# Supplementary File

# Development of an efficient conjugation-based genetic manipulation system for *Pseudoalteromonas*

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**Table S1.** Antibiotic resistance of *Pseudoalteromonas* strains.

| **Strain** | **Amp** | **Apr** | **Cm** | **Ery** | **Gm** | **Kan** | **Spc** | **Tet** |
|---|---|---|---|---|---|---|---|---|
| A37-1-2 | + | ++ | - | - | +++ | +++ | +++ | +++ |
| DSM 16099 | + | + | - | - | ++ | ++ | + | +++ |
| DSM 16098 | + | ++ | - | - | +++ | +++ | +++ | +++ |
| DSM 6842 | - | +++ | - | - | +++ | +++ | ++ | +++ |
| SM20429 | - | + | - | - | +++ | +++ | +++ | +++ |
| SM9913 | + | ++ | - | - | +++ | +++ | +++ | +++ |
| TAC125 | - | + | - | - | ++ | ++ | +++ | +++ |
| SCSIO 04301 | +++ | +++ | - | - | + | ++ | + | +++ |
| SCSIO 11900 | ++ | +++ | - | - | ++ | ++ | + | +++ |

Amp (ampicillin, 100 μg/ml); Apr (apramycin, 100 μg/ml); Cm (chloramphenicol, 30 μg/ml); Ery (erythromycin, 25 μg/ml); Gm (gentamycin, 10 μg/ml); Kan (kanamycin, 50 μg/ml); Spc (spectinomycin, 100 μg/ml); Tet (tetracycline, 10 μg/ml).

(-, no growth; +, growth)

**Table S2. Primers used in this study.**

| Primers | Sequence (5'-3') | Purpose | Expected size (bp) |
|---|---|---|---|
| oriT-F | CGGGATCCGGGTATGTGGTCGAAGGCTG | Construction of pWD2-oriT | 1506 |
| oriT-R | CGGGATCCCAGCTGGCAATTCCGGTTCG | | |
| pWD2-S | GAATTTCAACGGCATTCAAGC | Detection of pWD2-oriT | 428 |
| pWD2-A | GACCTTTTAGATCCAGCTCC | | |
| Ery-F | CGGGATCCCCAGTGACTAATCTTATGAC | Construction of pK18*mobsacB*-Ery and pWD2Ery-oriT | 914 |
| Ery-R | CGGAATTCCTCCATTCCCTTTAGTAACG | | |
| bsmA-up-S | CCCAAGCTTCTTTCTGATGTGACAGGCGAG | Upstream homologous fragment of *bsmA* | 753 |
| bsmA-up-A | ACATGCATGCCTGGTTGTTTAATGCGGTC | | |
