rpoN-*Tm*^{R},	This study

BDP038	DS40M4, pMMB67EH-tfox-kanR, ΔflrA, Δ*luxB*::flrA-*Tm*^{R},	This study
BDP054	DS40M4, pMMB67EH-tfox-kanR, ΔflrC, Δ*luxB*::Ptac-flrC-Spec^R,	This study
BDP055	DS40M4, pMMB67EH-tfox-kanR, ΔfliA, Δ*luxB*::Ptac-fliA-SpecR,	This study
AY05	DS40M4, pMMB67EH-tfox-kanR, ΔluxB::specR, ΔlafK	This study
AY11	DS40M4, pMMB67EH-tfox-kanR, ΔluxB::specR, ΔflrA, ΔlafK	This study
BDP024	BB120, ΔflrA	This study
BDP025	BB120, ΔflrC	This study
BDP026	BB120, ΔfliA	This study
BDP027	BB120, ΔflrA, ΔlafK	This study
AY01	BB120, ΔlafK	This study

Table S2. Plasmids used in this study.

Name	Description	Reference
pMMB67EH-tfoX-kanR	*kan*^{R}, P*tac-tfoX*, *lacI*, IncQ origin	(3)
pJV298	*CM*^{R}, P*tac-gfp*, *lacI*, p15a origin	(4)
pRE112	gene deletion vector, cmR (chloramphenicol resistant), sacB (sucrose intolerant), R6Kg origin	(5, 6)
pBB11	BB120 *flrA* deletion construct in pRE112	This study
pBB12	BB120 *flrC* deletion construct in pRE112	This study
pBB13	BB120 *fliA* deletion construct in pRE112	This study
pAY01	BB120 *lafK* deletion construct in pRE112	This study

Table S3. Oligonucleotides used in this study.

Name	Sequence	Notes
Oligos for MuGENT or SOE products		
CAS0148	CGTGCTCAAGTCTTCACTGATGATG	DS40M4 Δ*luxB* F1
CAS0149	GTCGACGGATCCCCGGAATGATGACTTGATCAGAAGAACGCTTTGA	DS40M4 Δ*luxB* R1
CAS0150	GAAGCAGCTCCAGCCTACACACTCGTAACGTTTAAACGATGCTGAG	DS40M4 Δ*luxB* F2
CAS0151	GGTGAATGGCCACAAGGTACCT	DS40M4 Δ*luxB* R2
BP145	GCGGCATGGATATCAATTCCATGGTC	DS40M4 delta FlrA F1

BP180	gctaattcagtttaagcggccatAAGCAGCTTTGCCAAACC TTGCATAAG	DS40M4 delta FlrA R1, universal overlap homology
BP182	atggccgcttaaactgaattagcTAGGGTCACCCTGGCGTT AAAGC	DS40M4 delta FlrA F2, universal overlap homology
BP148	AGAAAGGGAAACATCGGCATCACC	DS40M4 delta FlrA R2
BP149	GGTATCTAGTAAGATCACACCTGC	detect delta FlrA DS40M4, R
BP150	CGCAGTATCAACCGCCTTGAAGG	DS40M4 delta RpoN F1
BP151	gctaattcagtttaagcggccatCATTCAGTGTTACTTACCT TGTAATGCTCAG	DS40M4 delta RpoN R1, universal overlap homology
BP152	atggccgcttaaactgaattagcTAGGCACCAATTGAAAAG GAAAGTCTATGC	DS40M4 delta RpoN F2 , universal overlap homology
BP153	GGAGACAGAGGCAGCGGCTA	DS40M4 delta RpoN R2
BP154	GAATCAATTGCTGCATACATACTTTCG	detect delta RpoN DS40M4, R
BP155	ACCTTACTTAATGAGTAGGGTGTTGTGCC	DS40M4 delta FlrC F1
BP156	gctaattcagtttaagcggccatTTGCGCCATTATGATTCTC CAGTAATTGTTATC	DS40M4 delta FlrC R1, universal overlap homology
BP157	atggccgcttaaactgaattagcCCGGCTTAGTATTCACAT AAATAGCCATAGG	DS40M4 delta FlrC F2 , universal overlap homology
BP158	AGCAATTTCTGAATACCTTGGTCGTCC	DS40M4 delta FlrC R2
BP159	TGATGGCTTTCGTTAGGAGATCA	detect delta FlrC DS40M4, R
BP160	GAAGTACAGAACCTATTGGAAATGCTCGG	DS40M4 delta FliAp F1
BP161	gctaattcagtttaagcggccatCACCAAAAGGGTCCTCTG TGAATTCAG	DS40M4 delta FliAp R1, universal overlap homology
BP263	atggccgcttaaactgaattagcAGCGAATCTCGCGTAAGC CAAATACTG	DS40M4 delta FliAp F2 , universal overlap homology
BP163	CCATACCTACGCCACGACCAGA	DS40M4 delta FliAp R2
BP164	CACGGATGTGTTTAAGCAGGT	detect delta FliAp DS40M4, R
BP234	gtcgacggatccccggaatCTAAAGTAGGCGTTTACGCT GACTCG	DS40M4 luxB::RpoN_TmR complement R, homology to TmR cassette
BP235	tcaaagcgttcttctgatcaagtcatcCTATCTGTAGAAGACA ACATCATGGCCGTT	DS40M4 luxB::RpoN_TmR complement F, 500 bp upstream included

