

Supplementary Materials for
**Interference of flagellar rotation up-regulates the expression of small RNA
contributing to *Bordetella pertussis* infection**

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Figs. S1 to S3
Table S1

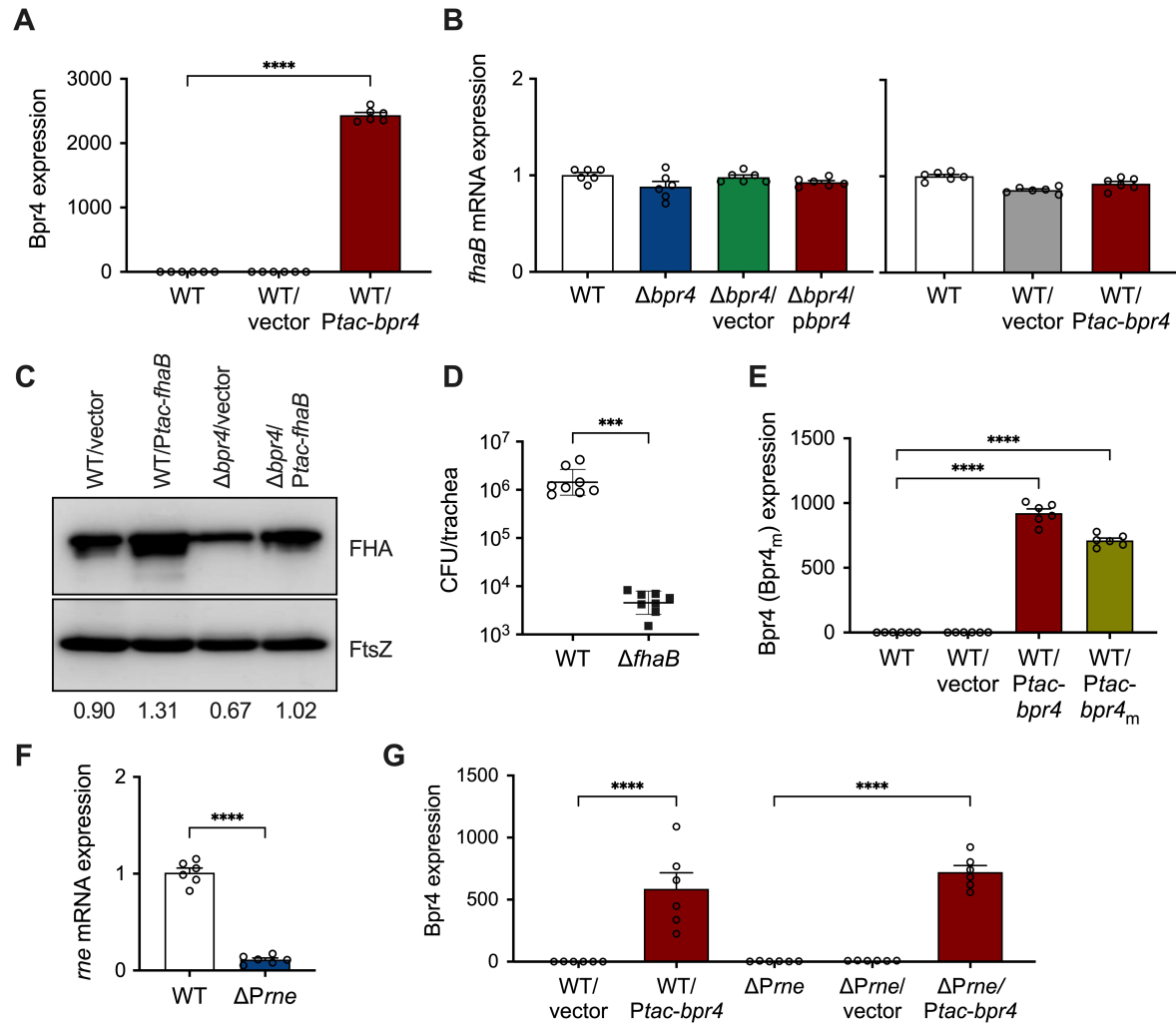


fig. S1. Characterization of *B. pertussis* 18323 mutant strains. (A, B, and E to G) The transcription levels of Bpr4 (A, E, and G) or Bpr4_m (E), *fhaB* (B), and *rne* encoding RNaseE (F) in *B. pertussis* 18323 WT and mutant strains. Total RNA was extracted from bacteria grown in SS broth for 12-14 h. Data represent fold changes in the expression of each transcript (mean + SEM) compared to WT (A, B, E, and F) or WT/vector (G) ($n = 6$). (C) Immunoblotting for FHA and FtsZ in bacterial lysates of *B. pertussis* 18323 mutant strains. FtsZ was used as the internal control for each sample of the bacterial lysates. The blot images are representatives of two independent experiments. The band intensity of FHA was measured using Fiji software and presented as a ratio relative to that of FtsZ. (D) Colonization of *B. pertussis* 18323 WT and $\Delta fhaB$ strains in mouse tracheas. Mice were intranasally inoculated with *B. pertussis* strains, and the numbers of bacteria recovered from the tracheas were counted on day 4. Each horizontal bar represents the geometric mean and geometric SD ($n = 8$). Data were obtained from two independent experiments and statistically analyzed by one-way ANOVA with Tukey's multiple-comparison test (A, B, E, and G), the Mann-Whitney test (D), or the unpaired *t* test (F). **** $P < 0.0001$, *** $P < 0.001$.