| bsmA-down-S | ACATGCATGCGAAGGTGGTGAGCTATTGG | Downstream homologous fragment of *bsmA* | 933 |
| bsmA-down-A | CGCGTCGACGGTGTAAACCCTTCTAAGTG | | |
| bsmA-wS | CCCAGAGCTAATGGAAGCTG | Confirmation of Δ*bsmA* | 2351[a] |
| bsmA-wA | GCGTGAGTGACTGTCATCAAG | | 1919[b] |
| bsmA-dS | GCCACCGCATTAAACAACCAG | Confirmation of the pK18Cm-*bsmA* integration | 627[a] |
| bsmA-dA | CCAGCAGTTAGTACAAAGCAATC | | 195[b] |
| pigM-up-S | GCTCTAGAGAATGCGGGTAAGTAAAGA | Upstream homologous fragment of *pigM-K* region | 966 |
| pigM-up-A | CGGAATTCAAACCATTGCGTAGTTGTAA | | |
| pigM-down-S | CGGAATTCCGCCCAGGTGCTGAATGTG | Downstream homologous fragment of *pigM-K* region | 815 |
| pigM-down-A | CCCAAGCTTCAGCCTGTGCCAGTTCCAT | | |
| pigM-wS | CAAGCCCTACAGCCAACAT | Confirmation of Δ*pigM-K* | 3963[a] |
| pigM-wA | CGGAACATCTGAAGATCCCAC | | 1938[b] |
| pigM-dS | TGCTGACGCCTTTGTGCTG | Confirmation of the pK18Ery-*pigM-K* integration | 2899[a] |
| pigM-dA | ATGCCATTTCGGGTTCCTG | | 874[b] |
| hmgA-up-S | GCTCTAGACTGAAATAGGGCTTACTGG | Upstream homologous fragment of *hmgA* | 1954 |
| hmgA-up-A | AACTGCAGTTGTGCATTTGCGCTACCATTT | | |
| hmgA-down-S | AACTGCAGAACACTTTCCGCCCACCCTATT | Downstream homologous fragment of *hmgA* | 2054 |
| hmgA-down-A | CCCAAGCTTCAATGTCACAAACGGCTCAA | | |
| hmgA-wS | AAGGTTTGGATTTTTCTTTTCG | Confirmation of Δ*hmgA* | 4788[a] |
| hmgA-wA | GTTGAAGTCACCTACTCACCTC | | 4197[b] |
| hmgA-dS | TCGCTTTCATCTCTTTTGTC | Confirmation of the pK18Ery-*hmgA* integration | 1560[a] |
| hmgA-dA | TAGGCATCGCTTTGCTGTTC | | 969[b] |
| fliFG-up-S | ACATGCATGCGCTAATAAAGTTGCTCGATC | Upstream homologous fragment of *fliFG* region | 1512 |
| fliFG-up-A | CGGAATTCAATAATTCTCCACTACCTAGTT | | |
| fliFG-down-S | CGGAATTCTCAAGAACAAAAACAACTACC | Downstream homologous | 1511 |

