|  |  |  |
| --- | --- | --- |
| Protein names | Gene name | -log Student's t-test p-value (FlhF vs GFP) |
| FlhF | VP2234 | 11.33 |
|  | VP0127 | 8.78 |
|  | VPA0809 | 8.59 |
| FliF | VP2249 | 8.43 |
|  | VPA0808 | 8.40 |
|  | VPA0807 | 7.85 |
|  | VP0700 | 7.42 |
| FliG | VP2248 | 7.39 |
| FliE | *fliE* | 6.72 |
| FlgB | VP0775 | 6.18 |
|  | VP1353 | 5.91 |
| FlhA | VP2235 | 5.87 |
|  | VP0944 | 5.65 |
|  | VPA0337 | 5.32 |
| FipA | VP2224 | 4.85 |
| FtsI | VP0454 | 4.77 |
| FlgC | VP0776 | 4.74 |
|  | VP0974 | 4.31 |
| DedD | VP2187 | 4.17 |
|  | VPA1077 | 4.00 |
| FliL | VP2243 | 3.97 |
|  | VP1100 | 3.24 |
|  | VP0646 | 3.17 |
| IbpA | VP0018 | 2.65 |
| SecD | secD | 2.61 |
| TolC | VP0425 | 2.51 |
| FlgF | VP0780 | 2.50 |
| DnaJ | dnaJ | 2.47 |

 **Supplementary file 1a**

**Supplementary file 1b**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Name | flhF | fipA | Flagellation pattern | Reference |
| Buchnera aphidicola JF99 | No | No | non motile | https://doi.org/10.1099/00207713-41-4-566 |
| Citrobacter freundii | No | No | peritrichous | https://doi.org/10.1128%2Fjb.23.2.167-182.1932 |
| Citrobacter rodentium | No | No | non motile | https://doi.org/10.1128%2Fjcm.33.8.2064-2068.1995 |
| Escherichia coli K12 MG1655 | No | No | peritrichous |  |
| Pantoea agglomerans C410P1 | No | No | peritrichous | https://doi.org/10.1099/00207713-39-3-337 |
| Proteus vulgaris | No | No | peritrichous | https://doi.org/10.1128/jb.90.5.1337-1354.1965 |
| Serratia marcescens WW4 | No | No | subpolar | https://dx.doi.org/10.1016%2Fj.resmic.2008.07.003 |
| Sodalis glossinidius | No | No | non motile | https://doi.org/10.1099/00207713-49-1-267 |
| Xenorhabdus nematophila  | No | No | peritrichous | https://doi.org/10.1099/00207713-29-4-352 |
| Yersinia enterocolitica WA | No | No | many |  |
| Aliivibrio fischeri ES114 | Yes | Yes | lophotrichous | https://doi.org/10.1128%2FJB.187.6.2058-2065.2005 |
| Grimontia hollisae | Yes | Yes | monotrichous | https://doi.org/10.1099/ijs.0.02660-0 |
| Photobacterium profundum | Yes | Yes | monotrichous | https://doi.org/10.1007/s007920050036 |
| vibrio cholerae O1 El Tor N16961 | Yes | Yes | monotrichous |  |
| Vibrio parahaemolyticus  | Yes | Yes | monotrichous |  |
| Alteromonas australica H 17 | Yes | Yes | monotrichous | doi.org/10.1099/00207713-45-4-755 |
| Idiomarina loihensis L2TR | Yes | Yes | monotrichous | https://doi.org/10.1099/ijs.0.02701-0 |
| Pseudoalteromonas atlantica | Yes | Yes | monotrichous | doi.org/10.1099/00207713-45-4-755 |
| Pseudoalteromonas haloplanktis | Yes | Yes | monotrichous | doi.org/10.1099/00207713-45-4-755 |
| Pseudoalteromonas luteoviolacea | Yes | Yes | monotrichous | doi.org/10.1099/00207713-45-4-755 |
| Pseudoalteromonas rubra | Yes | Yes | monotrichous | doi.org/10.1099/00207713-45-4-755 |
| Catenovulum sp. CCB-QB4 | Yes | Yes | peritrichous | https://doi.org/10.1099/ijs.0.027565-0 |
| Salinimonas sp. HMF8227 | Yes | Yes | monotrichous | https://doi.org/10.1099/ijs.0.63279-0 |
| Shewanella putrefaciens  | Yes | Yes | monotrichous |  |
| Moritella viscosa | Yes | Yes | monotrichous | https://doi.org/10.1099/00207713-50-2-479 |
| Psychromonas ingrahamii | No | No | non motile | https://doi.org/10.1099/ijs.0.64068-0 |
| Aeromonas salmonicida  | Yes | Yes | monotrichous | https://doi.org/10.1099/00207713-17-3-273 |
| Tolumonas auensis | No | No | non motile | https://doi.org/10.1099/00207713-46-1-183 |
| Hahella chejuensis | Yes | Yes | monotrichous | https://doi.org/10.1099/00207713-51-2-661 |
| Marinomonas sp. MWYL1 | Yes | Yes | monotrichous or amphitrichous | https://doi.org/10.1128/jb.110.1.402-429.1972 |
| Cobetia marina | No | No | subpolar | https://doi.org/10.1099/00221287-62-2-159 |
| Halomonas elongata | No | No | lophotrichous or peritrichous | https://doi.org/10.1099/00207713-30-2-485 |
| Azotobacter vinelandii DJ | Yes | No | peritrichous | https://dx.doi.org/10.1099%2Fmic.0.2008%2F017665-0 |
| Pseudomonas aeruginosa PAO1 | Yes | Yes | lophotrichous |  |
| Pseudomonas fluorescens | Yes | Yes | lophotrichous | https://doi.org/10.1099/mic.0.27362-0 |
| Pseudomonas putida F1 | Yes | Yes | lophotrichous |  |
| cellvibrio japonicus | Yes | Yes | monotrichous | https://doi.org/10.1099/ijs.0.02271-0 |
| Microbulbifer aggregans | No | No | non motile | https://doi.org/10.1099/ijsem.0.002258 |
| Saccharophagus degradans | Yes | Yes | monotrichous | https://doi.org/10.1099/ijs.0.63627-0 |
| Teredinibacter turnerae  | Yes | Yes | monotrichous | https://doi.org/10.1099/00207713-52-6-2261 |
| Dasania marina (taxid:471499) | Yes | No | monotrichous | https://pubmed.ncbi.nlm.nih.gov/18176532/ |
| Acinetobacter baumanii  | No | No | non motile | https://doi.org/10.1007/978-1-4939-9118-1\_17 |
| Alkanindiges illinoisensis | No | No | non motile | https://doi.org/10.1099/ijs.0.02568-0 |
| Moraxella catarrhalis BBH18 | No | No | non motile | https://doi.org/10.1099/00221287-51-3-387 |
| Perlucidibaca piscinae | No | No | monotrichous | https://doi.org/10.1099/ijs.0.65039-0 |
| Psychrobacter cryohalolentis | No | No | non motile | https://doi.org/10.1099/ijs.0.64043-0 |
| Thioalkalivibrio sp. K90 mix | Yes | Yes | monotrichous | https://doi.org/10.1099/00207713-51-2-565 |
| Allochromatium vinosum | Yes | Yes | monotrichous | https://doi.org/10.1099/00207713-48-4-1129 |
| Solimonas sp. K1W22B-7 | Yes | No | non motile | https://doi.org/10.1099/ijs.0.64938-0 |
| Stenotrophomonas maltophilia R5513 | Yes | No | lophotrichous | https://doi.org/10.1099/00221287-26-1-123 |
| Bordetella bronchiseptica RB50 | Yes | No | peritrichous |  |
| Burkholderia mallei ATCC 23344 | Yes | No | degenerate flagellum |  |
| Burkholderia pseudomallei  | Yes | No | monotrichous | https://doi.org/10.1128/IAI.71.4.1622-1629.2003 |
| Caulobacter crescentus | No | No | monotrichous |  |
| Hyphomonas neptunium | Yes | No | monotrichous |  |
| Magnetospirillum magneticum | Yes | No | amphitrichous | https://doi.org/10.1099/00207713-31-4-452 |
| Thalassospira xiamenensis | Yes | No | monotrichous | https://doi.org/10.1099/ijs.0.64544-0 |
| Rhodospirillum rubrum  | Yes | No | amphilophotrichous |  |
| Enhydrobacter aerosaccus  | No | No | non flagellated | https://doi.org/10.1099/00207713-37-3-289 |
| Phycisphaera mikurensis | Yes | No | monotrichous | https://doi.org/10.2323/jgam.55.267 |
| Gimesia maris | Yes | No | polar to subpolar | https://doi.org/10.1186/1944-3277-9-10 |
| Planctopirus ephydatiae | Yes | No | monotrichous | https://doi.org/10.1016/j.syapm.2019.126022 |
| Rubinisphaera brasiliensis | Yes | No | monotrichous | https://doi.org/10.1016/S0723-2020(89)80008-6 |
| schlesneria paludicola | Yes | No | subpolar | https://doi.org/10.1186/1944-3277-9-10 |
| Thermogutta terrifontis | Yes | No | monotrichous | https://doi.org/10.1099/ijs.0.000009 |
| Brachyspira hyodysenteriae WA1 | Yes | No | spirochaete |  |
| Leptospira interrogans  | Yes | No | spirochaete |  |
| Borreliella bavariensis | Yes | No | spirochaete |  |
| Spirochaeta africana | Yes | No | spirochaete | https://doi.org/10.1099/00207713-46-1-305 |
| Salinispira pacifica | Yes | No | spirochaete |  |
| Oceanispirochaeta sp. K2 | Yes | No | spirochaete | https://doi.org/10.1099/ijsem.0.002130 |
| Lentibacillus amyloliquefaciens | No | No | non motile | https://doi.org/10.1007/s10482-015-0618-9 |
| Virgibacillus halodenitrificans  | Yes | No | polar and lateral | https://doi.org/10.1099/00207713-39-2-145 |
| Bacillus subtilis | Yes | No | peritrichous |  |
| Halobacillus halophilus | Yes | No | peritrichous | https://doi.org/10.1016/s0723-2020(83)80007-1 |
| Psychrobacillus sp. AK 1817 | Yes | No | peritrichous | https://doi.org/10.1128/jb.94.4.889-895.1967 |
| Clostridioides difficile 630 | Yes | No | peritrichous | https://doi.org/10.1128/iai.69.12.7937-7940.2001 |
| Selenomonas sputigena | No | No | mid cell | https://doi.org/10.1128%2FMMBR.18.3.165-169.1954 |
| Selenomonas ruminantium | Yes | No | mid cell | https://doi.org/10.1128/aem.00286-11 |