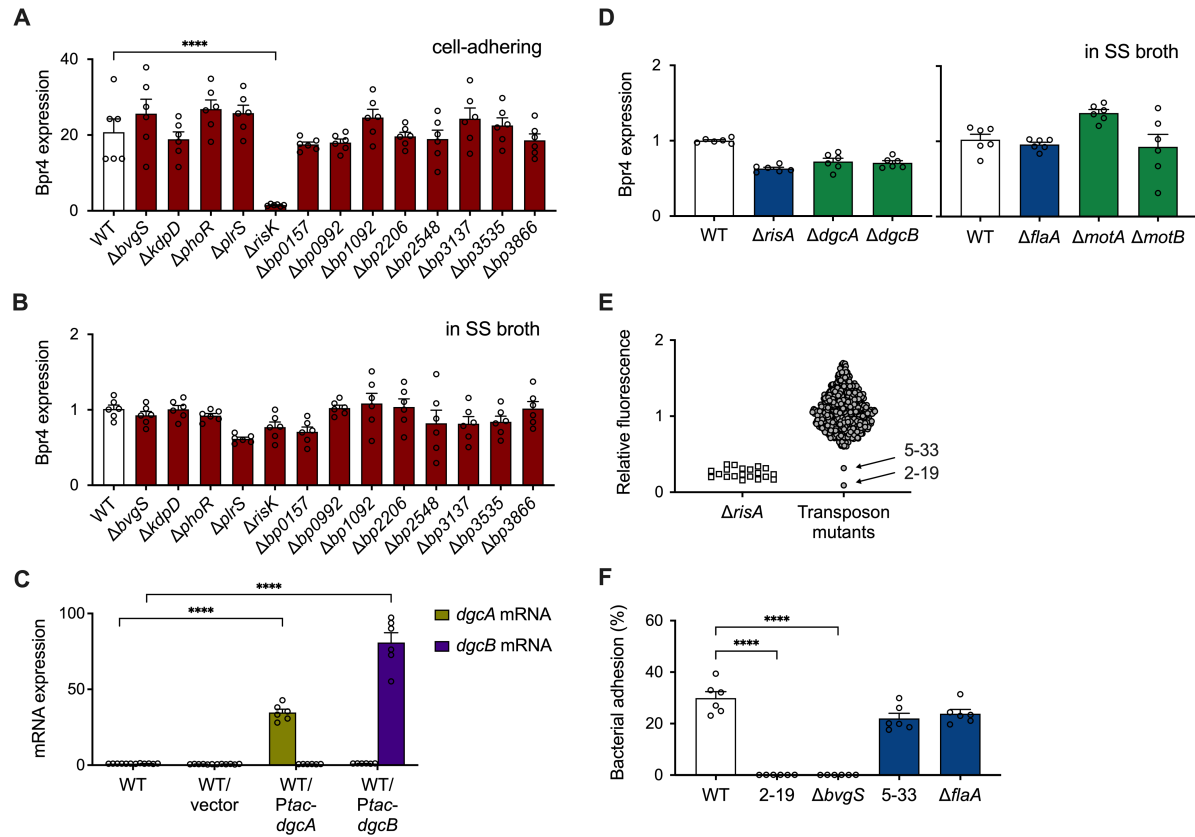


fig. S2. Screening for *B. pertussis* mutants deficient of Bpr4 upregulation during cell adherence. (A to D) Total RNA was extracted from *B. pertussis* 18323 WT and mutant strains incubated with Calu-3 cells for 1 h (A) or grown in SS broth for 12-14 h (B to D). Data represent fold changes in the expression of Bpr4, *dgcA* and *dgcB* (mean + SEM) compared to each strain grown in SS broth (A) or WT (B to D) (n = 6). (E) GFP reporter assay for *Pbpr4* activity. Calu-3 cells were incubated with *B. pertussis* 18323 WT, *ΔrisA*, and 800 transposon-integrated mutants harboring *Pbpr4-gfp*, and GFP expression of the bacteria was measured after a 1 h-incubation. Relative fluorescence levels of *ΔrisA* and the transposon-integrated mutants are represented as relative fluorescence units normalized to those of WT. Each data point of transposon mutants represents the mean of one mutant (n = 2). GFP reporter assay for the screening was performed using 20 microplates (40 mutants in a plate), and *ΔrisA* was used as the positive control for each plate (n = 20). (F) Adhesion of *B. pertussis* 18323 WT and mutant strains to Calu-3 cells. The numbers of bacteria adhered to the cells were counted after 1 h post-infection and are represented as the percentage of CFU of the input bacteria (mean + SEM) (n = 6). Data were obtained from two independent experiments and statistically analyzed by one-way ANOVA with Tukey's multiple-comparison test. *****P* < 0.0001.