		homology to luxB UP, v2
BP241	tcaaagcgttcttctgatcaagtcatcGCGTAGCTGTCTATCTATGGAAGATGGTG	DS40M4 luxB::FlrA_TmR complement F, 500 bp upstream included, homology to luxB UP
BP242	gtcgacggatccccggaatCTAGCGTTGAAGGTTGTACTTACGCAT	DS40M4 luxB::FlrA_TmR complement R, homology to TmR cassette
BP256	aattcggcgtaagcttaaggagatatacatATGGCGCAAAGCAAAGTGCTG	DS40M4 FlrC Complement F, homology to upstream+Ptac from pJV298
BP244	gtcgacggatccccggaatCTAAGCCGGAATATCGATACCAGCGTC	DS40M4 luxB::FlrC_TmR complement R, homology to TmR cassette
BP257	attcggcgtaagcttaaggagatatacatGTGAATAAAGCGATTACCTATGACCAACATG	DS40M4 FliAp Complement F, homology to upstream+Ptac from pJV298
BP246	gtcgacggatccccggaatTTAGTCATTTTGTGTCCACGCACTGAG	DS40M4 luxB::FliAp_TmR complement R, homology to TmR cassette
BP247	cgcctggagagtgtatagcgattac	detect delta luxB DS40M4, F
BP248	catctcagttgcctccttcattattagctg	detect delta luxB DS40M4, R
BP264	GCCGTTTTGCCGCTTCAGTTAC	DS40M4 delta FlgM F1
BP265	gtcgacggatccccggaatTATACCTGCCATATTTCAACCTTAAACTCG	DS40M4 delta FlgM R1, Ab overlap homology
BP266	gaagcagctccagcctacaTAACTGACTTAGGCCCAAGGTAAGC	DS40M4 delta FlgM F2 , Ab overlap homology
BP267	TGTTCATTACGTCGCCAATCTGC	DS40M4 delta FlgM R2
BP268	CCTGACTTAGGCTTTCGTCTTCGG	detect delta FlgM DS40M4, R
BP374	CACTTGCTCATTCAAATAAGGCTGAATCG	DS40M4 delta fliK F1
BP375	gctaattcagtttaagcggccatGTGGTTTCAAAGCGTGATGGAATTAG	DS40M4 delta fliK R1, universal overlap homology

BP376	atggccgcttaaactgaattagcCATATAGAAACTCTCGTCATCGGCAGC	DS40M4 delta fliK F2, universal overlap homology
BP377	CTGACAGAAGAACAGATTGAGTTGATCAAG	DS40M4 delta fliK R2
BP378	ATTGATTGAGCAAGGTCTCTGTTTACTC	DS40M4 delta fliK detect, R, 250 bp downstream
BP379	CAGCGCTGCGGTTCGATCATC	DS40M4 delta fliE F1
BP380	gctaattcagtttaagcggccatGTAATTTTAGGTAATAAGTGTGGCAGACAAGTC	DS40M4 delta fliE R1, universal overlap homology
BP381	atggccgcttaaactgaattagcTGTCACCTCTAAGCCAAAAGTTTGAC	DS40M4 delta fliE F2 , universal overlap homology
BP382	CGATCTGGACCACAACATGAATGC	DS40M4 delta fliE R2
BP383	TGGAAACGCTGATCGAATGCAACGG	detect delta fliE DS40M4, R, 250 bp downstream
BP384	TTTTGAACGATTCATCAGCAACGCC	DS40M4 delta flhA F1
BP385	gctaattcagtttaagcggccatTAACCCGCGCATCATTAATGGATTTATAG	DS40M4 delta flhA R1, universal overlap homology
BP386	atggccgcttaaactgaattagcCATACTGCGAGGTGTCTCAATCGG	DS40M4 delta flhA F2 , universal overlap homology
BP387	CGTGGTGGTGATGTCCATCTTGCG	DS40M4 delta flhA R2
BP388	CGTCACTAATGAAAATAATTGCATGGTGTG	detect delta flhA DS40M4, R, 250 bp downstream
BP389	AACAAGCTGCTCAGAAAGCAGCTC	DS40M4 delta flhF F1
BP390	gctaattcagtttaagcggccatGGGCGGCGGACTACTATGACTG	DS40M4 delta flhF R1, universal overlap homology
BP391	atggccgcttaaactgaattagcCAAACTATAAATCCATTAATGATGCGCGGG	DS40M4 delta flhF F2 , universal overlap homology
BP392	GCGTCGCAAGCGGATTTGATTCAG	DS40M4 delta flhF R2
BP393	CCGGTATTGAACCGGGATTGGCTG	detect delta flhF DS40M4, R, 250 bp downstream
BP394	ATCACGACCATGTCTGGTTGTGCG	DS4 delta flgK F1
BP395	gctaattcagtttaagcggccatATCTGACGCCATACATGCCCC	DS4 delta flgK R1, universal overlap homology
BP396	atggccgcttaaactgaattagcAGGAGGCCTTAGATGTCAACGCG	DS4 delta flgK F2, universal overlap homology
BP397	GGCTAGGTTGGTAGCCAGAGCC	DS4 delta flgK R2
BP398	ACTTCTTGGTTTTCTAGACGGTTACGAAC	DS4 delta flgK detect, R, 250bp downstream

BP399	ACTGAGCCTTGACCAGTCAATGTGC	DS4 delta flrB (flaL) F1
BP400	gctaattcagtttaagcggccatTAAGCGGCTTTTGTCGATAACAATTACTG	DS4 delta flrB (flaL) R1, universal overlap homology
BP401	atggccgcttaaactgaattagcCATTCACTGCCTGTCGAAATATCGC	DS4 delta flrB (flaL) F2, universal overlap homology
BP402	TTAGAAATCAACTATGACATGTTGGATCGC	DS4 delta flrB (flaL) R2
BP403	TAGGGATGCGACGCACAACACTG	DS4 delta flrB (flaL) detect, R, 250bp downstream
BP404	TAAGTGAACACAAACCGTTTGATAGCAC	DS4 delta motX F1
BP405	gctaattcagtttaagcggccatCGCGACACCTTCTGGTAGATGTACTC	DS4 delta motX R1, universal overlap homology
BP406	atggccgcttaaactgaattagcGTGTAGATGGGGACAGGCGC	DS4 delta motX F2, universal overlap homology
BP407	CGATTCGGTCGTGTCTTAAAAGACAATAAC	DS4 delta motX R2
BP408	AAAATGCGACTCACTGTGGCAAATATTTG	DS4 delta motX detect, R, 250bp downstream
BP409	CTATGGTATCTGCGCCCGACCTAGG	DS4 delta motY F1
BP410	gctaattcagtttaagcggccatCGCACCCAGGTTTGATCTTAAATATTCAC	DS4 delta motY R1, universal overlap homology
BP411	atggccgcttaaactgaattagcCATCTAGGTACTTCTCTCAAACGGCAG	DS4 delta motY F2, universal overlap homology
BP412	ACATGATCCAATGTGCAGCTGGTG	DS4 delta motY R2
BP413	AAACCGCAATTTGGGAGAAATGTAAAATTC	DS4 delta motY detect, R, 250bp downstream
BP414	ATATCGGTTTTATTCATGACATCAGTTCCC	DS4 delta flgL F1
BP415	gctaattcagtttaagcggccatCATCTAAGGCCTCCTTTAGCGCAAC	DS4 delta flgL R1, universal overlap homology
BP416	atggccgcttaaactgaattagcTAACGCGCCTATAGTCTATGCAGTGC	DS4 delta flgL F2, universal overlap homology
BP417	TGATAGCCACAGATTCTTGTGCGCC	DS4 delta flgL R2
BP418	ATAAACAACAAGTTATAGCGCATATGGGG	DS4 delta flgL detect, R, 250bp downstream
BP419	ATTGTCTAATGGCTTGATCACCACTTC	DS4 delta cheY (DSB67_11290) F1
BP420	gctaattcagtttaagcggccatTTTACGGCTGCAACGCTAAAAGAAAAAC	DS4 delta cheY DSB67_11290) R1, universal overlap homology
BP421	atggccgcttaaactgaattagcGCGCATTGTTGAGAAATCATCAACAATAAGG	DS4 delta cheY (DSB67_11290) F2,