**Supplementary file 1c**

|  |  |  |
| --- | --- | --- |
| **Strain** | **Genotype** | **Reference** |
| ***SM10λpir*** | KmR, thi-1, thr, leu, tonA, lacY, supE, recA::RP4-2-Tc::Mu, pir | Simon et al, 1983 |
| ***DH5pir*** | *sup E44, ΔlacU169 (ΦlacZΔM15), recA1, endA1, hsdR17, thi-1, gyrA96, relA1, λpir phage lysogen* | Miller & Mekanalos, 1988 |
| ***BTH101*** | *F-, cya-99, araD139, galE15, galK16, rpsL1 (StrR), hsdR2, mcrA1, mcrB1, relA1* | Euromedex |
| **WM3064** | *thrB1004 pro thi rpsL hsdS lacZ ΔM15 RP4‐1360 Δ(araBAD) 567ΔdapA 1341: [erm pir(wt)]* | W. Metcalf, University of Illinois, Urbana‐Champaign |

***Escherichia coli***

***Vibrio parahaemolyticus***

|  |  |  |
| --- | --- | --- |
| **Strain**  | **Genotype** | **Reference** |
| **RIMD 2210633** | Clinical isolate, wild type | Makino et al, 2003 |
| **EP12** | *Δvp2234 (ΔflhF), Δvpa1548 (ΔlafA)* | Arroyo Pérez et al., 2021 |
| **SR58** | *Δvp2225 (ΔcheW)* | Ringgaard et al., 2014  |
| **JH2** | *Δvpa1548 (ΔlafA)* | Arroyo Pérez et al., 2021  |
| **EP15** | *Δvp2224 (ΔfipA), Δvpa1548 (ΔlafA)* | this study |
| **PM60** | *Δvp2234 (ΔflhF)* | Arroyo Pérez et al., 2021 |
| **SW01** | *Δvp2224 (ΔfipA)* | this study |
| **JH4** | *Δvp2191 (ΔhubP)* | Arroyo Pérez et al., 2021  |
| **PM69** | *Δvp2234::vp2234-sfgfp (ΔflhF::flhF-sfgfp)* | Arroyo Pérez et al., 2021  |
| **PM77** | *Δvp2234::vp2234-sfgfp (ΔflhF::flhF-sfgfp), Δvp2224 (fipA)* | this study |
| **EP11** | *Δvp2234::vp2234-sfgfp (ΔflhF::flhF-sfgfp), Δvp2191 (hubP)* | Arroyo Pérez et al., 2021  |
| **EP09** | *Δvp2234::vp2234-sfgfp (ΔflhF::flhF-sfgfp), Δvp2224 (fipA), Δvp2191 (hubP)* | this study |
| **PM65** |  *vp2224 (fipA) L129A* | this study |
| **PM66** | *vp2224 (fipA) G110A* | this study |
| **EP16** | *Δvp2234::vp2234-sfgfp (ΔflhF::flhF-sfgfp), vp2224 (fipA) L129A* | this study |
| **EP17** | *Δvp2234::vp2234-sfgfp (ΔflhF::flhF-sfgfp), vp2224 (fipA) G110A* | this study |
| **EP13** | *vp2224 L129A, Δvpa1548(lafA)* | this study |
| **EP14** | *vp2224 G110A, Δvpa1548(lafA)* | this study |
| **PM64** | *Δvp2224::vp2224-sfgfp (ΔfipA::fipA-sfgfp)* | this study |
| **PM68** | *Δvp2224::vp2224-sfgfp (ΔfipA::fipA-sfgfp), Δvp2234 (flhF)* | this study |
| **PM71** | *Δvp2224::vp2224 L129A-sfgfp (ΔfipA L129A ::fipA-sfgfp)* | this study |
| **PM72** | *Δvp2224::vp2224 G110A-sfgfp (ΔfipA G110A ::fipA-sfgfp)* | this study |

***Pseudomonas putida***

|  |  |  |
| --- | --- | --- |
| Strain | Genotype | Reference |
| Wild type | Wild-type strain of *P. putida* KT2440 | Nelson et al, 2002 |
| FliC S267C | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378) | Hintsche et al, 2017 |
| FliC S267C Δ*flhF* | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378) and deletion of the gene *flhF* (*PP\_4343*) | this study |
| Δ*fipA* | deletion of the gene *fipA* (*PP\_4331*) | this study |
| FliC S267C Δ*fipA* | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378) and deletion of the gene *fipA* (*PP\_4331*) | this study |
| FipA -DILEL-sfGFP | C-terminal sfGFP tag of FipA (PP\_4331) linked with Asp, Ile, Leu, Glu and Leu | this study |
| FliC S267C Δ*flhF* FipA-DILEL-sfGFP | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378), deletion of the gene *flhF* (*PP\_4343*) and C-terminal sfGFP tag of FipA (PP\_4331) linked with Asp, Ile, Leu, Glu and Leu | this study |
| FlhF-GS-mCherry | C-terminal mCherry tag of FlhF (PP\_4343) linked with Gly and Ser | this study |
| FliC S267C Δ*fimV* FipA -DILEL-sfGFP | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378), deletion of the gene *fimV* (*PP\_1992*) and C-terminal sfGFP tag of FipA (PP\_4331) linked with Asp, Ile, Leu, Glu and Leu | this study |
| ΔfipA FlhF-GS-mCherry | deletion of the gene *fipA* (*PP\_4331*) and C-terminal mCherry tag of FlhF (PP\_4343) linked with Gly and Ser | this study |
| FliC S267C FipA G104A | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378) and in-frame substitution of Gly104 to Ala in the protein FipA (Sputcn32\_4331) | this study |
| FipA L123A | markerless in-frame substitution of Leu123 to Ala in the protein FipA (Sputcn32\_4331) | this study |
| FliC S267C FipA L123A | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378) and in-frame substitution of Leu123 to Ala in the protein FipA (Sputcn32\_4331) | this study |
| FliC S267C FipA-DILEL-sfGFP | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378) and C-terminal sfGFP tag of FipA (PP\_4331) linked with Asp, Ile, Leu, Glu and Leu | this study |
| FliC S267C FipA L116A | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378) and in-frame substitution of Leu116 to Ala in the protein FipA (Sputcn32\_4331) | this study |
| FliC S267C FipA G104A-DILEL-sfGFP | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378) and in-frame substitution of Gly104 to Ala in the protein FipA (Sputcn32\_4331) with C-terminal sfGFP tag linked with Asp, Ile, Leu, Glu and Leu | this study |
| FliC S267C FipA L123A-DILEL-sfGFP | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378) and in-frame substitution of Leu123 to Ala in the protein FipA (Sputcn32\_4331) with C-terminal sfGFP tag linked with Asp, Ile, Leu, Glu and Leu | this study |
| FliC S267C FipA L116A-DILEL-sfGFP | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378) and in-frame substitution of Leu116 to Ala in the protein FipA (Sputcn32\_4331) with C-terminal sfGFP tag linked with Asp, Ile, Leu, Glu and Leu | this study |
| FlhF-GS-mCherry ΔfimV | C-terminal mCherry tag of FlhF (PP\_4343) linked with Gly and Ser and deletion of the gene *fimV* (*PP\_1992*) | this study |
| FliC S267C ΔfipA FipA KI | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378), deletion of the gene *fipA* (*Sputcn32\_4331*) and reconstitution of the gene *fipA* (*Sputcn32\_4331*) | this study |
| FlhF-GS-mCherry ΔfimV ΔfipA | C-terminal mCherry tag of FlhF (PP\_4343) linked with Gly and Ser and deletion of the genes *fimV* (*PP\_1992*) and *fipA* (*Sputcn32\_4331*) | this study |
| FlhF-GS-mCherry FipA L123A | C-terminal mCherry tag of FlhF (PP\_4343) linked with Gly and Ser and markerless in-frame substitution of Leu123 to Ala in the protein FipA (Sputcn32\_4331) | this study |
| FlhF-GS-mCherry FipA L116A | C-terminal mCherry tag of FlhF (PP\_4343) linked with Gly and Ser and markerless in-frame substitution of Leu116 to Ala in the protein FipA (Sputcn32\_4331) | this study |
| FlhF-GS-mCherry FipA G104A | C-terminal mCherry tag of FlhF (PP\_4343) linked with Gly and Ser and markerless in-frame substitution of Gly104 to Ala in the protein FipA (Sputcn32\_4331) | this study |
| FliC S267C FlhF D362A-GS-mCherry | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378) in-frame substitution of Asp362 to Ala in FlhF (PP\_4343) with C-terminal mCherry tag linked with Gly and Ser | this study |
| FliC S267C FlhF-GS-mCherry FipA ΔTMD | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378) and in-frame deletion of the N-terminal transmembrane domain in the protein FipA (Sputcn32\_4331) | this study |
| FliC S267C FlhF-GS-mCherry FipA ΔTMD | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378), C-terminal mCherry tag of FlhF (PP\_4343) linked with Gly and Ser and in-frame deletion of the N-terminal transmembrane domain in the protein FipA (Sputcn32\_4331) | this study |
| FliC S267C FipA ΔTMD-DILEL-sfGFP | markerless in-frame substitution of Ser267 to Cys in the flagellin protein FliC (PP\_4378) and in-frame deletion of the N-terminal transmembrane domain in the protein FipA (Sputcn32\_4331) with C-terminal sfGFP tag linked with Asp, Ile, Leu, Glu and Leu | this study |