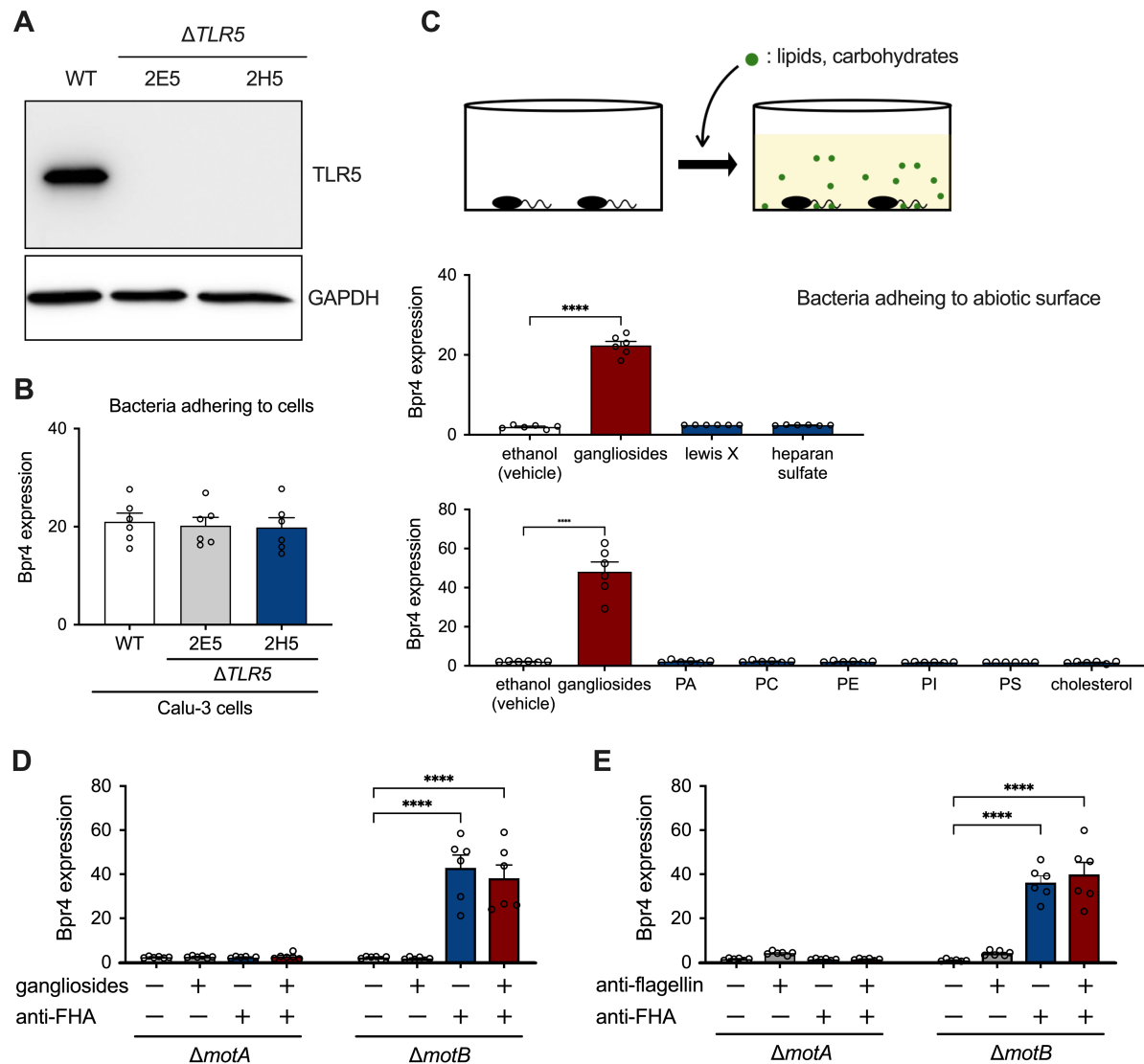


fig. S3. Involvement of host factors in Bpr4 upregulation. (A) Immunoblotting for TLR5 and GAPDH in lysates from Calu-3 WT cells and two distinct clones, 2E5 and 2H5, of $\Delta TLR5$ cells. GAPDH was used as the internal control for each sample of the cell lysates. The blot images are representatives of two independent experiments. (B to E) Bpr4 expression of *B. pertussis* adhering to TLR5-knockout cells or abiotic surfaces. Calu-3 WT and $\Delta TLR5$ cells were incubated with *B. pertussis* 18323 WT for 1 h (B). *B. pertussis* 18323 WT was added to the wells of the culture plates, and the plate-adhering bacteria were treated with 1 μ g/ml gangliosides, lewis X, heparan sulfate, PA, PC, PE, PI, PS, and cholesterol, or ethanol (vehicle) for 1 h (C). *B. pertussis* 18323 mutant strains were added to wells of plastic plates coated with gangliosides, anti-FHA antiserum, and/or anti-flagellin antiserum (D and E). Total RNA was extracted from the cell- and plate-adhering bacteria. Data represent fold changes in Bpr4 expression (mean + SEM) compared to bacteria grown in SS broth (n = 6). Data were obtained from two independent experiments and statistically analyzed by one-way ANOVA with Tukey's multiple-comparison test. **** $P < 0.0001$.

table S1. Primers used in this study.

Name	Sequence (5' to 3')	Application
bpr4-U-S	CGACTAGTGGATCCGATATGAAGGCAATGTCGGCGATAC	<i>Δbpr4</i> -pABB-CRS2-Gm
bpr4-U-AS	CGCAGGGCTAGGTATGAACTGTCTCAAGCGGATTGCGCGGCCGATC	
bpr4-D-S	GATCGGCCGCGCAATCCGCTTGAGACAGTTCATACCTAGCCCTGCG	
bpr4-D-AS	TGGAATTCCCGGGAGGAGATGCACCGCCTGATCGAAAAC	
bpr8-U-S	CGACTAGTGGATCCGGTCATCCAGAGCAAGTACAAGG	<i>Δbpr8</i> -pABB-CRS2-Gm
bpr8-U-AS	GCATCACGGGCGGGGAGCCTCCGACGCAGCGCCTCAAAATGTTGG	