| | | | |
|---|---|---|---|
| fliFG-down-A | ACGCGTCGACGTACCAGCTGTCTTTCTGCT | fragment of *fliFG* region | |
| fliFG-wS | GCTAAGCCATTTGCACCAG | Confirmation of Δ*fliFG* | 4884[a] |
| fliFG-wA | GGCTATGATTGCTTCGCAA | | 3168[b] |
| fliFG-dS | GCCGCTTTATCTACACCAT | Confirmation of the | 1908[a] |
| fliFG-dA | GCTTCACTGAGCATAGTGGCT | pK18Ery-*fliFG* integration | 192[b] |
| hmgA-pWD2-oriT-F | ACATGCATGCTCGACCATATCGCTGCCATCACG | Complementation of Δ*hmgA* | 1755 |
| hmgA-pWD2-oriT-R | AACTGCAGTTATACGTCCTGTTTACCTGTA | | |

Restriction sites included in oligonucleotide sequences are underlined.

[a] the size of the wild-type.

[b] the size of the deletion mutant.

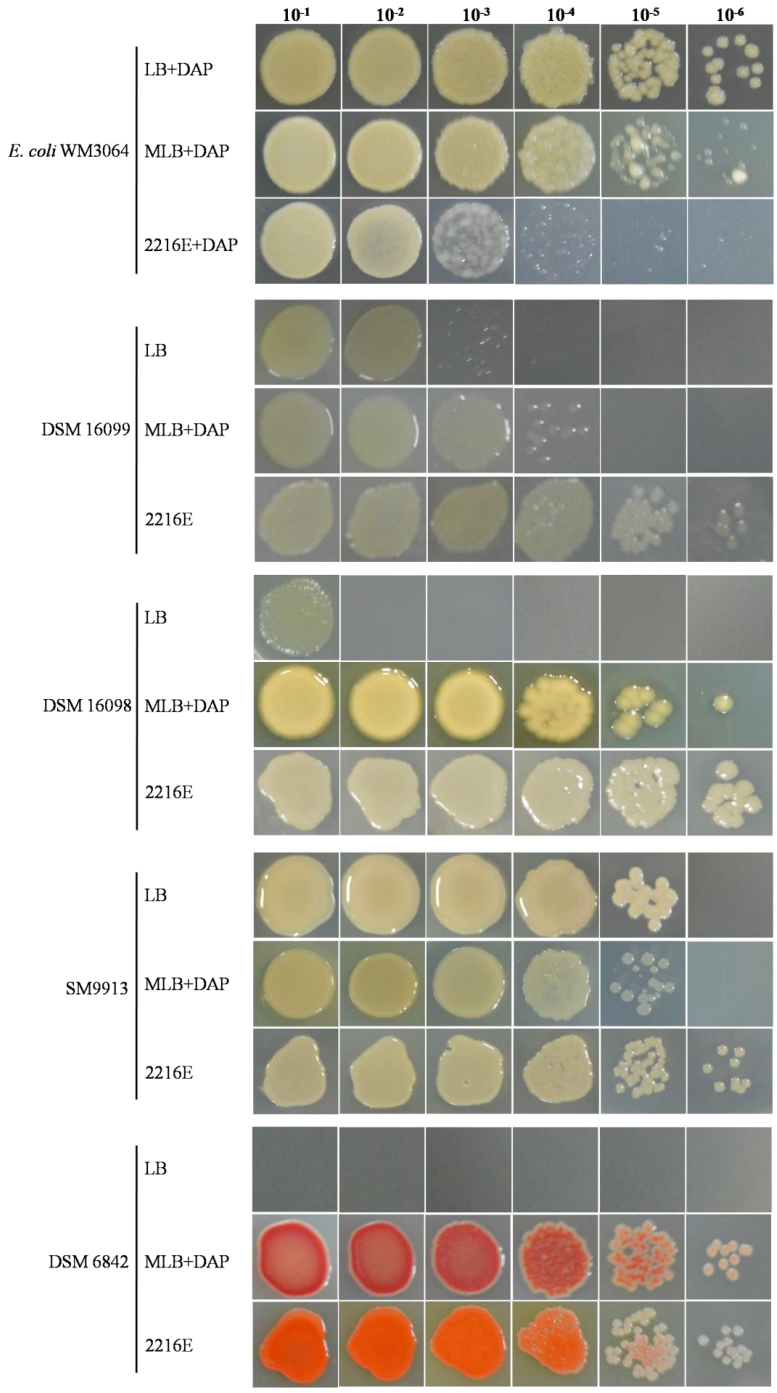


**Fig. S1.** Growth test of the *E. coli* WM3064 donor strain and four recipient strains (DSM 16099, DSM 16098, SM9913, and DSM 6842) in LB with or without DAP, modified LB medium (MLB) with DAP, and 2216E with or without DAP.