***Shewanella putrefaciens***

|  |  |  |
| --- | --- | --- |
| Strain | Genotype | Reference |
| wild type | wild type strain of *S. putrefaciens* CN-32 | Fredrickson et al, 1998 |
| Δ*flhF* | deletion of the gene *flhF* (*Sputcn32\_2561*) | Rossmann et al, 2015 |
| CheA-mCherry | C-terminal mCherry tag of CheA (Sputcn32\_2556) | this study |
| *flgE1* T183C | markerless in-frame substitution of Thr183 to Cys in the polar hook protein FlgE1 (Sputcn32\_2594), fully functional and suitable for maleimide staining | Rossmann et al, 2019 |
| *flaB1* T166C *flaA*1 T174C Δ*flagL* | markerless in-frame substitution of Thr166 to Cys in the polar major flagellin protein FlaB1 (Sputcn32\_2585) and Thr174 to Cys in the polar minor flagellin protein FlaA1 (Sputcn32\_2586), fully functional and suitable for maleimide staining and deletion of the lateral gene cluster (Sputcn32*\_3444*-Sputcn32*\_3485*) | Kühn et al, 2017 |
| Δ*fipA* | deletion of the gene *fipA* (*Sputcn32\_2550*) | this study |
| CheA-mCherry Δf*ipA* | C-terminal mCherry tag of CheA (Sputcn32\_2556) and deletion of the gene *fipA* (*Sputcn32\_2550*) | this study |
| *flaB1* T166C *flaA1* T174C Δ*flagL* Δ*fipA* | markerless in-frame substitution of Thr166 to Cys in the polar major flagellin protein FlaB1 (Sputcn32\_2585) and Thr174 to Cys in the polar minor flagellin protein FlaA1 (Sputcn32\_2586), fully functional and suitable for maleimide staining, deletion of the lateral gene cluster (Sputcn32*\_3444*-Sputcn32*\_3485*) and deletion of the gene *fipA* (*Sputcn32\_2550*) | this study |
| FlhF-GS-mVenus | C-terminal mVenus tag of FlhF (Sputcn32\_2561) linked with Gly and Ser | this study |
| FipA-DILEL-sfGFP | C-terminal sfGFP tag of FipA (Sputcn32\_2550) linked with Asp, Ile, Leu, Glu and Leu | this study |
| FlhF-GS-mVenus Δ*fipA* | C-terminal mVenus tag of FlhF (Sputcn32\_2561) linked with Gly and Ser and deletion of the gene *fipA* (*Sputcn32\_2550*) | this study |
| FlhF-GS-mVenus Δ*flhG* | C-terminal mVenus tag of FlhF (Sputcn32\_2561) linked with Gly and Ser and deletion of the gene *flhG* (*Sputcn32\_2560*) | this study |
| FipA-DILEL-sfGFP Δ*flhF* | C-terminal sfGFP tag of FipA (Sputcn32\_2550) linked with Asp, Ile, Leu, Glu and Leu and deletion of the gene *flhF* (*Sputcn32\_2561*) | this study |
| FlhF-GS-mVenus Δ*hubP* | C-terminal mVenus tag of FlhF (Sputcn32\_2561) linked with Gly and Ser and deletion of the gene *hubP* (*Sputcn32\_2442*) | this study |
| FlhF-GS-mVenus Δ*fipA* Δ*hubP* | C-terminal mVenus tag of FlhF (Sputcn32\_2561) linked with Gly and Ser and deletion of the genes *fipA* (*Sputcn32\_2550*)and *hubP* (*Sputcn32\_2442*) | this study |
| *flgE1* T183C Δ*flagL* | markerless in-frame substitution of Thr183 to Cys in the polar hook protein FlgE1 (Sputcn32\_2594), fully functional and suitable for maleimide staining and deletion of the lateral gene cluster (Sputcn32*\_3444*-*Sputcn32\_3485*) | Hook et al, 2020 |
| FipA-DILEL-sfGFP Δ*hubP* | C-terminal sfGFP tag of FipA (Sputcn32\_2550) linked with Asp, Ile, Leu, Glu and Leu and deletion of the gene *hubP* (*Sputcn32\_2442*) | this study |
| HubP-mCherry FlhF-GS-Venus | C-terminal mCherry tag of HubP (Sputcn32\_2442) and C-terminal mVenus tag of FlhF (Sputcn32\_2561) linked with Gly and Ser | this study |
| FlhF-GS-mVenus Δ*hubP* *ΔSputcn32\_3157* | C-terminal mVenus tag of FlhF (Sputcn32\_2561) linked with Gly and Ser and deletion of the genes hubP (*Sputcn32\_2442*) and *Sputcn32\_3157* | this study |
| *flaB1* T166C *flaA*1 T174C Δ*flagL* FipA-DILEL-sfGFP | markerless in-frame substitution of Thr166 to Cys in the polar major flagellin protein FlaB1 (Sputcn32\_2585) and Thr174 to Cys in the polar minor flagellin protein FlaA1 (Sputcn32\_2586), fully functional and suitable for maleimide staining, deletion of the lateral gene cluster (Sputcn32*\_3444*-Sputcn32*\_3485*) and C-terminal sfGFP tag of FipA (Sputcn32\_2550) linked with Asp, Ile, Leu, Glu and Leu | this study |
| *flaB1* T166C *flaA*1 T174C Δ*flagL* Δ*flhF* | markerless in-frame substitution of Thr166 to Cys in the polar major flagellin protein FlaB1 (Sputcn32\_2585) and Thr174 to Cys in the polar minor flagellin protein FlaA1 (Sputcn32\_2586), fully functional and suitable for maleimide staining, deletion of the lateral gene cluster (Sputcn32*\_3444*-Sputcn32*\_3485*) and deletion of the gene *flhF* (*Sputcn32\_2561*) | this study |
| Δ*fipA* *fipA* (*Sputcn32\_2550*) KI | deletion of the gene *fipA* (*Sputcn32\_2550*) and reconstitution of the gene *fipA* (*Sputcn32\_2550*) | this study |
| FipA L118A | markerless in-frame substitution of Leu118 to Ala in the protein FipA (Sputcn32\_2550) | this study |
| FipA G106A | markerless in-frame substitution of Gly106 to Ala in the protein FipA (Sputcn32\_2550) | this study |
| FipA L118-DILEL-sfGFP | markerless in-frame substitution of Leu118 to Ala in the protein FipA (Sputcn32\_2550) with a C-terminal sfGFP tag linked with Asp, Ile, Leu, Glu and Leu | this study |
| FipA G106A-DILEL-sfGFP | markerless in-frame substitution of Gly106 to Ala in the protein FipA (Sputcn32\_2550) with a C-terminal sfGFP tag linked with Asp, Ile, Leu, Glu and Leu | this study |
| *flgE1* T183C Δ*flagL* FliM1-GS-sfGFP | markerless in-frame substitution of Thr183 to Cys in the polar hook protein FlgE1 (Sputcn32\_2594), fully functional and suitable for maleimide staining, deletion of the lateral gene cluster (Sputcn32*\_3444*-*Sputcn32\_3485*) and C-terminal sfGFP tag of FliM1 (Sputcn32\_2569) linked with Gly and Ser | Hook et al, 2020 |
| FipA L125A | markerless in-frame substitution of Leu125 to Ala in the protein FipA (Sputcn32\_2550) | this study |
| *flaB1* T166C *flaA*1 T174C Δ*flagL* Δ*fipA* Δ*hubP* | markerless in-frame substitution of Thr166 to Cys in the polar major flagellin protein FlaB1 (Sputcn32\_2585) and Thr174 to Cys in the polar minor flagellin protein FlaA1 (Sputcn32\_2586), fully functional and suitable for maleimide staining and deletion of the lateral gene cluster (Sputcn32*\_3444*-Sputcn32*\_3485*) and the genes *fipA* (*Sputcn32\_2550*) and *hubP* (*Sputcn32\_2442*) | this study |
| FipA L125A-DILEL-sfGFP | markerless in-frame substitution of Leu125 to Ala in the protein FipA (Sputcn32\_2550) with a C-terminal sfGFP tag linked with Asp, Ile, Leu, Glu and Leu | this study |
| FlhF-mCherry FipA-DILEL-sfGFP | C-terminal mCherry tag of FlhF (Sputcn32\_2561) and C-terminal sfGFP tag of FipA (Sputcn32\_2550) linked with Asp, Ile, Leu, Glu and Leu | this study |
| FipA L118A FlhF-GS-mVenus | markerless in-frame substitution of Leu118 to Ala in the protein FipA (Sputcn32\_2550) and C-terminal mVenus tag of FlhF (Sputcn32\_2561) linked with Gly and Ser | this study |
| FipA G106A FlhF-GS-mVenus | markerless in-frame substitution of Gly106 to Ala in the protein FipA (Sputcn32\_2550) and C-terminal mVenus tag of FlhF (Sputcn32\_2561) linked with Gly and Ser | this study |
| FipA L125A FlhF-GS-mVenus | markerless in-frame substitution of Leu125 to Ala in the protein FipA (Sputcn32\_2550) and C-terminal mVenus tag of FlhF (Sputcn32\_2561) linked with Gly and Ser | this study |
| FipA ΔTMD | in-frame deletion of the N-terminal transmembrane domain in the protein FipA (Sputcn32\_2550) | this study |
| FlhF-GS-mVenus FipA ΔTMD | markerless in-frame deletion of the N-terminal transmembrane domain in the protein FipA (Sputcn32\_2550) and C-terminal mVenus tag of FlhF (Sputcn32\_2561) linked with Gly and Ser | this study |
| FipA ΔTMD-DILEL-sfGFP  | in-frame deletion of the N-terminal transmembrane domain in the protein FipA (Sputcn32\_2550) and C-terminal sfGFP tag of FipA (Sputcn32\_2550) linked with Asp, Ile, Leu, Glu and Leu | this study |

