

Table S2. List and application of primers used in this work.

| Primer name | Sequence 5'-3' ^a | 5' Position | Remarks |
|----------------------------------|--|-------------|--|
| pPsv48B (accession no. FR820586) | | | |
| mobB_pB_F | <u>GGATCCT</u> GCCTGTTCTTGGTTTGTG | 37661 | Cloning <i>mobB</i> into pJET1.2 |
| mobB_pB_R | <u>GGATCCT</u> GAGCGTCACGAAGTAGCTC | 41869* | |
| ext_mobBpB_F | TCATCGTGGCACCCATATC | 37598 | Check marker exchange mutagenesis of <i>mobB</i> |
| ext_mobBpB_R | GACCTGCGTCACTCATAGCC | 41938* | |
| virB4_F | <u>GGATCCT</u> TTAACCCGCAATGGAATG | 17559 | Cloning <i>virB4</i> into pJET1.2 |
| virB4_R | <u>GGATCC</u> GGCATCAACGAAATTGAAC | 21264* | |
| ext_virB4_F | GAGCCTCAAGTACGGCAAAC | 17523 | Check marker exchange mutagenesis of <i>virB4</i> |
| ext_virB4_R | CACATCGTCCCGATGAGC | 21337* | |
| oriT_pB_F | TAT <u>GGTACCT</u> GTGTCATCGAGAGACATTCAG | 37837 | Cloning <i>oriT</i> into pKMAGJ |
| oriT_pB_R | TATCTGCAGCCTCCGACGATCAATGAC | 38057* | |
| bit_F | <u>GGATCC</u> GCGCCGTCTGTTCTTTTC | 15310* | Check possession of pB and construct pB-specific probe |
| bit_R | <u>GGATCCT</u> AAGCGTGGTCGCTGATATG | 14062 | |
| pPsv48C (accession no. FR820587) | | | |
| mobB_pC_F | <u>TACGTA</u> AGCGTTGTTGTTGTCGTTG | 21202 | Cloning <i>mobB</i> into pJET1.2 |
| mobB_pC_R | <u>TACGTAT</u> GAGCGTCACGAAGTAGCTC | 25436* | |

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|-------------------------|------------------------------------|--|--|
| ext_mobBpC_R | GCCTCTTCCAGCCCTACAC | 25488* | Check marker exchange mutagenesis of <i>mobB</i> with primer oriT_pC_F |
| traA_F | GCGATCTGGTGCTGAGATTC | 37100 | Cloning <i>traA</i> into pJET1.2 |
| traA_R | CATGTGGCTTTCTCCAGTTG | 39630* | |
| ext_traA_F | TTCCGGACTGTTTTTGATCC | 37003 | Check marker exchange mutagenesis of <i>traA</i> |
| ext_traA_R | GCTGCTTGTTTCATCGGTTTC | 39680* | |
| oriT_pC_F | TATGGTACCTAGGTGCAGTGTGCACTTGTC | 21047 | Cloning <i>oriT</i> into pKMAGJ |
| oriT_pC_R | TATCTGCAGCCACAAGATCACCTCCTG | 21266* | |
| Other purposes | | | |
| Km_R | CTAGCGAACCCCAGAGTCC | Used to amplify the Km ^R cassette from pK18 <i>mob</i> sacB | |
| pk18mob_R | CAGGAAACAGCTATGACA | | |
| pk18mob-km-sac_F | <u>GATATC</u> TTATGGACAGCAAGCGAACC | Used to amplify the Km ^R - <i>sacB</i> cassette from pK18 <i>mob</i> sacB | |
| pk18mob-km-sac_R | <u>GATATC</u> TCGTGGACTATGAGCACGTC | | |
| Gm_pJQ200_F | GCAAAAGAAAATGCCGATG | Used to amplify the Gm ^R cassette from pJQ200SK | |
| Gm_pJQ200_R | CCTTGCCGTAGAAGAACAGC | | |
| 3335_F1 | TTTGCGCACAGCTTAGTACG | | |

| | | |
|--------------------------------------|---|--|
| 3335_R1 | <u>GAATTC</u> CTCGGGGCACAACAGGAC | Used to amplify the downstream region of pseudogene PSA3335_RS06050 from chromosome of NCPPB 3335 for insertion of Tc ^R cassette by marker exchange |
| 3335_F2 | <u>GAATTC</u> CCCCGGGACATTGGCCAGTCCGAAGAC | |
| 3335_R2 | CTTCGCACCCTTTGTTTCAG | Used to amplify the upstream region of pseudogene PSA3335_RS06050 from chromosome of NCPPB 3335 for insertion of Tc ^R cassette by marker exchange |
| 3335_ext_F | ATTCGTTCGGCATCGTTC | Check insertion of Tc ^R cassette into the pseudogene PSA3335_RS06050 from chromosome of NCPPB 3335 |
| 3335_ext_R | CGAGGGCGACGAAAGATG | |
| Check insertions and marker exchange | | |
| Km_Insrt_chk | ACCTGCGTGCAATCCATC | |
| Km_chk_F | GAATGGGCTGACCGCTTC | |
| Kmsac_chk_F | GGTACCGAGCGAAATGACC | |
| pJQ200SK_F | AGGTCTGGATTTCGATCACG | |
| Gm_pJQ200_R1 | TACGTGCAAGCAGATTACGG | |
| Gm_pJQ200_F1 | ACGAACCGAACAGGCTTATG | |

^a Restriction sites introduced in primers are underlined.

* The primer anneals in the reverse strand of the DNA molecule.