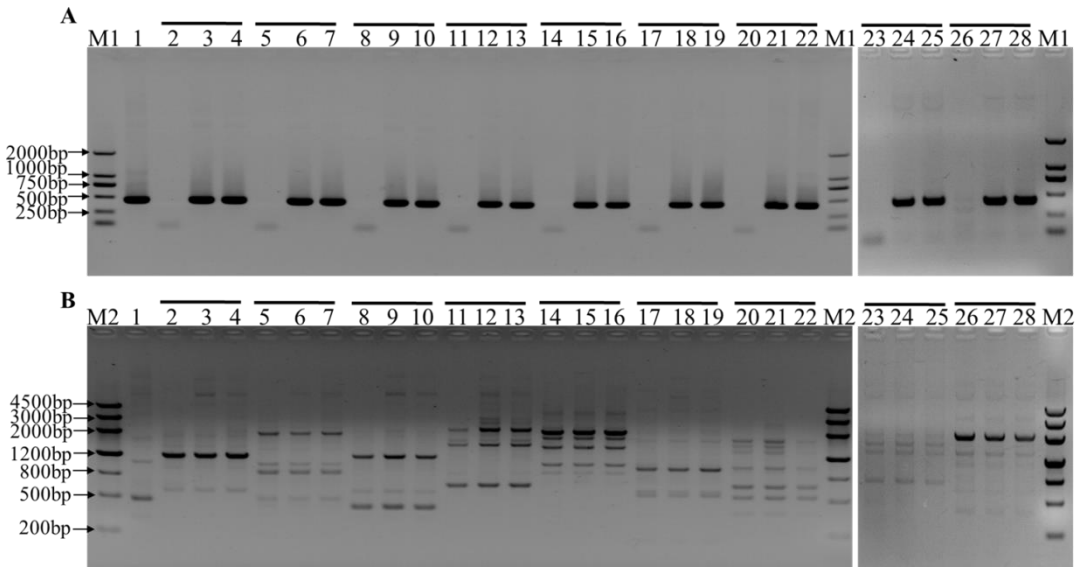


**Fig. S2.** Confirmation of transconjugation by PCR. (A) PCR amplification with the pWD2-S/pWD2-A primer pair to detect pWD2-oriT in the transconjugants. (B) RAPD-PCR to confirm the host strain of the transconjugants. The templates in (A) and (B) are: Lane 1, donor strain *E. coli* WM3064/pWD2-oriT; Lane 2, SM9913; Lane 3-4, SM9913/pWD2-oriT; Lane 5, A37-1-2; Lane 6-7, A37-1-2/pWD2-oriT; Lane 8, DSM 16099; Lane 9-10, DSM 16099/pWD2-oriT; Lane 11, DSM 16098; Lane 12-13, DSM 16098/pWD2-oriT; Lane 14, SM20429; Lane 15-16, SM20429/pWD2-oriT; Lane 17, TAC125; Lane 18-19, TAC125/pWD2-oriT; Lane 20, DSM 6842; Lane 21-22, DSM 6842/pWD2-oriT. Lane 23, SCSIO 04301; Lane 24-25, SCSIO 04301/pWD2-oriT; Lane 26, SCSIO 11900; Lane 27-28, SCSIO 11900/pWD2-oriT; M1, DL2K marker; M2, DNA Marker III.

**Construction of the Δ*bsmA* strain in SM9913.**

The suicide plasmid used for *bsmA* gene knockout was based on pK18*mobsacB*-Cm; the schematic is shown in **Fig. 3.** Two primer pairs (bsmA-up-S/bsmA-up-A and bsmA-down-S/bsmA-down-A) were used to amplify the upstream and downstream DNA sequences of *bsmA* from SM9913 genomic DNA. The 753 bp and 933 bp PCR fragments were digested with *Hin*dIII/*Sph*I and *Sph*I/*Sal*I, respectively, and cloned into the *Hin*dIII/*Sal*I sites of pK18*mobsacB*-Cm. These were transformed into *E.coli* WM3064 to yield the WM3064/pK18Cm-*bsmA* recombinant strain for *bsmA* gene knockout.

The pK18Cm-*bsmA* suicide vector was mobilized from *E. coli* WM3064 into SM9913 by intergeneric conjugation. After mating, the cells were spread on 2216E plates containing chloramphenicol (30 μg/ml) to screen for clones in which the suicide plasmid pK18Cm-*bsmA* had inserted into the SM9913 genome via a single crossover event. Mutants were then grown at 20°C with shaking in 2216E medium without antibiotics for 8 h. To select the mutants in which the second recombination had occurred, the culture was diluted and spread on 2216E containing 10% sucrose and grown at 20°C for about 36-48 h. Single colonies were then transferred onto 2216E and 2216E containing chloramphenicol (30 μg/ml) plates simultaneously, and the colonies sensitive to chloramphenicol were collected and confirmed by PCR followed by DNA sequencing.