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**Supplementary file 1d**

**General constructions**

|  |  |  |
| --- | --- | --- |
| Plasmid | Description | Reference |
| pDM4 | Suicide vector for gene deletions in *Vibrio* sp. | Milton et al., 1996  |
| pJH036 | pBAD33 derivative for sfGFP C-terminal fusion | Iyer et al, 2020 |
| pNPTS138-R6KT | mobRP4+ ori-R6K *sacB;* β-galactosidase fragment alpha; suicide vector for in-frame deletions or integrations in *P. putida* and *S. putrefaciens*; Kanr | Lassak et al, 2010 |
| pKT25 | *plac* ori p15A vector for protein-protein interaction analysis; MCS downstream from T25 fragment encoding region; Kanr | Karimova et al, 1998 |
| pKNT25 | *plac* ori p15A vector for protein-protein interaction analysis; MCS upstream from T25 fragment encoding region; Kanr | Karimova et al, 1998 |
| pUT18 | *plac* ori Col E1 vector for protein-protein interaction analysis; MCS downstream from T18 fragment encoding region; Ampr | Karimova et al, 1998 |
| pUT18C | *plac* ori Col E1 vector for protein-protein interaction analysis; MCS upstream from T18 fragment encoding region; Ampr | Karimova et al, 1998 |

**Plasmids for *Vibrio***

|  |  |  |
| --- | --- | --- |
| **Plasmids** | **Description** | **Reference** |
| **pJH003** | For deletion of vpa1548(lafA) | Heering & Ringgaard, 2016  |
| **pSW022** | For deletion of vp2224(fipA) | This work |
| **pPM188fip** | For insertion of vp2234-sfgfp (flhF-sfgfp), replacing native flhF | Arroyo Pérez et al., 2021 |
| **pPM178** | For insertion of vp2224-sfgfp (fipA-sfgfp), replacing native fipA | this work |
| **pPM179** | For insertion of vp2224 (fipA) G110A point mutation in the chromosome in the native locus | this work |
| **pPM180** | For insertion of vp2224 (fipA) L129A point mutation in the chromosome in the native locus | this work |
| **pPM191** | For insertion of vp2224 (fipA) G110A fused to sfGFP in the chromosome in the native locus | this work |
| **pPM187** | For insertion of vp2224 (fipA) L129A fused to sfGFP in the chromosome in the native locus | this work |
| **pPM039** | For deletion of vp2191 (hubP) | Arroyo Pérez et al., 2021  |
| **pPM194** | For overexpression of VP2224(FipA)Δ7-27 -sfGFP | this work |
| **pPM146** | For overexpression of VP2224(FipA) | this work |
| **pPM159** | For overexpression of VP2224(FipA)-sfGFP | this work |

**Plasmids for *Pseudomonas putida***

|  |  |  |
| --- | --- | --- |
| 1. Plasmid
 | 1. Description
 | 1. Reference
 |
| pNPTS138-R6KT *flhF* KO (*PP\_4343*) | plasmid for deletion of the *flhF* gene (*PP\_4343*) in *P. putida* KT2440; Kanr | 1. this study
 |
| pNPTS138-R6KT FlhF K235A (PP\_4343) | plasmid for in frame complementation of *flhF* (*PP\_4343*) with FlhF K235A mutant in *P. putida* KT2440; Kanr | 1. this study
 |  |
| pNPTS138-R6KT FlhF-GS-mCherry (PP\_4343) | plasmid for in frame complementation of *flhF* (*PP\_4343*) with FlhF-GS-mCherry in *P. putida* KT2440; Kanr | 1. this study
 |
| pNPTS138-R6KT FlhF K235A-GS-mCherry (PP\_4343) | plasmid for in frame complementation of *flhF* (*PP\_4343*) with FlhF K235A-GS-mCherry mutant in *P. putida* KT2440; Kanr | 1. this study
 |
| pNPTS138-R6KT FlhF D301A-GS-mCherry (PP\_4343) | plasmid for in frame complementation of *flhF* (*PP\_4343*) with FlhF D301A-GS-mCherry mutant in *P. putida* KT2440; Kanr | 1. this study
 |
| pNPTS138-R6KT FlhF D362A-GS-mCherry (PP\_4343) | plasmid for in frame complementation of *flhF* (*PP\_4343*) with FlhF D362A-GS-mCherry mutant in *P. putida* KT2440; Kanr | 1. this study
 |
| pNPTS138-R6KT *fipA* KO (*PP\_4331*) | plasmid for deletion of the *fipA* gene (*PP\_4331*) in *P. putida* KT2440; Kanr | 1. this study
 |
| pNPTS138-R6KT *fipA* KI (*PP\_4331*) | plasmid for in frame complementation of *fipA* (*PP\_4331*) with wild type *fipA* in *P. putida* KT2440; Kanr | 1. this study
 |
| pNPTS138-R6KT FipA ΔTMD (AS5-22) (PP\_4331) | plasmid for in frame complementation of *fipA* (*PP\_4331*) with FipA ΔTMD mutant in *P. putida* KT2440; Kanr | 1. this study
 |
| pNPTS138-R6KT FipA G104A (PP\_4331) | plasmid for in frame complementation of *fipA* (*PP\_4331*) with FipA G104A mutantin *P. putida* KT2440; Kanr | 1. this study
 |
| pNPTS138-R6KT FipA L116A (PP\_4331) | plasmid for in frame complementation of *fipA* (*PP\_4331*) with FipA L116A mutantin *P. putida* KT2440; Kanr | 1. this study
 |
| pNPTS138-R6KT FipA L123A (PP\_4331) | plasmid for in frame complementation of *fipA* (*PP\_4331*) with FipA L123A mutantin *P. putida* KT2440; Kanr | 1. this study
 |
| pNPTS138-R6KT FipA-DILEL-sfGFP (PP\_4331) | plasmid for in frame complementation of *fipA* (*PP\_4331*) with FipA-DILEL-sfGFP in *P. putida* KT2440; Kanr | 1. this study
 |
| pNPTS138-R6KT FipA ΔTMD-DILEL-sfGFP (AS5-22) (PP\_4331) | plasmid for in frame complementation of *fipA* (*PP\_4331*) with FipA ΔTMD-DILEL-sfGFP mutant in *P. putida* KT2440; Kanr | 1. this study
 |
| pNPTS138-R6KT FipA G104A-DILEL-sfGFP (PP\_4331) | plasmid for in frame complementation of *fipA* (*PP\_4331*) with FipA G104A-DILEL-sfGFP mutant in *P. putida* KT2440; Kanr | 1. this study
 |
| pNPTS138-R6KT FipA L116A-DILEL-sfGFP (PP\_4331) | plasmid for in frame complementation of *fipA* (*PP\_4331*) with FipA L116A-DILEL-sfGFP mutant in *P. putida* KT2440; Kanr | 1. this study
 |
| pNPTS138-R6KT FipA L123A-DILEL-sfGFP (PP\_4331) | plasmid for in frame complementation of *fipA* (*PP\_4331*) with FipA L123A-DILEL-sfGFP mutant in *P. putida* KT2440; Kanr | 1. this study
 |