		universal overlap homology, v2
BP422	GGCGCAGCTCAACCAAATCG	DS4 delta cheY (DSB67_11290) R2, v2
BP423	AAGCACTGGTTGAATCAATAAAACAACTTC	DS4 delta cheY (DSB67_11290) detect, R, 250bp downstream
BP424	GAATCGACGTCATCTCTTCGCGCATG	DS4 delta flhB F1
BP425	gctaattcagtttaagcggccatCGTCACTAATGAAAATAATTGCATGGTGTG	DS4 delta flhB R1, universal overlap homology
BP426	atggccgcttaaactgaattagcCAATCCAGCCTCCTAGCAATCCAAG	DS4 delta flhB F2, universal overlap homology
BP427	AGATAAAATGGCGGTACAAATCTCCG	DS4 delta flhB R2
BP428	TTAAGACGGCACTCAGTATGTCGTTG	DS4 delta flhB detect, R, 250bp downstream
BP429	AGCTGCTTCTAGCGCGTTACCTAAG	DS4 delta pomA F1
BP430	gctaattcagtttaagcggccatACAAAGCACTCCTTTCGCTATACTTAAAC	DS4 delta pomA R1, universal overlap homology
BP431	atggccgcttaaactgaattagcTCCGGAGGTCATCTGATGGATGAAG	DS4 delta pomA F2, universal overlap homology
BP432	CGTTGTTGCGATGTTGTGTTTGC	DS4 delta pomA R2
BP433	AACTCTTGCGCAATGATGCTGGTG	DS4 delta pomA detect, R, 250bp downstream
BP434	ATCAAGAACTCAGATCCAGCGTACG	DS4 delta flgT F1
BP435	gctaattcagtttaagcggccatGCAACACGTATAATGCCTTGCGATATG	DS4 delta flgT R1, universal overlap homology
BP436	atggccgcttaaactgaattagcTGTTAGTAACCGTTCGGGTATAAATATTGC	DS4 delta flgT F2, universal overlap homology
BP437	ACAAAGTACGCTAAGTCTATCGGCATTTTG	DS4 delta flgT R2
BP438	CTTTACCATTGTAAATTGGTGAGTAGGCG	DS4 delta flgT detect, R, 250bp downstream
BP439	GACAACCTAGGTGACTTTGACCAAAC	DS4 delta flgO F1
BP440	gctaattcagtttaagcggccatGCCATCTTTTCATGGTTTGATTCTCCAG	DS4 delta flgO R1, universal overlap homology
BP441	atggccgcttaaactgaattagcGCCCGTAGGAAACTAGCATGAAGAAG	DS4 delta flgO F2, universal overlap homology
BP442	CTTCTTGTGCCATTTCAAGCAGC	DS4 delta flgO R2
BP443	TGATCGGCAAGTTCTGCACGCC	DS4 delta flgO detect, R, 250bp downstream
BP444	GGTGAAAACAACCCAATCGCTTCAAAC	DS4 delta flgP F1

BP445	gctaattcagtttaagcggccatTGCTAGTTTCCTACGGGCGGAGAATC	DS4 delta flgP R1, universal overlap homology
BP446	atggccgcttaaactgaattagcAGCAGACCTTGTTCTAATACAACTTCAC	DS4 delta flgP F2, universal overlap homology
BP447	TTGCGGCCAGTCACGACATCAC	DS4 delta flgP R2
BP448	GGTAAGGTCGGCATGACCTACAATG	DS4 delta flgP detect, R, 250bp downstream
BP449	AACCGCGACACCTGCTATATAGC	DS4 delta flgN F1
BP450	gctaattcagtttaagcggccatAACTCTTTGAATCAATTCCACGACTACTC	DS4 delta flgN R1, universal overlap homology
BP451	atggccgcttaaactgaattagcGAATGCTTACCTTGGGCCTAAGTC	DS4 delta flgN F2, universal overlap homology
BP452	CAAATTTGTCCGTTAAACCCGTCAGTG	DS4 delta flgN R2
BP453	AAAAGCGCCAGCACAACAAGACG	DS4 delta flgN detect, R, 250bp downstream
BP454	GTTTTGCCGTCAAAGACCTTCATCTC	DS4 delta flgA F1
BP455	gctaattcagtttaagcggccatGGGTTGGCTGTTGTACGGCAAATC	DS4 delta flgA R1, universal overlap homology
BP456	atggccgcttaaactgaattagcACTGTTTCTCTGGTATTATGCCTGCC	DS4 delta flgA F2, universal overlap homology
BP457	GCAGCAGCATTTGTTTACTAGAATCCG	DS4 delta flgA R2
BP458	CTAGTGGATGCAGATTTGGCATCG	DS4 delta flgA detect, R, 250bp downstream
BP459	GAAAGAGCAGTCACACCTAAGATTATCTG	DS4 delta cheV F1
BP460	gctaattcagtttaagcggccatCGTCATATGCTCATCTCCATCTCAAAC	DS4 delta cheV R1, universal overlap homology
BP461	atggccgcttaaactgaattagcGGTAACGCGGTTAAATCTGCATTAACAC	DS4 delta cheV F2, universal overlap homology
BP462	TGTAAATGCTTGAACCTTCGTGGAAC	DS4 delta cheV R2
BP463	CCACAATGTTTCGTTAGTCGTCATCG	DS4 delta cheV detect, R, 250bp downstream
BP464	GGCATTGAAAGTACATCGGGCGC	DS4 delta cheR F1
BP465	gctaattcagtttaagcggccatAAAGTCACGATACTCTTGATCACTGATTG	DS4 delta cheR R1, universal overlap homology
BP466	atggccgcttaaactgaattagcCAACACGCTTACTAGCTGACTCTTTTAATG	DS4 delta cheR F2, universal overlap homology
BP467	AACACGCACTAGCGCCACACG	DS4 delta cheR R2
BP468	AAGCCAAAGTGATTGATGGAGTGTC	DS4 delta cheR detect, R, 250bp downstream
BP469	TTTTATTGATCGATTTGCCGTACAACAGC	DS4 delta flgB F1