bpr8-D-S	CCAACATTTTGAGGCGCTGCGTCGGAGGCTCCCCCGCCCGTGATGC	
bpr8-D-AS	TGGAATTCCCGGGAGTTGTCGTTGCCATCCAGCGAAAG	
bpr9-U-S	CGACTAGTGGATCCGTACTTTTCCCGTAGCAGGAAC	<i>Δbpr9</i> -pABB-CRS2-Gm
bpr9-U-AS	CCAGGATAGCGATGAGGTTACGCCCATATACACCAGGACTCGG	
bpr9-D-S	CCGAGTCCTGGTGTATATGGGCGTGAACCTCATCGCTATCCTGG	
bpr9-D-AS	TGGAATTCCCGGGAGCATGACCAGGGGGATCAATACCG	
risA-U-S	TTAACCGACTAGTGGATCCGCAGGGAAGGATTGGACCTG	<i>ΔrisA</i> -pABB-CRS2-Gm
risA-U-AS	CTGCCCCGGGGCGGGCCATTAGGGGCCGGAATGTAACAGTGAC	
risA-D-S	GTCACTGTTACATTTCCGGCCCCATATGGCCCCCGCCCGGCGAG	
risA-D-AS	ATTTGTGGAATTCCCGGGAGCCGGCGCGCGTATTCATCAG	
dgcA-U-S	TTAACCGACTAGTGGATCCGCCGAACAGCGACGAGAAGAAATC	<i>ΔdgcA</i> -pABB-CRS2-Gm
dgcA-U-AS	GATCGCTACAGCGTAGCGCAAGCGGGCTCCGGAGGGTGTTC	
dgcA-D-S	GAACACCCCTCCGGAGCCCGCTTGCGCTAGCTGTAGCGATC	
dgcA-D-AS	ATTTGTGGAATTCCCGGGAGCTCGGACTTCATGCTCAGCAC	
dgcB-U-S	TTAACCGACTAGTGGATCCGATGTTGACGTCGGTCTGCATG	<i>ΔdgcB</i> -pABB-CRS2-Gm
dgcB-U-AS	CGCTGACGAAGCGCGCCGGTTGTATCCAGCAGGGGGGTG	
dgcB-D-S	CACCCCCCTGCTGGATACAACCGCGGCGCGCTTCGTCAGCG	
dgcB-D-AS	ATTTGTGGAATTCCCGGGAGGGTGCGAAAATTGCCCTGTG	
flaA-U-S	TTAACCGACTAGTGGATCCGATCCGACGTCAGGTGTAAGG	<i>ΔflaA</i> -pABB-CRS2-Gm
flaA-U-AS	CGCCCGGGTGTCGCGATCCCTAGCCGTTCCAGCCCCCTG	
flaA-D-S	CAGGGGCTGGAACGGCTAGGCATCGGCACACCCCGGGCG	
flaA-D-AS	ATTTGTGGAATTCCCGGGAGTCATGCTCAAGACCAAGACG	
motA-U-S	TTAACCGACTAGTGGATCCGCAGCCGATAGTTCCCTTGCTC	<i>ΔmotA</i> -pABB-CRS2-Gm
motA-U-AS	GTGCTCATGCGGCGGCTCCGATCAGGCCCTGCCAACGAAG	
motA-D-S	CTTCGTTGGCAGAGGCCTGATCGGAGCCGCCGATGAGCAC	
motA-D-AS	ATTTGTGGAATTCCCGGGAGCCTCTCCCTATCGCTACCTG	
motB-U-S	TTAACCGACTAGTGGATCCGTCCAAATAGGCCAAAAGCAC	<i>ΔmotB</i> -pABB-CRS2-Gm
motB-U-AS	CGTCATGCCCTCTCCCTATCGGCGGCGGCTCCGTCAGGCGC	
motB-D-S	CGCCCTGACGGAGCCGCCGCCGATAGGGAGAGGCATGACG	
motB-D-AS	ATTTGTGGAATTCCCGGGAGGTCGAGACCTTGCTGAAAC	
Prne-U-S	TTAACCGACTAGTGGATCCGCGTTTCTTGCGGAGCATCAGCAG	<i>ΔPrne</i> -pABB-CRS2-Gm