**Plasmids for *Shewanella putrefaciens***

|  |  |  |
| --- | --- | --- |
| Plasmid | Description | Reference |
| pNPTS138-R6KT polar flagellar cluster KO(*Sputcn32\_2548-2608*) | plasmid for deletion of the polar flagellar gene cluster (*Sputcn32\_2548-2608*) in *S. putrefaciens* CN-32; Kanr | this study |
| pNPTS138-R6KT lateral flagellar cluster KO (*Sputcn32\_3444-3485*) | plasmid for deletion of the lateral flagellar gene cluster (*Sputcn32\_3444-3485*) in *S. putrefaciens* CN-32; Kanr | Lassak et al, 2010 |
| pNPTS138-R6KT *flagL* KO (*Sputcn32\_3455*, *Sputcn32\_3456*) | plasmid for deletion of the lateral flagellin genes (*Sputcn32\_3455*, *Sputcn32\_3456*) in *S. putrefaciens* CN-32; Kanr | Rossmann et al, 2015 |
| pNPTS138-R6KT *hubP* KO (*Sputcn32\_2442*) | plasmid for deletion of the *hubP* gene (*Sputcn32\_2442*) in *S. putrefaciens* CN-32; Kanr | Rossmann et al, 2015 |
| pNPTS138-R6KT *flhF* KO(*Sputcn32\_2561*) | plasmid for deletion of the *flhF* gene (*Sputcn32\_2561*) in *S. putrefaciens* CN-32; Kanr | Rossmann et al, 2015 |
| pNPTS138-R6KT *flhG* KO (*Sputcn32\_2560*) | plasmid for deletion of the *flhG* gene (*Sputcn32\_2560*) in *S. putrefaciens* CN-32; Kanr | Schuhmacher et al, 2015 |
| pNPTS138-R6KT FlhF-GS-Venus (Sputcn32\_2561) | plasmid for in frame complementation of *flhF* (*Sputcn32\_2561*) with FlhF-GS-mVenus in *S. putrefaciens* CN-32; Kanr | this study |
| pNPTS138-R6KT *fipA* KO (*Sputcn32\_2550*) | plasmid for deletion of the *fipA* gene (*Sputcn32\_2550*) in *S. putrefaciens* CN-32; Kanr | this study |
| pNPTS138-R6KT *fipA* KI (*Sputcn32\_2550*) | plasmid for in frame complementation of *fipA* (*Sputcn32\_2550*) with wild type *fipA* in *S. putrefaciens* CN-32; Kanr | this study |
| pNPTS138-R6KT FipA ΔTMD (AS5-23) (Sputcn32\_2550) | plasmid for in frame complementation of *fipA* (*Sputcn32\_2550*) with FipA ΔTMD mutant in *S. putrefaciens* CN-32; Kanr | this study |
| pNPTS138-R6KT FipA G106A (Sputcn32\_2550) | plasmid for in frame complementation of *fipA* (*Sputcn32\_2550*) with FipA G106A mutant in *S. putrefaciens* CN-32; Kanr | this study |
| pNPTS138-R6KT FipA L118A (Sputcn32\_2550) | plasmid for in frame complementation of *fipA* (*Sputcn32\_2550*) with FipA L118A mutant in *S. putrefaciens* CN-32; Kanr | this study |
| pNPTS138-R6KT FipA L125A (Sputcn32\_2550) | plasmid for in frame complementation of *fipA* (*Sputcn32\_2550*) with FipA L125 mutant in *S. putrefaciens* CN-32; Kanr | this study |
| pNPTS138-R6KT FipA-DILEL-sfGFP (Sputcn32\_2550) | plasmid for in frame complementation of *fipA* (*Sputcn32\_2550*) with FipA-DILEL-sfGFP in *S. putrefaciens* CN-32; Kanr | this study |
| pNPTS138-R6KT FipA ΔTMD-DILEL-sfGFP (AS5-23) (Sputcn32\_2550) | plasmid for in frame complementation of *fipA* (*Sputcn32\_2550*) with FipA ΔTMD-DILEL-sfGFP mutant in *S. putrefaciens* CN-32; Kanr | this study |
| pNPTS138-R6KT FipA G106A-DILEL-sfGFP (Sputcn32\_2550) | plasmid for in frame complementation of *fipA* (*Sputcn32\_2550*) with FipA G106A-DILEL-sfGFP mutant in *S. putrefaciens* CN-32; Kanr | this study |
| pNPTS138-R6KT FipA L116A-DILEL-sfGFP (Sputcn32\_2550) | plasmid for in frame complementation of *fipA* (*Sputcn32\_2550*) with FipA L116A-DILEL-sfGFP mutant in *S. putrefaciens* CN-32; Kanr | this study |
| pNPTS138-R6KT FipA L125A-DILEL-sfGFP (Sputcn32\_2550) | plasmid for in frame complementation of *fipA* (*Sputcn32\_2550*) with FipA L125A mutant in *S. putrefaciens* CN-32; Kanr | this study |
| pNPTS138-R6KT FliM1-GS-sfGFP (Sputcn32\_2569) | plasmid for in frame complementation of *fliM1* (*Sputcn32\_2569*) with FliM1-GS-sfGFP in *S. putrefaciens* CN-32; Kanr | Hook et al, (2020)  |

**BACTH plasmids for *V. parahaemolyticus***

|  |  |  |
| --- | --- | --- |
| **Plasmid** | 1. **Description**
 | 1. **Reference**
 |
| 1. **pSW74**
 | 1. T25-vp2224(fipA)Δ1-27
 | 1. this study
 |
| 1. **pSW119**
 | 1. T18-vp2224(fipA)Δ1-27
 | 1. this study
 |
| 1. **pPM118**
 | 1. vp2224(fipA)Δ1-27-T18
 | 1. this study
 |
| 1. **pPM119**
 | 1. vp2224(fipA)Δ1-27-T25
 | 1. this study
 |
| 1. **pPM124**
 | 1. vp2234(flhF)-T18
 | 1. this study
 |
| 1. **pPM128**
 | 1. vp2234(flhF)-T25
 | 1. this study
 |
| 1. **pPM132**
 | 1. T18-vp2234(flhF)
 | 1. this study
 |
| 1. **pPM136**
 | 1. T25-vp2234(flhF)
 | 1. this study
 |
| 1. **pPM160**
 | 1. T18-vp2224(fipA)Δ1-27 G110A
 | 1. this study
 |
| 1. **pPM161**
 | 1. T18-vp2224(fipA)Δ1-27 E126A
 | 1. this study
 |
| 1. pPM162
 | 1. T18-vp2224(fipA)Δ1-27 L129A
 | 1. this study
 |

**BACTH plasmids for *P. putida***

|  |  |  |
| --- | --- | --- |
| Plasmid | Description | reference |
| pKT25 FlhF (PP\_4343) | plasmid for BACTH assay carrying T25-FlhF (PP\_4343); Kanr | this study |
| pKNT25 FlhF (PP\_4343) | plasmid for BACTH assay carrying FlhF-T25 (PP\_4343); Kanr | this study |
| pUT18 FlhF (PP\_4343) | plasmid for BACTH assay carrying FlhF-T18 (PP\_4343); Ampr | this study |
| pUT18C FlhF (PP\_4343) | plasmid for BACTH assay carrying T18-FlhF (PP\_4343); Ampr | this study |
| pKT25 FlhF K235A (PP\_4343) | plasmid for BACTH assay carrying T25-FlhF K235A (PP\_4343); Kanr | this study |
| pKNT25 FlhF K235A (PP\_4343) | plasmid for BACTH assay carrying FlhF K235A -T25 (PP\_4343); Kanr | this study |
| pUT18 FlhF K235A (PP\_4343) | plasmid for BACTH assay carrying FlhF K235A -T18 (PP\_4343); Ampr | this study |
| pUT18C FlhF K235A (PP\_4343) | plasmid for BACTH assay carrying T18-FlhF K235A (PP\_4343); Ampr | this study |
| pKT25 FipA (PP\_4331) | plasmid for BACTH assay carrying T25-FipA (PP\_4331); Kanr | this study |
| pKNT25 FipA (PP\_4331) | plasmid for BACTH assay carrying FipA-T25 (PP\_4331); Kanr | this study |
| pUT18 FipA (PP\_4331) | plasmid for BACTH assay carrying FipA-T18 (PP\_4331); Ampr | this study |
| pUT18C FipA (PP\_4331) | plasmid for BACTH assay carrying T18-FipA (PP\_4331); Ampr | this study |
| pKT25 FipA G104A (PP\_4331) | plasmid for BACTH assay carrying T25-FipA G104A (PP\_4331); Kanr | this study |
| pKNT25 FipA G104A (PP\_4331) | plasmid for BACTH assay carrying FipA G104A -T25 (PP\_4331); Kanr | this study |
| pUT18 FipA G104A (PP\_4331) | plasmid for BACTH assay carrying FipA G104A -T18 (PP\_4331); Ampr | this study |
| pUT18C FipA G104A (PP\_4331) | plasmid for BACTH assay carrying T18-FipA G104A (PP\_4331); Ampr | this study |
| pKT25 FipA L116A (PP\_4331) | plasmid for BACTH assay carrying T25-FipA L116A (PP\_4331); Kanr | this study |
| pKNT25 FipA L116A (PP\_4331) | plasmid for BACTH assay carrying FipA L116A -T25 (PP\_4331); Kanr | this study |
| pUT18 FipA L116A (PP\_4331) | plasmid for BACTH assay carrying FipA L116A -T18 (PP\_4331); Ampr | this study |
| pUT18C FipA L116A (PP\_4331) | plasmid for BACTH assay carrying T18-FipA L116A (PP\_4331); Ampr | this study |
| pKT25 FipA L125A (PP\_4331) | plasmid for BACTH assay carrying T25-FipA L123A (PP\_4331); Kanr | this study |
| pKNT25 FipA L125A (PP\_4331) | plasmid for BACTH assay carrying FipA L123A -T25 (PP\_4331); Kanr | this study |
| pUT18 FipA L125A (PP\_4331) | plasmid for BACTH assay carrying FipA L123A -T18 (PP\_4331); Ampr | this study |
| pUT18C FipA L125A (PP\_4331) | plasmid for BACTH assay carrying T18-FipA L123A (PP\_4331); Ampr | this study |