BP470	gctaattcagtttaagcggccatTGTTTGCCTCTACAGTAAGAACTGACC	DS4 delta flgB R1, universal overlap homology
BP471	atggccgcttaaactgaattagcAGCAATCAAAGGGGAATAATTTAGATGAGC	DS4 delta flgB F2, universal overlap homology
BP472	AGTTATGTCCTGGACGTTCCGTCATG	DS4 delta flgB R2
BP473	GTTGTACTCCGCATTCAGCGGTTTG	DS4 delta flgB detect, R, 250bp downstream
BP474	ACAATCATTGGTACAGACGCTGAGATC	DS4 delta flgC F1
BP475	gctaattcagtttaagcggccatAGCTCATCTAAATTATTCCCCTTTGATTGC	DS4 delta flgC R1, universal overlap homology
BP476	atggccgcttaaactgaattagcGCAGATGGGTCAATAAGGATAAGGGG	DS4 delta flgC F2, universal overlap homology
BP477	TTCAATCGCACCGGTTTGGATGC	DS4 delta flgC R2
BP478	CTTGCCGATGCCATCTACGGTCG	DS4 delta flgC detect, R, 250bp downstream
BP479	AAAAGTTTGAGATGGAGATGAGCATATGAC	DS4 delta flgD F1
BP480	gctaattcagtttaagcggccatTTCCGGCCATACGCTACCCC	DS4 delta flgD R1, universal overlap homology
BP481	atggccgcttaaactgaattagcTTCAATAGCGCGTCAGGTTAGGAG	DS4 delta flgD F2, universal overlap homology
BP482	TTCTGACCGATTGGCTCAAGACCAC	DS4 delta flgD R2
BP483	AATGCTTGAACCTTCGTGGAACTG	DS4 delta flgD detect, R, 250bp downstream
BP484	TGGTCATTTCAGATATCGAAATGCCAG	DS4 delta flgE F1
BP485	gctaattcagtttaagcggccatTTCCAAAATCTCCTAACCTGACGCG	DS4 delta flgE R1, universal overlap homology
BP486	atggccgcttaaactgaattagcAGCGCTAGTTAGATAGCTCTATCATCTG	DS4 delta flgE F2, universal overlap homology
BP487	GGCAATGTTGCCGTTACCATAACTG	DS4 delta flgE R2
BP488	TAAATTGAGGGTTTTCAGATGGCAATTCAG	DS4 delta flgE detect, R, 250bp downstream
BP489	TTCCTGCCACTACAACCTTGGTCG	DS4 delta flgF F1
BP490	gctaattcagtttaagcggccatCGCGATCCATAAATTACTCCAAAAGTTCTC	DS4 delta flgF R1, universal overlap homology
BP491	atggccgcttaaactgaattagcCAGAGTTAGAGGTTCACAATGCATCC	DS4 delta flgF F2, universal overlap homology
BP492	TGACGGTAGAACTTTTCGTTTTGGC	DS4 delta flgF R2
BP493	TGCGTCGCAACCACTTTAGAACCG	DS4 delta flgF detect, R, 250bp downstream
BP494	AAGCAATTGTCTCAGCAGGACCC	DS4 delta flgG F1

BP495	gctaattcagtttaagcggccatGCATTGTGAACCTCTAACTCTGTTAACTC	DS4 delta flgG R1, universal overlap homology
BP496	atggccgcttaaactgaattagcTTACTAAGATCGGGATTTAGGGAGCC	DS4 delta flgG F2, universal overlap homology
BP497	TAGCAAAAGGTGTGCCAGTTTAGG	DS4 delta flgG R2
BP498	TTTTGTTTAGGATGAATCGGTGCCC	DS4 delta flgG detect, R, 250bp downstream
BP499	TTTACACCAACAACCCAATGGATCTG	DS4 delta flgH F1
BP500	gctaattcagtttaagcggccatTCATTGGCTCCCTAAATCCCGATC	DS4 delta flgH R1, universal overlap homology
BP501	atggccgcttaaactgaattagcGACGTCGAAAAGACGGCAAATTGAC	DS4 delta flgH F2, universal overlap homology
BP502	TGAACATCTGGTGAGCCATCAATCATC	DS4 delta flgH R2
BP503	AATGCCGAAGTTTTGCAGCATTGCC	DS4 delta flgH detect, R, 250bp downstream
BP504	GTGTGGCGCTAGTGCGTGTTC	DS4 delta flgI F1
BP505	gctaattcagtttaagcggccatTGCTTACTCAATCTCTTGTAGTCAATTTGC	DS4 delta flgI R1, universal overlap homology
BP506	atggccgcttaaactgaattagcTCGATTAGGAGAGAAACCATGGTCAAG	DS4 delta flgI F2, universal overlap homology
BP507	TGAAGGTGTCATTGGCTGCCTGC	DS4 delta flgI R2
BP508	ATTTGACGGTAGAACTTTTCGTTTTGGC	DS4 delta flgI detect, R, 250bp downstream
BP509	TTTAAAGATACCAATGGCTTATTCCGCC	DS4 delta flgJ F1
BP510	gctaattcagtttaagcggccatACCATGGTTTCTCTCCTAATCGATTAGATG	DS4 delta flgJ R1, universal overlap homology
BP511	atggccgcttaaactgaattagcTTCACCCGATTACGAATGCTTGATAGAC	DS4 delta flgJ F2, universal overlap homology
BP512	ATGCGTCAGATACTGAGCTCTCTG	DS4 delta flgJ R2
BP513	AGAAATGTTATGACCAGTCGTATTCAACTG	DS4 delta flgJ detect, R, 250bp downstream
BP514	CTCATGAGTCTTCTTCATTGCTTCTGC	DS4 delta ORF3 (flrD) F1
BP515	gctaattcagtttaagcggccatCGCGGAAGACGTCGATAAAGATTG	DS4 delta ORF3 (flrD) R1, universal overlap homology
BP516	atggccgcttaaactgaattagcGATTCATCAGCCAATTACAGGTGAGC	DS4 delta ORF3 (flrD) F2, universal overlap homology
BP517	GTCCGATGGCAGCGAAATTCCG	DS4 delta ORF3 (flrD) R2