Prne-U-AS	CGCATTAACAGCATGCGCTTCATTTAGAGACGCTTGCGGCTTGC	
Prne-D-S	CTTGCAAGCCGCAAGCGTCTCTGAAATGAAGCGCATGCTGTTTAATG	
Prne-D-AS	ATTTGTGGAATTCCCGGGAGATGAAGTCGATCAGCATCAGG	
bvgS-U-S	GATCCGAGCTCTCCCATCCAGTGCCATAGTCTGG	<i>ΔbvgS</i> -pABB-CRS2-Gm
bvgS-U-AS	TTAGCTTCCTTAGCTAGCCAGGCAGATCAGAC	
bvgS-D-S	CGCGCCATTTAAATGCAAGATCAGCCCTGAAGCC	
bvgS-D-AS	ATTTGTGGAATTCCCGCGGACATGTGAGTTGTATC	
kdpD-U-S	GATCCGAGCTCTCCCCACCACCTTCAGCGTCG	<i>ΔkdpD</i> -pABB-CRS2-Gm
kdpD-U-AS	TTAGCTTCCTTAGCTGCCGGGTCGGCCAT	
kdpD-D-S	CGCGCCATTTAAATGGTAAGGCGCCTGCTTCGTCTTC	
kdpD-D-AS	ATTTGTGGAATTCCCCCATTTGCGCACAGTCG	
phoR-U-S	GATCCGAGCTCTCCCGTTTCATGACGTCGTAGCG	<i>ΔphoR</i> -pABB-CRS2-Gm
phoR-U-AS	TTAGCTTCCTTAGCTGCGCAGCCAGATCATCG	
phoR-D-S	CGCGCCATTTAAATGGAAGATCCGGACCGCTGAG	
phoR-D-AS	ATTTGTGGAATTCCCGCAGCACTTCGTCGTCG	
plrS-U-S	GATCCGAGCTCTCCCCCTTCGATGCGGTGC	<i>ΔplrS</i> -pABB-CRS2-Gm
plrS-U-AS	TTAGCTTCCTTAGCTGGCAAGGCGTAGAAACATTCTC	
plrS-D-S	CGCGCCATTTAAATGATAATGCGGCTACGCAATAGG	
plrS-D-AS	ATTTGTGGAATTCCCAAACTCGATCAGGCTGTGC	
risK-U-S	GATCCGAGCTCTCCCCCTGGAGATATCCGGCG	<i>ΔrisK</i> -pABB-CRS2-Gm
risK-U-AS	TTAGCTTCCTTAGCTCATAGGGCGAAAACCGG	
risK-D-S	CGCGCCATTTAAATGCTGGTGCGCATTACCTTG	
risK-D-AS	ATTTGTGGAATTCCCCGATCCCTCGTTCTGGATG	

bp0157-U-S	GATCCGAGCTCTCCCGAGGCGCTCTACAGCGTG	<i>Δbp0157</i> -pABB-CRS2-Gm
bp0157-U-AS	TTAGCTTCCTTAGCTCCGTTGCATCGACGGC	
bp0157-D-S	CGCGCCATTTTAAATGCAAACGCGTAATCCGGGC	
bp0157-D-AS	ATTTGTGGAATTCACGCGGGTGTGGAGAACG	
bp0992-U-S	GATCCGAGCTCTCCAGACCTTGCGGTTGCTGG	<i>Δbp0992</i> -pABB-CRS2-Gm
bp0992-U-AS	TTAGCTTCCTTAGCTGCTGGACATGGACTTCTTG	
bp0992-D-S	CGCGCCATTTTAAATGACAGAACGACCAGTCCG	
bp0992-D-AS	ATTTGTGGAATTCACGCGGCATTGCTGATGTG	
bp1092-U-S	GATCCGAGCTCTCCAGGCCGACTGGATGAAGC	<i>Δbp1092</i> -pABB-CRS2-Gm
bp1092-U-AS	TTAGCTTCCTTAGCTGGACATGACGAAGCAGG	