**BACTH plasmids for *S. putrefaciens***

|  |  |  |
| --- | --- | --- |
| 1. Plasmid
 | 1. purpose/description
 | 1. reference
 |
| 1. pKT25 FlhF (Sputcn32\_2561)
 | 1. plasmid for BACTH assay carrying T25-FlhF (Sputcn32\_2561); Kanr
 | 1. this study
 |
| 1. pKNT25 FlhF (Sputcn32\_2561)
 | 1. plasmid for BACTH assay carrying FlhF-T25 (Sputcn32\_2561); Kanr
 | 1. this study
 |
| 1. pUT18 FlhF (Sputcn32\_2561)
 | 1. plasmid for BACTH assay carrying FlhF-T18 (Sputcn32\_2561); Ampr
 | 1. this study
 |
| 1. pUT18C FlhF (Sputcn32\_2561)
 | 1. plasmid for BACTH assay carrying T18-FlhF (Sputcn32\_2561); Ampr
 | 1. this study
 |
| 1. pKT25 FipA (Sputcn32\_2550)
 | 1. plasmid for BACTH assay carrying T25-FipA (Sputcn32\_2550); Kanr
 | 1. this study
 |
| 1. pKNT25 FipA (Sputcn32\_2550)
 | 1. plasmid for BACTH assay carrying FipA-T25 (Sputcn32\_2550); Kanr
 | 1. this study
 |
| 1. pUT18 FipA (Sputcn32\_2550)
 | 1. plasmid for BACTH assay carrying FipA-T18 (Sputcn32\_2550); Ampr
 | 1. this study
 |
| 1. pUT18C FipA (Sputcn32\_2550)
 | 1. plasmid for BACTH assay carrying T18-FipA (Sputcn32\_2550); Ampr
 | 1. this study
 |
| pKT25 FipA G106A (Sputcn32\_2550) | 1. plasmid for BACTH assay carrying T25-FipA G106A (Sputcn32\_2550); Kanr
 | 1. this study
 |
| pKNT25 FipA G106A (Sputcn32\_2550) | 1. plasmid for BACTH assay carrying FipA G106A -T25 (Sputcn32\_2550); Kanr
 | 1. this study
 |
| pUT18 FipA G106A (Sputcn32\_2550) | 1. plasmid for BACTH assay carrying FipA G106A -T18 (Sputcn32\_2550); Ampr
 | 1. this study
 |
| pUT18C FipA G106A (Sputcn32\_2550) | 1. plasmid for BACTH assay carrying T18-FipA G106A (Sputcn32\_2550); Ampr
 | 1. this study
 |
| pKT25 FipA L116A (Sputcn32\_2550) | 1. plasmid for BACTH assay carrying T25-FipA L116A (Sputcn32\_2550); Kanr
 | 1. this study
 |
| pKNT25 FipA L116A (Sputcn32\_2550) | 1. plasmid for BACTH assay carrying FipA L116A -T25 (Sputcn32\_2550); Kanr
 | 1. this study
 |
| pUT18 FipA L116A (Sputcn32\_2550) | 1. plasmid for BACTH assay carrying FipA L116A -T18 (Sputcn32\_2550); Ampr
 | 1. this study
 |
| pUT18C FipA L116A (Sputcn32\_2550) | 1. plasmid for BACTH assay carrying T18-FipA L116A (Sputcn32\_2550); Ampr
 | 1. this study
 |
| pKT25 FipA L125A (Sputcn32\_2550) | 1. plasmid for BACTH assay carrying T25-FipA L125A (Sputcn32\_2550); Kanr
 | 1. this study
 |
| pKNT25 FipA L125A (Sputcn32\_2550) | 1. plasmid for BACTH assay carrying FipA L125A -T25 (Sputcn32\_2550); Kanr
 | 1. this study
 |
| pUT18 FipA L125A (Sputcn32\_2550) | 1. plasmid for BACTH assay carrying FipA L125A -T18 (Sputcn32\_2550); Ampr
 | 1. this study
 |
| pUT18C FipA L125A (Sputcn32\_2550) | 1. plasmid for BACTH assay carrying T18-FipA L125A (Sputcn32\_2550); Ampr
 | 1. this study
 |

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**Supplementary file 1e**

**Oligos used for *V. parahaemolyticus* work**

|  |  |  |
| --- | --- | --- |
| Name | Sequence | Purpose |
| VP2224-del-a | CCCCC tctaga ACGTTGTCATGCTTGGTGAAAGCA | KO |
| VP2224-del-b | AGTCTCTTCAGCCATCGTCATTC | KO |
| VP2224-del-c | gaatgacgatggctgaagagact cgacgataaagagaataaaaagaagc | KO |
| VP2224-del-d | CCCCC tctaga ACGCGACGCTGCTGACCCGCAGAA | KO |
| VP2224-check | acaaactccgtggggatgaatac | CP |
| vp2224 AA1-6/28-end w/o Stop | ccccc ctcaga atg gctgaagagacttttctgcgc | KI |
| pUT18C/pKT25-vp2234-cw | ccccc tctaga G aaaataaagcgattttttgccaaagac | BACTH |
| pUT18C/pKT25-vp2234-ccw | ccccc ggtacc ctagagtccttcgttgtcactg | BACTH |
| vpa1548-del-d | Ccccc ctcgag TTATGTGTTCCGCCTTCCTCTC | CP |
| vpa1548-del-chk | aagtagccacatcccaaacgc | CP |
| VP2191-del-d | ccccc tctaga GACAATGCGCTGCACGGAAT | CP |
| VP2191-del-chk | gatggaaaacggctacacca | CP |
| del vp2234(FlhF)-d | CCCCC tctaga GAATACATGCTACGAGCTCAAGG | CP |
| del vp2234(FlhF)-chk | GTTTACGGCATGATTGATGGCG | CP |
| vp2224-Gly110Ala-cw | gagcaaccaaaatggtgcagttaGCGgctgatatcaacgagctaatcg | KI |
| vp2224-Gly110Ala-ccw | CGATTAGCTCGTTGATATCAGCcgcTAACTGCACCATTTTGGTTGCTC | KI |
| vp2224-Glu126Ala-cw | agagtgtgaactgccaaaagcaGCAgcagagttgatgctctctttgc | KI |
| vp2224-Glu126Ala-ccw | GCAAAGAGAGCATCAACTCTGctgCTGCTTTTGGCAGTTCACACTCT | KI |
| vp2224-Leu129Ala-cw | tgaactgccaaaagcagaagcagag GC gatgctctctttgcagaaaaaactg | KI |
| vp2224-Leu129Ala-ccw | CAG TTT TTT CTG CAA AGA GAG CAT CGC CTC TGC TTC TGC TTT TGG CAG TTC A | KI |
| C-term sfGFP-vp2224-a | CCCCC actagt ATGGCTGAAGAGACTTTTTTATCTGTAC | KI |
| C-term sfGFP-vp2224-b | gagctcgaggatgtc TCGTCGACGCCCACGTGG | KI |
| C-term sfGFP-vp2224-c | gacatcctcgagctc atgagcaaaggagaagaacttttcac | KI |
| C-term sfGFP-vp2224-d | tta tttgtagagctcatccatgcc | KI |
| C-term sfGFP-vp2224-e | ggcatggatgagctctacaaa taa AGAGAATAAAAAGAAGCTTCGG | KI |
| C-term sfGFP-vp2224-f | ccccc gcatgc TTTGTTTGTCGATTGCTGTTAGTGG | KI |
| del AA7-27 vp2224-b | AAAAGTCTCTTCAGCCATCGTCATTC | KO |
| del AA7-27 vp2224-c | GAATGACGATGGCTGAAGAGACTTTT CTGCGCATTCGTGCTAGTTTGC | KO |
| vp2224-cw-pBAD | CCCCC tctaga atggctgaagagacttttttatctg | KI |
| vp2224-ccw-pBAD | CCCCC gcatgc ttatcgtcgacgcccacg | KI |
| vp2224 cw restore deletion | ACCTATAATTGGCTGAATGACG ATGGCTGAAGAGACTTTTTTATCTGTAC | KI |
| downstream vp2224 cw | AGAGAATAAAAAGAAGCTTCGGC | CP |
| pUT18/pKNT25- vp2224-cw | ccccc TCTAGA atggctgaagagacttttttatctgtac | BACTH |
| pUT18/pKNT25- tr-vp2224-cw | ccccc TCTAGA ATG cgcattcgtgctagtttgc | BACTH |
| pUT18/pKNT25-vp2222 -ccw | ccccc GGTACC CG tcgtcgacgcccacgtg | BACTH |
| pUT18C/pKT25-vp2224-cw | ccccc tctaga G gctgaagagacttttttatctgtac | BACTH |
| pUT18C/pKT25-vp2224-ccw | ccccc ggtacc ttatcgtcgacgcccacgtg | BACTH |
| tr2224 put18C cw | ccccc tctaga G ATG cgcattcgtgctagtttgcaaaa | BACTH |
| sfGFP-1-ccw | ccccc tctaga tttgtagagctcatccatgccatg | BACTH |
| vp2224 C-term PhoA-LacZ cw | CCCCC tctaga g atggcccggacaccagaaatg | TOP |
| end -LacZ w/o STOP ccw | gcgccattcgccattcaggctgc | TOP |
| LacZ to vp2224 w/o ATG | CCT GAA TGG CGA ATG GCG C GCT GAA GAG ACT TTT TTA TCT GTA CC | TOP |
| end vp2224 ccw | CCCCC aagctt ttatcgtcgacgcccacgtgg | TOP |
| vp2224 ccw restore deletion | GCCGAAGCTTCTTTTTATTCTCT TTATCGTCGACGCCCACGTG | KI |