BP518	AACACGCGTATCATCGTGATTGAG	DS4 delta ORF3 (flrD) detect, R, 250bp downstream
BP519	TTTCAACAATGCATCCCCACGACGG	DS4 delta cheW F1
BP520	gctaattcagtttaagcggccatATTGGCTGATGAATCATGGCTGAAG	DS4 delta cheW R1, universal overlap homology
BP521	atggccgcttaaactgaattagcAGTTAATCCTCGTTAATGTCGTTCCCG	DS4 delta cheW F2, universal overlap homology
BP522	AGATGGCGATTAAAGTACTAGTCGTTGATG	DS4 delta cheW R2
BP523	ACGTCGTCATGCTTGGTGAAAGCATG	DS4 delta cheW detect, R, 250bp downstream
BP524	GTGCCCCACATCGGTTTGCC	DS4 delta ORF2 F1
BP525	gctaattcagtttaagcggccatTGCTAAACGCAGGTTTAGATGTAAAATCAC	DS4 delta ORF2 R1, universal overlap homology
BP526	atggccgcttaaactgaattagcTTATTGCTCATCGAACGCTAACCTCTC	DS4 delta ORF2 F2, universal overlap homology
BP527	GACGAAGAAATGTCGAAAGCGGTAG	DS4 delta ORF2 R2
BP528	GTGGCTTCAAATTGACTATCGTTCCG	DS4 delta ORF2 detect, R, 250bp downstream
BP529	GTTCAAGATATCCGCTTGACGGCG	DS4 delta ORF1 F1
BP530	gctaattcagtttaagcggccatGATGAGCAATAACGACATACTTTCCAGTG	DS4 delta ORF1 R1, universal overlap homology
BP531	atggccgcttaaactgaattagcGATCTTTCCTACGCTAAGCCCACTTC	DS4 delta ORF1 F2, universal overlap homology
BP532	GAAGTGGTCGCTACAGTTGAAGCGC	DS4 delta ORF1 R2
BP533	GGTTCCGCTGCAAAAGTCTACGG	DS4 delta ORF1 detect, R, 250bp downstream
BP534	TTTTCAATCTTTATCGACGTCTTCCGC	DS4 delta cheB F1
BP535	gctaattcagtttaagcggccatGGAAAGATCATGATCGTTTGGAGTGTAG	DS4 delta cheB R1, universal overlap homology
BP536	atggccgcttaaactgaattagcCTTATTCCTTTTATCGACTCCAGCTTCG	DS4 delta cheB F2, universal overlap homology
BP537	CGCTCGTTCAGATGCTAGAAAGTGG	DS4 delta cheB R2
BP538	GGCCACGGCCATGTAGTTATCGTAC	DS4 delta cheB detect, R, 250bp downstream
BP539	AGTGATTTTACATCTAAACCTGCGTTTAGC	DS4 delta cheA F1
BP540	gctaattcagtttaagcggccatAGCGAAGCTGGAGTCGATAAAAGG	DS4 delta cheA R1, universal overlap homology

BP541	atggccgcttaaactgaattagcACGTTACCTTTAAAATCCAAGACTGGAC	DS4 delta cheA F2, universal overlap homology
BP542	AATACATGATTGCGAAAGCAAATGAACTG	DS4 delta cheA R2
BP543	GTCAAATCATTCGTCGCGTGATCAC	DS4 delta cheA detect, R, 250bp downstream
BP544	CACTTGGATTTTGCAAAGCGTGTTG	DS4 delta cheZ F1
BP545	gctaattcagtttaagcggccatAAGGTAACGTATGAGCTACGATTTAGACG	DS4 delta cheZ R1, universal overlap homology
BP546	atggccgcttaaactgaattagcCTCTAGTGAGATCATCCTGAATAGACCC	DS4 delta cheZ F2, universal overlap homology
BP547	AAGAGGTAGAGCGTCGTGAGCC	DS4 delta cheZ R2
BP548	ATGATCACAGCGGAAGCAAAGCG	DS4 delta cheZ detect, R, 250bp downstream
BP549	CCACACCAAGCTGAAGTTGGTCAGG	DS4 delta fliR F1
BP550	gctaattcagtttaagcggccatGGAGGCTGGATTGGCAGAGTCAG	DS4 delta fliR R1, universal overlap homology
BP551	atggccgcttaaactgaattagcACGCGCCTCTAGTAAAGCACCTG	DS4 delta fliR F2, universal overlap homology
BP552	GATTGTCCGTGGTCGGATGCCG	DS4 delta fliR R2
BP553	TTCCGTGACGCACTGTGGATGG	DS4 delta fliR detect, R, 250bp downstream
BP554	ATGTCTTCATCAGTATTTTGACGCGTTAC	DS4 delta fliQ F1
BP555	gctaattcagtttaagcggccatGAGGCGCGTATGGAGTATCCGAC	DS4 delta fliQ R1, universal overlap homology
BP556	atggccgcttaaactgaattagcGTCTTACCCTCTTCTCTCTGTGCC	DS4 delta fliQ F2, universal overlap homology
BP557	CGGTAGAGCAACTGCGTTCACCAAC	DS4 delta fliQ R2
BP558	CGGCATTCATTACCTCTGAGCTCAAG	DS4 delta fliQ detect, R, 250bp downstream
BP559	TGGTCAATCAAACCAGCGTTCAAG	DS4 delta fliP F1
BP560	gctaattcagtttaagcggccatGATTGGCACAGAGAGAAGAGGGTAAG	DS4 delta fliP R1, universal overlap homology
BP561	atggccgcttaaactgaattagcGGCGGATTGAGTTATCCTTTGTCATG	DS4 delta fliP F2, universal overlap homology
BP562	CTCAGCAGCAAGCGCGTGTAGATG	DS4 delta fliP R2
BP563	TGTTGAAACGCATGCAAGTGCCAG	DS4 delta fliP detect, R, 250bp downstream
BP564	CAGAACTTGTGCCACGGCAGTAAATAAAC	DS4 delta fliO F1
BP565	gctaattcagtttaagcggccatCATGACAAAGGATAACTCAATCCGCC	DS4 delta fliO R1, universal overlap homology