**Construction of the Δ*hmgA* strain in SCSIO 04301 and complementation of Δ*hmgA*.** The suicide plasmid used for *hmgA* gene knockout was based on pK18*mobsacB*-Ery; the schematic is shown in **Fig. 3**. Two primer pairs (hmgA-up-S/hmgA-up-A and hmgA-down-S/hmgA-down-A) were used to amplify the upstream and downstream DNA sequences of *hmgA* from SCSIO 04301

genomic DNA. The 1954 bp and 2054 bp PCR fragments were digested with *Xba*I/*Pst*I and *Pst*I/*Hin*dIII, respectively, and cloned into the XbaI/*Hin*dIII sites of pK18*mobsacB*-Ery. These were transformed into *E.coli* WM3064 to yield the WM3064/pK18Ery-*hmgA* recombinant strain for *hmgA* gene knockout.

The pK18Ery-*hmgA* suicide vector was mobilized from *E. coli* WM3064 into SCSIO 04301 by intergeneric conjugation. After mating, the cells were spread on 2216E plates containing erythromycin (25 μg/ml) to screen for clones in which the suicide plasmid pK18Ery-*hmgA* had inserted into the SCSIO 04301 genome via a single crossover event. Mutants were then grown at 25°C with shaking in 2216E medium without antibiotics for 8 h. To select the mutants in which the second recombination had occurred, the culture was diluted and spread on 2216E containing 10% sucrose and grown at 25°C for about 36-48 h. Single colonies were then transferred onto 2216E and 2216E containing erythromycin (25 μg/ml) plates simultaneously, and the colonies sensitive to erythromycin were collected and confirmed by PCR using appropriate primers and sequencing.

For complementation of Δ*hmgA*, the *hmgA* gene with its promoter was amplified from SCSIO 04301 using the primers hmgA-pWD2-oriT-F and hmgA-pWD2-oriT-R. The 1755 bp PCR fragment was digested with *Sph*I and *Pst*I, and cloned into the corresponding sites of pWD2-oriT. These were transformed into *E.coli* WM3064 to yield the WM3064/pWD2-oriT-*hmgA* recombinant strain. The pWD2-oriT-*hmgA* complementation vector was mobilized from *E. coli* WM3064 into Δ*hmgA* by intergeneric conjugation. After mating, the cells were spread on 2216E plates containing chloramphenicol (30 μg/ml) to screen for clones in which the pWD2-oriT-*hmgA* had transferred into Δ*hmgA*. As a control, the empty vector pWD2-oriT was transferred into the Δ*hmgA* as the similar

steps. Colony PCR using pWD2-oriT-specific primers was used to confirm the presence of the transferred plasmid.

**Construction of the Δ*fliFG* mutant strain in SCSIO 11900.** The suicide plasmid used for deletion of a DNA region containing *fliF and fliG* genes was based on pK18*mobsacB*-Ery. The schematic is shown in **Fig. 3**. Two primer pairs (fliFG-up-S/fliFG-up-A and fliFG-down-S/fliFG-down-A) were used to amplify the upstream and downstream DNA sequences of the target region in SCSIO 11900 genomic DNA. The 1512 bp and 1511 PCR fragments were digested with *Sph*I/*Eco*RI and *Eco*RI/*Sal*I, respectively, and cloned into the *Sph*I/*Sal*I sites of pK18*mobsacB*-Ery. These were transformed into *E. coli* WM3064 to yield the WM3064/pK18Ery-*fliFG* recombinant strain.

The suicide plasmid pK18Ery-*fliFG* was mobilized from *E. coli* WM3064 into SCSIO 11900 by intergeneric conjugation. After mating, cells were spread on 2216E plates containing erythromycin (25 μg/ml) to screen for clones in which the suicide vector pK18Ery-*fliFG* had inserted into the SCSIO 11900 genome via a single crossover event. The mutants were then grown at 25°C with shaking in 2216E medium without antibiotics for 8 h. To select mutants in which the second recombination had occurred, the culture was diluted and spread on 2216E medium containing 10% sucrose and grown at 25°C for about 24-36 h. Single colonies were transferred onto 2216E and 2216E containing erythromycin (25 μg/ml) plates simultaneously, and colonies sensitive to erythromycin (25 μg/ml) were collected and confirmed by PCR followed by DNA sequencing.