**Oligonucleotides used for *Pseudomonas* and *Shewanella* work**

|  |  |  |
| --- | --- | --- |
| Name | Sequence | Purpose |
| M13 | TGTAAAACGACGGCCAGTCC | CP/SP |
| M13r | CACACAGGAAACAGCTATGACC | CP/SP |
| flhF1-flhG1 fwd  | GCGCTGAGTGTGTTGATCCAAA | CP |
| EcoRV FliM1 N-term fwd | GCGAATTCGTGGATCCAGATGCTCATTGAAGATGCTCTCCTG | KI |
| EcoRV FliM1 N-term rev | GCCAAGCTTCTCTGCAGGATAATAAAACTGCGGCCCACTTCC | KI |
| Check-GFP FliM1-fwd | GCAGTTCAGATGAGTCATCCTC | CP |
| Check-GFP FliM1 KO-rev | GACATTTTGGCAGTTGATGCGAC | CP |
| OL FliM1 GFP rev | GAAAAGTTCTTCTCCTTTGCTGCTGCCTAATTCAGATATATCTCTAGCTTTGCCTTTGC | KI |
| OL FliM1 GFP fwd | GGATGAGCTCTACAAAGGATCCTAAGGTGAAGCAAGATGAGCACAGAAGATA | KI |
| EcoRV FlhF C-term fwd | GCGAATTCGTGGATCCAGATGCAAGAAATGGTTGGACAGCCT | KI |
| EcoRV FlhF C-term rev | GCCAAGCTTCTCTGCAGGATGCCACATCTAAAAATCGGTCGG | KI |
| Check-FlhF-FLAG-fwd | GCATCAGTCAATGCAAGCAACC | CP |
| OL-FlhF-Venus rev | CACGCTGCCCTCAAATGCACAGGCCATATTATCTG | KI |
| OL\_Venus fwd | GCATTTGAGGGCAGCGTGAGCAAGGGCGAGGAGCTGTT | KI |
| OL\_Venus rev | GTCATAACTTTACTTGTACAGCTCGTCCATGCC | KI |
| OL-FlhF-Venus fwd | TACAAGTAAAGTTATGACCCTGGATCAAGCAAG | KI |
| FlhF-Ven Seq\_Primer | GCTGAGTTAGTACGAGCACTAC | SP |
| FlhG-Ven Seq\_Primer | CGATATTATTGTCCGTGGGCCT | SP |
| FlhF-Ven Seq\_Primer fwd | GCTGTTGTAGTTGTACTCCAGC | SP |
| EcoRV FlhF C-term rev | GCCAAGCTTCTCTGCAGGATGCCACATCTAAAAATCGGTCGG | KI |
| EcoRV-2550-GFP-fwd | GCGAATTCGTGGATCCAGATGCCATCAATAACGGAAAAGGGG | KI/ CP |
| OL-2550-GFP-rev | GAAAAGTTCTTCTCCTTTGCTCAGTTCCAGAATATCTTTACGATGTAACCGGATCAATAATTCAGC | KI/CP |
| OL-2550-GFP-fwd | GGATGAGCTCTACAAAGGATCCTAACGAAGTGTAGGGGCTAAGACG | KI |
| EcoRV-2550-GFP-rev | GCCAAGCTTCTCTGCAGGATGCCTTTGTTTATATGCTCGACGG | KI |
| Check-2550-GFP-fwd | CGATGAAGAATGGGCTGAACTC | KI/CP |
| Check-2550-GFP-rev | CGAAGGATGCGAGAATGACGAA | KI/CP |
| OL-2069\_FlhF-rev | AATCTTCACTAGCATCCCCGTACATTGAACTC | KI |
| OL-FlhF-Ven-fwd | GGGATGCTAGTGAAGATTAAACGATTTTTTGCCAAAGAC | KI |
| OL-FlhF-Ven-rev | AACATTAGCTTACTTGTACAGCTCGTCCATGC | KI |
| OL-2068-fwd | TACAAGTAAGCTAATGTTTTAGGGTCTTACGCG | KI |
| BACTH 2550 pkT25 fwd | CAGGGTCGACTCTAGAGGGCGATGAATTTTTGATCGCGG | BACTH |
| BACTH 2550 pkT25 rev | TTAGTTACTTAGGTACCCGGGGTTTACGATGTAACCGGATCAATAATTCAGC | BACTH |
| BACTH 2550 fwd | CTGCAGGTCGACTCTAGAGGGCGATGAATTTTTGATCGCGG | BACTH |
| BACTH 2550 rev | GAGCTCGGTACCCGGGGTTTACGATGTAACCGGATCAATAATTCAGC | BACTH |
| OL\_FliM1 mCh rev | TTTGTATAACTCATCCATACCA | KI |
| FlhF-Ven Seq\_Primer rev | GCTGGAGTACAACTACAACAGC | SP |
| OL-GFP-fwd | AGCAAAGGAGAAGAACTTTTC | KI |
| OL-GFP-rev | GGATCCTTTGTAGAGCTCATCC | KI |
| OL -mCherry fwd | GTTTCCAAAGGGGAAGAGGACA | KI |
| pKT25-for | CACTGACGGCGGATATCGACATGTT | CP/SP |
| pKT25-rev | CCGCCGGACATCAGCGCCATTC | CP/SP |
| pUT18-for | CCAGGCTTTACACTTTATGCTTCC | CP/SP |
| pUT18-rev | GACGCGCCTCGGTGCCCACTGC | CP/SP |
| pKNT25-for | CCCAGGCTTTACACTTTATGCTTCC | CP/SP |
| pKNT25-rev | GTTTTTTTCCTTCGCCACGGCCTTG | CP/SP |
| pUT18C-for | CGGCGTGCCGAGCGGACGTTCG | CP/SP |
| pUT18C-rev | TCAGCGGGTGTTGGCGGGTGTC | CP/SP |
| FlhF Seq\_Primer fwd | GCCCACTTTGGATCAACACACT | SP |
| FlhF Seq\_Primer rev | CGTGCTCACAAAACTCGATGAA | SP |
| EcoRV FliFG1 KO fwd | GCGAATTCGTGGATCCAGATGCCGAAAACTTGTGGCTGAAAA | KO |
| OL- FliFG1 KO rev | ATCGCCACCCCCGACAATCATTTCTGTGCTC | KO |
| OL- FliFG1 KO fwd | ATTGTCGGGGGTGGCGATGAGTTCCTCTAAT | KO |
| EcoRV FliFG1 KO rev | GCCAAGCTTCTCTGCAGGATGCAACCTAATAGTCACTGCTTG | KO |
| OL-fipA L118A rev | AGCTTCAGCTTTGGGCGCTTCACA | KI |
| OL-fipA L118A fwd | ATAAAAGAGTGTGAAGCGCCCAAA | KI |
| OL-fipA G106A rev | TTCATCGACTCCCGCGGCAAGTCC | KI |
| OL-fipA G106A fwd | AAAATGGTCGGACTTGCCGCGGGA | KI |
| OL-PPfipA G104A rev | CATCGATACTCGCAGCCATCCC | KI |
| OL-PPfipA G104A fwd | GCTGGTGGGGATGGCTGCGAGT | KI |
| OL-PPfipA L123A rev | ACACCTTGCTCATCGCCTCCGC | KI |
| OL-PPfipA L123A fwd | GGCCGAGGCGGAGGCGATGAGC | KI |
| EcoRV-flhF KO-fwd | GCCAAGCTTCTCTGCAGGATGCATAGGCGTCGGTGATTGAGG | KO |
| OL-flhF KO-rev | TAAGTGAAGGCATTTGAGTAGAGTTATGACCCTGG | KO |
| OL-flhF KO-fwd | CTCAAATGCCTTCACTTATGCGTCCTCTACTGG | KO |
| EcoRV-flhF KO-rev | GCGAATTCGTGGATCCAGATGCTAAGCATTCTCCTAAGCTTGTTG | KO |
| OL-fipA L125A rev | TAACCGGATCAAGGCTTCAGCTTC | KI |
| OL-fipA L125A fwd | GCTGAAGCTGAAGCCTTGATCCGG | KI |
| EcoRV FlhF sub rev | GCCAAGCTTCTCTGCAGGATGCTCGTCACATACAACGACTAG | KI |