BP566	atggccgcttaaactgaattagcGCTTCAATCGAGTTTTGATTGAACTGC	DS4 delta fliO F2, universal overlap homology
BP567	CCGGTACAAGAATTAGCGCAATTCG	DS4 delta fliO R2
BP568	CGACACCATCATGGACATCCCAGTC	DS4 delta fliO detect, R, 250bp downstream
BP569	ACGGAATATCGATCGCCACGACG	DS4 delta fliN F1
BP570	gctaattcagtttaagcggccatTGATCAGCCAAACTGAACGCATC	DS4 delta fliN R1, universal overlap homology
BP571	atggccgcttaaactgaattagcTTTTCTGTCCTGTGTTAAACCTTACTTGC	DS4 delta fliN F2, universal overlap homology
BP572	AAAAGAAACATTGGATGCAAATGGTAAACC	DS4 delta fliN R2
BP573	ACTTACCGCGTGAAGATGGGGC	DS4 delta fliN detect, R, 250bp downstream
BP574	ACGATCAGCACTGATGCCGATGC	DS4 delta fliM F1
BP575	gctaattcagtttaagcggccatAAACCCTTACTCAATACCATCGCCTC	DS4 delta fliM R1, universal overlap homology
BP576	atggccgcttaaactgaattagcTGGCTTAATAGATCGGTCACGTTATACC	DS4 delta fliM F2, universal overlap homology
BP577	TTCTAAGCGTTTCTCATCGTTATATTGCG	DS4 delta fliM R2
BP578	AATCAAAGCCCAGCTGATGGTGC	DS4 delta fliM detect, R, 250bp downstream
BP579	TTCGTTGATAGAGGTTGCCGCTTGG	DS4 delta fliL F1
BP580	gctaattcagtttaagcggccatGGTATAACGTGACCGATCTATTAAGCCAAG	DS4 delta fliL R1, universal overlap homology
BP581	atggccgcttaaactgaattagcTTCTGCCATTACTGTCTCTTATTGTTCTG	DS4 delta fliL F2, universal overlap homology
BP582	CATGAAAGAGTCTGTGCCTTATGACATG	DS4 delta fliL R2
BP583	AAATGTTGTCTCAGCAAGGCATGC	DS4 delta fliL detect, R, 250bp downstream
BP584	TTTTCAATCACTGGCTTACCCACCG	DS4 delta fliJ F1
BP585	gctaattcagtttaagcggccatAGCGTTTCTCATCGTTATATTGCGTTAG	DS4 delta fliJ R1, universal overlap homology
BP586	atggccgcttaaactgaattagcACGCTATTACCCACCCAAGATGCTC	DS4 delta fliJ F2, universal overlap homology
BP587	CTGGTGGCTGCTCTGGGTGAG	DS4 delta fliJ R2
BP588	TTATGCCACAAATCACCACAGAAGAG	DS4 delta fliJ detect, R, 250bp downstream
BP589	TTTTCCTTTTGGTGCATCCGCACC	DS4 delta fliI F1
BP590	gctaattcagtttaagcggccatATAGCGTATGAATAATGCGATGGAATTCC	DS4 delta fliI R1, universal overlap homology

BP591	atggccgcttaaactgaattagcTTGCATGGACTACTCGCCCC	DS4 delta fliI F2, universal overlap homology
BP592	ATTACGGTAACCGATCAACACGGTC	DS4 delta fliI R2
BP593	CGCTGCCTATTTCTGGTCATCC	DS4 delta fliI detect, R, 250bp downstream
BP594	TGCGCCACGTTGCTTGTTTGTG	DS4 delta fliH F1
BP595	gctaattcagtttaagcggccatGTCCATGCAAGCCTTGGCCG	DS4 delta fliH R1, universal overlap homology
BP596	atggccgcttaaactgaattagcCCATGCTTAACCTACAATCTGTCTATGTTG	DS4 delta fliH F2, universal overlap homology
BP597	AGTGTTGCCTTCGATGCCACTGTAC	DS4 delta fliH R2
BP598	GCGACGATATCGAAGCAATGCCTCC	DS4 delta fliH detect, R, 250bp downstream
BP599	GCAAAACTGAACAATGGTTAACGTGG	DS4 delta fliG F1
BP600	gctaattcagtttaagcggccatATCCTTTCTTGTTCTTCCTGTTTAGGC	DS4 delta fliG R1, universal overlap homology
BP601	atggccgcttaaactgaattagcTCGTTAGCCATTTTCGTTCATCCAG	DS4 delta fliG F2, universal overlap homology
BP602	GAAAGGTGCATTTACTGGCGCGG	DS4 delta fliG R2
BP603	TGCTCAACCCAGCAAGTGGC	DS4 delta fliG detect, R, 250bp downstream
BP604	GCCATTTCACGAGACAAGACAATGTG	DS4 delta fliF F1
BP605	gctaattcagtttaagcggccatAATGGCTAACGATATCGTACCTCAAGATG	DS4 delta fliF R1, universal overlap homology
BP606	atggccgcttaaactgaattagcGCCACACTTATTACCTAAAATTACACTGGC	DS4 delta fliF F2, universal overlap homology
BP607	ATAGCTTGGTACGCTAATTGCTTGC	DS4 delta fliF R2
BP608	GTGCGAAAGTTGGGGCAGATTTTGG	DS4 delta fliF detect, R, 250bp downstream
BP609	AATTGTTATCGACAAAAGCCGCTTACTG	DS4 delta fliS F1
BP610	gctaattcagtttaagcggccatGCAGCAGAAGTCGGCATTTAATCG	DS4 delta fliS R1, universal overlap homology
BP611	atggccgcttaaactgaattagcCAAAGAACCGCGCATAGTATTCCTC	DS4 delta fliS F2, universal overlap homology
BP612	TCGAAGAGCCGTATTAAAGATACTGACTAC	DS4 delta fliS R2
BP613	TTAATACTGAAGAAATCCTTCACTTGGTCG	DS4 delta fliS detect, R, 250bp downstream
BP614	GCGCGGTTTCGACCATTGGC	DS4 delta fliT F1
BP615	gctaattcagtttaagcggccatAAAGAGGAATACTATGCGCGGTTCTTTG	DS4 delta fliT R1, universal overlap homology