bp1092-D-S	CGCGCCATTTTAAATGCTTCGTGATGACCTTGCCG	
bp1092-D-AS	ATTTGTGGAATTCCTTGCGCAACCATTGGCC	
bp2206-U-S	GATCCGAGCTCTCCCGCATGACATGTTCGCC	<i>Δbp2206</i> -pABB-CRS2-Gm
bp2206-U-AS	TTAGCTTCCTTAGCTTCAGGATGCGTCGCTTGAG	
bp2206-D-S	CGCGCCATTTTAAATGGCGGCTAGCTGTGAAGATTC	
bp2206-D-AS	ATTTGTGGAATTCCTCGTACACCTGTTGAGAGAG	
bp2548-U-S	GATCCGAGCTCTCCCGACCGCTGTCTAGCC	<i>Δbp2548</i> -pABB-CRS2-Gm
bp2548-U-AS	TTAGCTTCCTTAGCTCCACAGGAGAAACAGCGG	
bp2548-D-S	CGCGCCATTTTAAATGCTACTCGGACCTGCCCCG	
bp2548-D-AS	ATTTGTGGAATTCGGACTGCTCAGGAAGAAGAG	
bp3137-U-S	GATCCGAGCTCTCCCTGGCAATCTGCATTACAACG	<i>Δbp3137</i> -pABB-CRS2-Gm
bp3137-U-AS	TTAGCTTCCTTAGCTAACAGGCCGTGCACTCATG	
bp3137-D-S	CGCGCCATTTTAAATGGGTTCTGTGCTGCGCTTTAC	
bp3137-D-AS	ATTTGTGGAATTCGCGCAGGATGATCACCAGGTTG	
bp3535-U-S	GATCCGAGCTCTCCGTCAGCATCGACGACCTG	<i>Δbp3535</i> -pABB-CRS2-Gm
bp3535-U-AS	TTAGCTTCCTTAGCTGGGAGGAAGGAATTCATGG	
bp3535-D-S	CGCGCCATTTTAAATGATCGGTACGCAACTCACC	
bp3535-D-AS	ATTTGTGGAATTCGCGAGGTGTGCAGGATCTG	
bp3866-U-S	GATCCGAGCTCTCCGAAACCGTATTTTCGGGCC	<i>Δbp3866</i> -pABB-CRS2-Gm
bp3866-U-AS	TTAGCTTCCTTAGCTCACCCTCTTCAGCATGCG	
bp3866-D-S	CGCGCCATTTTAAATGTCCGCGACGAAATTGTGG	
bp3866-D-AS	ATTTGTGGAATTCCTATTGCGGCGAAGTCATCG	
fhaB-U-S	GATCCGAGCTCTCCAAGAACGGCACCTGGAC	<i>ΔfhaB</i> -pABB-CRS2-Gm
fhaB-U-AS	TTAGCTTCCTTAGCTAAATAGGTAGTCGCGGCC	
fhaB-D-S	CGCGCCATTTTAAATGATTCCGACCAGCGAAGTG	
fhaB-D-AS	ATTTGTGGAATTCCTCAGCTTCTGCAGCAGG	
CmR-S	AGCTAAGGAAGCTAAAATGGAG	<i>Cm^r</i> gene
CmR-AS	CATTTAAATGGCGCGCCTT	
pABB-inverse-S	GGGAATTCACAAATTGTTATCCG	Inverse PCR of pABB-CRS2-Gm
pABB-inverse-AS	GGGAGAGCTCGGATCCACTA	
Pbpr4-BamHI-S	CGCTCTAGAACTAGTGGATCACAACCGGATGTTTCGGCGATC	<i>pbpr4</i>
Tbpr4-EcoRI-AS	GATAAGCTTGATATCGAATTCATCCGCGCGGCTCCTTG	
bpr4m-S	CACCTGGCGCGGTCTCAGGCGGATCCCCGCGCAATCCGCTTG	<i>pbpr4_m</i>
bpr4m-AS	CAAGCGGATGCGCGGGGGATCCGCCTGAGGACCGCGCCAGGTG	