| BACTH 2550 L125A pkT25 rev | TTAGTTACTTAGGTACCCGGGGTTTACGATGTAACCGGATCAAGGCTTCAGC | BACTH |
| BACTH 2550 L125A rev | GAGCTCGGTACCCGGGGTTTACGATGTAACCGGATCAAGGCTTCAGC | BACTH |
| OL-FipA L125A-GFP-rev | GAAAAGTTCTTCTCCTTTGCTCAGTTCCAGAATATCTTTACGATGTAACCGGATCAAGGCTTCAGC | KI |
| BACTH FlhF pkT25 fwd | CAGGGTCGACTCTAGAGAAGATTAAACGATTTTTTGCCAAAGACA | BACTH |
| BACTH FlhF pkT25 rev | TTAGTTACTTAGGTACCCGGGGCTCAAATGCACAGGCCATATTATCT | BACTH |
| BACTH FlhF fwd | CTGCAGGTCGACTCTAGAGAAGATTAAACGATTTTTTGCCAAAGACA | BACTH |
| BACTH FlhF rev | GAGCTCGGTACCCGGGGCTCAAATGCACAGGCCATATTATCT | BACTH |
| BACTH FlhF GTG fwd | CTGCAGGTCGACTCTAGAGGTGAAGATTAAACGATTTTTTGCCAAAG | BACTH |
| EcoRV\_FipA KO fwd | GCGAATTCGTGGATCCAGATTTTTAGGTATCATTAACTTACGTGGTAATGT | KO |
| OL-FipA KO rev | ACACTTCGCTATTTACGATGATCGCCCATTAAAAATCCTTATGCA | KO |
| OL-FipA KO fwd | AAGGATTTTTAATGGGCGATCATCGTAAATAGCGAAGTGTAGGG | KO |
| EcoRV-FipA KO rev | GCCAAGCTTCTCTGCAGGATGAACTGATCGCCTTTGTTTATATGC | KO |
| Check-FipA KO fwd | AAGAAATGTCGCAGCCGTAGC | CP |
| Check-FipA KO rev | CCAGTTGCGACAATCTTCGGAG | CP |
| OL-PPfipA L116A rev | CATCAACTCCGCCTCGGCCTGGGTCGCGCCGCAGCTCTGGGT | KI |
| OL-PPfipA L1164A fwd | GAGTTGACCCAGAGCTGCGGCGCGACCCAGGCCGAGGCG | KI |
| Check-PP\_4331 (FipA) fwd | GCTTACGAACAGAACGCAAGGC | CP |
| Check-PP\_4331 (FipA) rev | GCAATACGTGATTTCGGTGCAG | CP |
| EcoRV-PP\_4331 KO-fwd | GCGAATTCGTGGATCCAGATGCAGATGCACGCCAAACAGAAA | KO |
| PP\_4331 KO-OL-rev | TCAAGGAGCTAGGATCAACTCAGATGTTCTCCAGC | KO |
| PP\_4331KO-OL-fwd | TTGATCCTAGCTCCTTGACGGGGTACCCTCG | KO |
| EcoRV-PP\_4331 KO-rev | GCCAAGCTTCTCTGCAGGATGCATGAATTGCCTGTACAACACCA | KO |
| Check-PP\_4331KO-fwd | GCGAAACGATCGATCAGGTCGA | CP |
| Check-PP\_4331KO-rev | GCACCGTAATCGAACACATGTG | CP |
| EcoRV-PP\_4331-GFP-fwd | GCGAATTCGTGGATCCAGATGCAGATGCACGCCAAACAGAAA | KI |
| PP\_4331-GFP-OL-rev | GAAAAGTTCTTCTCCTTTGCTCAGTTCCAGAATATCAGGAGCCCGGTACACCTTGCTC | KI |
| PP\_43310-GFP-OL-fwd | GGATGAGCTCTACAAAGGATCCTGACGGGGTACCCTCGGCAGCA | KI |
| EcoRV-PP\_4331-GFP-rev | GCCAAGCTTCTCTGCAGGATGCATGAATTGCCTGTACAACACCA | KI |
| EcoRV-FlhF-mCh-fwd | GCGAATTCGTGGATCCAGATGCATGGACAGCTTCCGTATCGG | KI |
| FlhF-mCh-OL-rev | CTCTTCCCCTTTGGAAACGCTGCCACCCGCTCGCCGTGGGTTGTGA | KI |
| FlhF-mCh-OL-fwd | ATGGATGAGTTATACAAATGACCATGAAGCGTGTGCAAAG | KI |
| EcoRV-FlhF-mCh-rev | GCCAAGCTTCTCTGCAGGATGCCAACACACGGAAACGGTTCA | KI |
| Check-PP\_4343 KO-fwd | GCCTGAAATCGAGCCGATCGAA | CP |
| Check-PP\_4343 KO-rev | GCGTCGGTAATCGAGGTAGGTT | CP |
| BACTH PP FipA pkT25 fwd | CAGGGTCGACTCTAGAGATCCTAGAGGTTGCTGTCATCT | BACTH |
| BACTH PP FipA pkT25 rev | TTAGTTACTTAGGTACCCGGGGAGGAGCCCGGTACACCTTGCTC | BACTH |
| BACTH PP FipA fwd | CTGCAGGTCGACTCTAGAGATCCTAGAGGTTGCTGTCATCT | BACTH |
| BACTH PP FipA rev | GAGCTCGGTACCCGGGGAGGAGCCCGGTACACCTTGCTC | BACTH |
| BACTH PP FlhF pkT25 fwd | CAGGGTCGACTCTAGAGCAAGTTAAGCGATTTTTCGCCGC | BACTH |
| BACTH PP FlhF pkT25 rev | TTAGTTACTTAGGTACCCGGGGACCCGCTCGCCGTGGGTTGTGA | BACTH |
| BACTH PP FlhF fwd | CTGCAGGTCGACTCTAGAGCAAGTTAAGCGATTTTTCGCCGC | BACTH |
| BACTH PP FlhF rev | GAGCTCGGTACCCGGGGACCCGCTCGCCGTGGGTTGTGA | BACTH |
| EcoRV FlhF sub fwd | GCGAATTCGTGGATCCAGATGCATCAGTCAATGCAAGCAACC | KI |
| Check-FlhF KI/O-fwd | GCCACTGGGTAGTGTCGTAAAA | CP |
| OL-FlhF D328A rev | CCCCATACCAGCGGTGGCTATCAATAC | KI |
| OL-FlhF D328A fwd | AAGCTAGTATTGATAGCCACCGCTGGT | KI |
| EcoRV PPFlhF sub fwd | GCGAATTCGTGGATCCAGATGCATGTTCTGGCGTATCAGGAA | KI |
| OL-FlhF K235A rev | GCGCGCGGCCAGCGCGGCCAGGGT | KI |
| OL-FlhF K235A fwd | GGCAAGACCACCACCCTGGCCGCGCTGGCCGCG | KI |
| EcoRV PPFlhF sub rev | GCCAAGCTTCTCTGCAGGATGCATGCTACCCATGTCTGTTCT | KI |
| Check-PP\_4343 KI-fwd | GCTACCAGTGATTACCCTGGAG | CP |
| EcoRV PPFlhF sub 1 rev | GCCAAGCTTCTCTGCAGGATGCGTCGGTAATCGAGGTAGGTT | KI |
| KT2440 FlhF Seq primer rev | GCTGGTGAGCATGGACAGCTTC | SP |
| EcoRV-FipA dTM fwd | GCGAATTCGTGGATCCAGATGCCGTAGCTGCAAGTAAAGATG | KI |
| OL-FipA dTM rev | CTGCTTTTGTTCATCGCCCATTAAAAATCCTTATGC | KI |
| OL-FipA dTM fwd | GGCGATGAACAAAAGCAGTTGAGTAAATTACGTAATAAAGTTG | KI |
| OL-PP\_FipA dTM rev | GCTGTAGTTCTCTAGGATCAACTCAGATGTTCTCC | KI |
| OL-PP\_FipA dTM fwd | ATCCTAGAGAACTACAGCAAGCGCCAGCGCG | KI |

**Abbreviations**: fwd: forward; rev: reverse; KO: knock-out (deletion) primer; KI: knock-in (integration) primer, CP: check primer, SP: sequencing primer, BACTH: Bacterial adenylate cyclase two-hybrid system primer