BP616	atggccgcttaaactgaattagcCTATTGGCCCAACGCGTTCATCAG	DS4 delta fliT F2, universal overlap homology
BP617	ATCAACGGTCAAAGCGAAGATGTAAAAG	DS4 delta fliT R2
BP618	GCGGTAACACAGGTTTCGCGAAAC	DS4 delta fliT detect, R, 250bp downstream
BP619	AACGCGTTTCCGTTAGATCGGTGATC	DS4 delta fliD F1
BP620	gctaattcagtttaagcggccatGGCCAATAGATGAAAGATGCACTCATAG	DS4 delta fliD R1, universal overlap homology
BP621	atggccgcttaaactgaattagcGGGCCTAAACTCATCAAATCACCTC	DS4 delta fliD F2, universal overlap homology
BP622	TTTGTCTTCAGGTTTCAAAATCAACAGC	DS4 delta fliD R2
BP623	AAAATGGTCGAGCAAATGAATGAATTTGTG	DS4 delta fliD detect, R, 250bp downstream
BP624	TTTGCGCAATTGAGTGTTAGAAAGGG	DS4 delta flaG F1
BP625	gctaattcagtttaagcggccatCGGTTTGTTAGTTGAAAAGGTGTAAGTCG	DS4 delta flaG R1, universal overlap homology
BP626	atggccgcttaaactgaattagcTACAATCTCCCTTCCACCTTATGAGC	DS4 delta flaG F2, universal overlap homology
BP627	AATGGTGAAGCAGCAGATCTTACAAC	DS4 delta flaG R2
BP628	CTAGACAACATTAACGAGAACGTGAACG	DS4 delta flaG detect, R, 250bp downstream
CAS0158	GTGGCTGTTCGTAACGCGAAC	F1 primer delta FlaE (DSB67_RS03825)
CAS0202	GTTCATCGCTGCAACCTATTCAGCTAATTCAGTTTAAGCGGCCAT	R1 for delta FlaE SOE (DSB67_RS03825)
CAS0160	atggccgcttaaactgaattagcTGAATAGGTTGCAGCGATGAACATCG	F2 primer delta FlaE (DSB67_RS03825)
CAS0161	CGTAACCGTTCTACCAAGCTCTGG	R2 primer delta FlaE (DSB67_RS03825)
CAS0162	CGTAACTCTAACTTCGCAGAAGTG	R delta FlaE detect primer
CAS0163	GGATGCCGAACATGGTGATCGTC	F1 primer delta FlaF (DSB67_RS11440)
CAS0164	gctaattcagtttaagcggccatCAGCATATCGGTTGCACTGGTC	R1 primer delta FlaF (DSB67_RS11440)
CAS0165	atggccgcttaaactgaattagcCTTGGTTAATGATTCGCACCACACG	F2 primer delta FlaF (DSB67_RS11440)
CAS0166	CCAGAGAATTCAACGTCACCTTCAAC	R2 primer delta FlaF (DSB67_RS11440)
CAS0189	CCAAGTAGTTCTCTCAAAGTTAC	R delta FlaF detect primer
CAS0168	CGGTACTTACGGTACTCAGTC	F1 primer delta FlaA (DSB67_RS11425)
CAS0169	gctaattcagtttaagcggccatCGCCATAGTTGATCTCCTTAAGGC	R1 primer delta FlaA (DSB67_RS11425)

CAS0170	atggccgcttaaactgaattagcGGCTAATTGGGTCGACTCAGCC	F2 primer delta FlaA (DSB67_RS11425)
CAS0171	GAAGCAATGTCACCACCATCTTCC	R2 primer delta FlaA (DSB67_RS11425)
CAS0172	CGATTCTTCATCGACTCGAAACGC	R delta FlaA detect primer
CAS0192	CGTGATTATGACGGTAGCGTGG	F1 delta FlaB (DSB67_03820)
CAS0193	gctaattcagtttaagcggccatCACTGCCATGGTGATTTCTCCAATTG	R1 delta FlaB (DSB67_03820)
CAS0194	atggccgcttaaactgaattagcCTAGGTTAATAAACCTAATCGACGTG	F2 delta FlaB (DSB67_03820)
CAS0195	CCTATTCATGGACCGTGATGCG	R2 delta FlaB (DSB67_03820)
CAS0196	CACCAACATAATGCTGTCCTC	delta FlaB detect primer
CAS0197	GGAGTGAGTAGAGACAGCGGTAG	F1 delta FlaD (DSB67_RS11430)
CAS0198	gctaattcagtttaagcggccatCACTGCCATGGTGATTTCTCC	R1 delta FlaD (DSB67_RS11430)
CAS0199	atggccgcttaaactgaattagcCCTAACTCAGCGCTAAGTCTTCTAGGT	F2 delta FlaD (DSB67_RS11430)
CAS0200	TCACGTACGAATCGGTTAGCGTTC	R2 delta FlaD (DSB67_RS11430)
CAS0201	CGCCATAGTTGATCTCCTTAAGGC	delta FlaD detect primer
BP090	TGaattcccgggagagctcgatatcgcatGTGTGATAGCACAACCAAAATACAT	BB120 FliA polar upstream homology, F, homology to pRE112
BP091	CAAAAGGGTCCTCTGTGAATTC	BB120 FliA polar upstream homology, R
BP092	ATCGTACTGAATTCACAGAGGACCCTTTTGACACACTGATTTTAAGATTCAGTGG	BB120 FliA polar downstream homology, F, homology to FliA upstream homology
BP093	CCCgatcccaagcttcttctagaggtaccGATTTCGGTCAGCTGACCGCG	BB120 FliA polar downstream homology, R, homology to pRE112
BP094	GTCACAGAGCGCTTCTTGAAT	BB120 sequence deletion of polar FliA
BP095	TCTCTGAGCAGCTTGATACCC	BB120 check deletion of FliA polar, F
BP096	GTCGTCGACTTCGTCCTGCG	BB120 check deletion of FliA polar, R
BP104	TGaattcccgggagagctcgatatcgcatGTAGAGAATCAAGTAGAGCGTTATAAG	BB120 FlaM upstream homology, F, homology to pRE112
BP105	TATGATTCTCCAGTATGTGCTTGC	BB120 FlaM upstream homology, R