bpr4-SmaI-S	ACACAGGAAACAGAATTCCCGATCGGCCGCGCAATCCGCTTG	<i>Ptac-bpr4</i> and <i>-bpr4_m</i>
bpr4-EcoRI-AS	TAGCTCCTGAAAATCGAATTCCTAGACGCCAAAGAAGCTCAC	
fhaB-SmaI-S	ACACAGGAAACAGAATTCCCATGAACACGAACCTGTACAG	<i>Ptac-fhaB</i>
fhaB-EcoRI-AS	TAGCTCCTGAAAATCGAATTCATTGTTGGTTTCATAGAAG	
dgcA-SmaI-S	ACACAGGAAACAGAATTCCCATGCCCCACCCCGCCGACCTG	<i>Ptac-dgcA</i>
dgcA-EcoRI-AS	TAGCTCCTGAAAATCGAATTCAGGCCGGGCATCGCCGGAC	
dgcB-SmaI-S	ACACAGGAAACAGAATTCCCATGAAGCCTTCGCCTGAAATC	<i>Ptac-dgcB</i>
dgcB-EcoRI-AS	TAGCTCCTGAAAATCGAATTCATGTGCTGGCCGACTGCGCCAC	
Pbpr4-AS	GTTCTTCTCCTTTACTCATCGCTGAGGACCGCGCCAGG	<i>Pbpr4-gfp</i>
gfp-S	CCTGGCGCGGTCTCAGGCGATGAGTAAAGGAGAAGAAC	
gfp-AS	TAGCTCCTGAAAATCGAATTC	
motA-U-AS2	CGTTCTCGGAGGAGGCCATATCAGGCCTCTGCCAACGAAG	<i>dsRed-motA</i> -pABB-CRS2-Gm
motA-5'-S	GATCCACCGGATCTAGATAAGTGTTGATTGTTATCGGTTATG	
motA-5'-AS	ATTTGTGGAATTCGCGGAGTCTTGCCCTGGCGCACATGC	
dsRed-S	CTTCGTTGGCAGAGGCCTGATATGGCCTCCTCCGAGAACG	
dsRed-AS	CATAACCGATAACAATCAACACTTATCTAGATCCGGTGGATC	
hfq-XhoI-S	CCACAACAAGCTCGAAATGAGCAATAAAGGGCAAAC	pColdII-HAT- <i>hfq</i>
hfq-EcoRI-AS	CGACAAGCTTGAATTATTATTACGCGGGAACCTCCAC	

flaA-XhoI-S	CATGCCCACAACAAGCTCGAGGCTGCAGTCATCAATACC	pColdII-HAT- <i>flaA</i>
flaA-EcoRI-AS	CAGGTCGACAAGCTTGAATTCTTAGCGCAGCAGCGACAGG	
bpr4-EcoRI-S	AGGGAGACCGGAATTGATCGGCCGCGCAATCCGCTTG	pSPT18- <i>bpr4</i> and- <i>bpr4_m</i>
bpr4-BamHI-AS	CGACTCTAGAGGATCCCTAGACGCCAAGAAGCTC	
bpr4 _m -EcoRI-S	AGGGAGACCGGAATTGATCCCCCGCGCAATCCGCTTG	
fhaB-5'UTR-EcoRI-S	AGGGAGACCGGAATTGATTTCAAAACCTAAAACG	pSPT18- <i>fhaB</i> -5'UTR
fhaB-5'UTR-BamHI-AS	CGACTCTAGAGGATCTCGTGTTCATATTCGACC	
pBBR1-TcR-S	ACCAATTAACCAATTCTGAAAATAGGCGTATCACGA	pMariT
pBBR1-TcR-AS	GTAATACAAGGGGTGTTTCAGGTCGAGGTGG	
pMariK-S	TCAGAATTGGTTAATTGGTTG	
pMariK-AS	AACACCCCTTGTATTACTG	
TcR-AS	TGAGCGCATGTGTAGATTTC	Determination of transposon insertion sites
Mari5	GACTCTAGACCATGGTTGTGTCT	
T18-S	TAAGAAGGAGATATACCATGGCCGCCAGCGAGGCCAC	pET21d-T18- <i>dgcA</i> and - <i>dgcB</i>
T18(dgcA)-AS	CAGGTCGGCGGGGTGGGGCGTTCCACTGCGCCAGCGAC	
T18(dgcB)-AS	GATTTTCAGGCGAAGGCTTGCGTTCCACTGCGCCAGCGAC	
dgcA-S	GTGCTGGGCGCAGTGGAACGCCCAACCCCGCGACCTG	
dgcA-HindIII-AS	CTCGAGTGCGGCCGCAAGCTTCAGGCCGGGCATCGCCGGAC	
dgcB-S	GTGCTGGGCGCAGTGGAACGCAAGCCTTCGCCTGAAATC	
dgcB-HindIII-AS	CTCGAGTGCGGCCGCAAGCTCTAGTGCTGGCCGACTGCGCCAC	
T25-S	TAAGAAGGAGATATACCATGCAGCAATCGCATCAGGC	pET28b-T25- <i>motA</i> and - <i>motB</i>
T25(motA)-AS	CATAACCGATAACAATCAACACCCGCCGCGTGCGGCCAGGTAATC	
T25(motB)-AS	CGGTGATTGTTGACCGTGCTCCGCCGCGTGCGGCCAGGTAATC	
motA-S	GATTACCTGGCGCGCACGCGCGGGTGTTGATTGTTATCGGTTATG	
motA-HindIII-AS	CTCGAGTGCGGCCGCAAGCTTCAGGCCGCGCCGCGTGCCG	
motB-S	GATTACCTGGCGCGCACGCGCGGAGCACGGTCAACAATCACCG	
motB-HindIII-AS	CTCGAGTGCGGCCGCAAGCTCTACCTGGCGCCCCCGTCCGAG	
TLR5-check-S	TAACCTTGCCCTTGGTATGC	Check for <i>TLR5</i> gene deletion
TLR5-check-S2	AACATGGGATGGTGCTTCTC	
TLR5-check-AS	ATAGCGATGGTCACCCAAAC	
qbpr4-S	ATCGGCCGCGCAATCCGCTTG	qRT-PCR for Bpr4
qbpr4-AS	CCGGGCCCGCTATTTATTCAC	
qfhaB-S	CGTTGAAGTCTGGAACCTTGTT	qRT-PCR for <i>fhaB</i>
qfhaB-AS	AAGGTTCCCGTTGTCAATATCG	
qrne-S	CCTTCCTGCTGAACGAAAAG	qRT-PCR for <i>rne</i>
qrne-AS	AGGTGCTTGTTGGGAATCAG	
qgfp-S	TTCCATGGCCAACACTTGTAC	qRT-PCR for <i>gfp</i>
qgfp-AS	GGGCATGGCACTCTTGAAAAAG	
qdgcA-S	GGCCTGGTCTGCACTTCTTC	qRT-PCR for <i>dgcA</i>
qdgcA-AS	CTTGCGACAGCAGGGTGGTG	
qdgcB-S	TCTTCCTGTCCGACCAGTTG	qRT-PCR for <i>dgcB</i>
qdgcB-AS	TACACCGATTCCAGCCAATC	
qflaA-S	TGGCTGCAGTCATCAATACC	qRT-PCR for <i>flaA</i>
qflaA-AS	GCCGATTGGGACTTGTTT	
qmotA-S	TGATCATGATGCGCAGGTAG	qRT-PCR for <i>motA</i>
qmotA-AS	TGAAGAATTTCGGCCACAG	
qrecA-S	CCAATGTGGTCGACAAGTCC	qRT-PCR for <i>recA</i>
qrecA-AS	ATGGCCATTTCCTTGCTGCTC	