BP106	GCGAGTGCAAGCACATACTGGAGAATCATATCTCCACATCAATAGCCATAGGA	BB120 FlaM downstream homology, F, homology to FlaM upstream homology
BP107	CCCgatcccaagcttcttctagaggtaccGAGAACACGCGCTTTACGCAC	BB120 FlaM downstream homology, R, homology to pRE112
BP108	CAGATAGATGTCTTTTTCCGCC	BB120 sequence deletion of FlaM
BP109	AAGTACAACCTTCAACGCTAGG	BB120 check deletion of FlaM, F
BP111	TGaattcccgggagagctcgatatcgcatGGATGCACTCATAGAAATTAGTGATATTG	BB120 FlaK upstream homology, F, homology to pRE112
BP112	AAGTAAGAATGATTGCCTTTATTATGG	BB120 FlaK upstream homology, R
BP113	ACCCCATAATAAAGGCAATCATTCTTACTTGGTCACCTTGGCGTTAAAGC	BB120 FlaK downstream homology, F, homology to FlaM upstream homology
BP114	CCCgatcccaagcttcttctagaggtaccGTCCGCAATTCACCGTTCACA	BB120 FlaK downstream homology, R, homology to pRE112
BP115	TTGTGCCAAATTGCATAGAAATGA	BB120 sequence deletion of FlrA
BP116	TCGCGAAACGCGTTGAAGAC	BB120 check deletion of FlrA, F
AY001	TGaattcccgggagagctcgatatcgcatGCTTCTATGTCAGGGAATTGTGATAAA	BB120 LafK upstream homology, F, homology to pRE112
AY002	TGAACCTTTACCTTCTCTTGTTGG	BB120 LafK upstream homology, R
AY003	ACAACCCCAACAAGAGAAGGTAAAGGTTCAAAGGAGAAAAGTAGATGGCAAGT	BB120 LafK downstream homology, F, homology to LafK upstream homology
AY004	CCCgatcccaagcttcttctagaggtaccGTTAAGATCTTCGCCCGGCTT	BB120 LafK downstream homology, R, homology to pRE112
AY005	TTAAATGTATCTCGCCAGCGA	BB120 sequence deletion of lateral LafK
AY006	CCTAGCAACTCTACATCAAGCA	BB120 check deletion of LafK, F
AY022	GTCGTGAATATCGGTCCAGACAGG	DS40M4, delta LafK F1
AY023	gctaattcagtttaagcggccatCATTGAACCTTTACCTTCTCTTGTTGGG	DS40M4, delta LafK R1, universal overlap homology

AY024	atggccgcttaaactgaattagcGCCTAAAAGGAGAAAAGTAGATGGCAAG	DS40M4, delta LafK F2, universal overlap homology
AY025	TACCACTGCACCACGCAGCT	DS40M4, delta LafK R2
AY026	ATGAAAGGCTCGCTTTTTGGCTG	DS40M4, detect delta LafK Reverse
RC373	cggtacctctagaagaagctt	Amplify pRE112 backbone(universal)
RC374	catgcgatatcgagctctcc	Amplify pRE112 backbone(universal)
RC287	GCGGCCTGAGTTTTTTAAGT	Sequence pRE112-upstream (universal)
RC058	aatgaattacaacagtactgcgatg	Sequence pRE112-downstream (universal)
qPCR Primers		
BP345	ATGGCTAAGGGGCAATCTCTAC	qRT-PCR DS40M4 Hfq F
BP346	CTTGCAGTTTGATACCGTTCAC	qRT-PCR DS40M4 Hfq R
BP330	ATGGCGATTAACGTTAATACTAACGTTTC	qRT-PCR DS40M4 flaA F
BP331	AAGACAAACGCTCCATTGATTTTTGTTG	qRT-PCR DS40M4 flaA R
BP358	ATGAATGTGAATTTATCCAATGTTTCTG	qRT-PCR DS40M4 fliK F
BP359	AAAAACCTTTGCTTTCAGGGT	qRT-PCR DS40M4 fliK R
BP650	ATGAAAAGATGGCTTGTTGCCG	qRT-PCR DS40M4 flgO F
BP651	ATTGGCTGCCTGAATACGGTTC	qRT-PCR DS40M4 flgO R
BP673	AGCACAACCAAAATACATGATTGCG	qRT-PCR DS40M4 flhF F
BP674	CTAGAGTCCTTCGCTGTCACTG	qRT-PCR DS40M4 flhF R
BP675	TTTAGACTTTGCTAAGGAATTGCAGG	qRT-PCR DS40M4 flgB F
BP676	GACCAAGGTTGTAGTGGCAGG	qRT-PCR DS40M4 flgB R

References

1. Bassler BL, Greenberg EP, Stevens AM. 1997. Cross-species induction of luminescence in the quorum-sensing bacterium Vibrio harveyi. J Bacteriol 179:4043 LP – 4045.
2. Dias GM, Thompson CC, Fishman B, Naka H, Haygood MG, Crosa JH, Thompson FL. 2012. Genome sequence of the marine bacterium Vibrio campbellii DS40M4, isolated from open ocean water. J Bacteriol 194:904.
3. Simpson CA, Podicheti R, Rusch DB, Dalia AB, van Kessel JC. 2019. Diversity in Natural Transformation Frequencies and Regulation across Vibrio Species. MBio 10:1–16.
4. Tschirhart T, Shukla V, Kelly EE, Schultzhaus Z, NewRingeisen E, Erickson JS, Wang Z, Garcia W, Curl E, Egbert RG, Yeung E, Vora GJ. 2019. Synthetic Biology Tools for the Fast-Growing Marine Bacterium Vibrio natriegens. ACS Synth Biol 8:2069–2079.
5. Chaparian RR, Olney SG, Hustmyer CM, Rowe-Magnus DA, van Kessel JC. 2016. Integration host factor and LuxR synergistically bind DNA to coactivate quorum-sensing genes in Vibrio harveyi. Mol Microbiol 101:823–840.
6. Edwards RA, Keller LH, Schifferli DM. 1998. Improved allelic exchange vectors and their use to analyze 987P fimbria gene expression. Gene 207:149–157.