Supplementary Information

Evolved plasmid-host interactions reduce plasmid interference cost

Hirokazu Yano^{1,2,4}, Katarznya Wegrzyn³, Wesley Loftie-Eaton^{1,2}, Jenny Johnson¹, Gail E. Deckert^{1,2}, Linda M. Rogers^{1,2}, Igor Konieczny³, and Eva M. Top^{1,2}

¹ Department of Biological Sciences, ² Institute for Bioinformatics and Evolutionary Studies, University of Idaho, Moscow, Idaho 83844, USA. ³ Department of Molecular and Cellular Biology, Intercollegiate Faculty of Biotechnology, University of Gdansk, 24 Kladki, 80-822 Gdansk, Poland.

⁴ Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305-8571 Japan

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Fig. S3. Loss of TrfA1 production improves persistence of pBP136Km in *P. putida* populations.

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Plasmid and strain constructions

1. Constructs used for fitness cost and plasmid copy number analysis.

DNA cloning was generally performed using *E. coli* strain EC100. EC100D *pir*+ cells were used for DNA cloning into pHY835LAC (Table S1). Phusion DNA polymerase (New England Biolab) was used for PCR. The *trfA* genes were amplified from pMS0506 and its evolved variants (pEvo-Sh1, pEvo-Sh11, pEvo-Sh5, pEvo-Sh13, pEvo-Sh3) by PCR, and the PCR products were cloned into pHSG398. All strains and plasmids are listed in Table S1. The M124L mutation was introduced into *trfA1* and its variants on the pHSG398 derivative, by amplifying the pHSG398 derivative with primers trfAM124LF, trfAM124LF (Table S2), phosphorylation and self-ligation of the PCR products. After the sequences were confirmed to be correct, the insert region in the pHSG398 derivatives was amplified using primers SacI-SD-pBP136trfA1F, and BamHindTrfAR. The PCR products were digested with *Sac*I and *Hin*dIII, and cloned into *SacI-Hin*dIII site region of pBBR1-MCS2. The *trfA2* genes were amplified using primers SacI-SD-pBP136trfA2, BamHindTrfAR using pMS0506 as template, and cloned into pBBR1-MCS2. This gave rise to pHY1010, pHY1011, pHY1012, pHY1014, pHY1015, pHY987, and pHY988. Using the same method, *trfA1* and its variants were cloned into the *SacI-Hin*dIII sites region of pHX835LAC. This gave rise to pHY884, pHY885, pHY886, pHY888, pHY890, pHY892, pHY897, and pHY895.

For construction of copy number reference plasmid pHY873, the 2-kb fragment containing *atp* genes of strain MR-1 was amplified from genomic DNA of MR-1 using primers EcoMR1oriCF, EcoMR1oriCR (Table S2). The PCR product was digested with *Eco*RI and the 1.8-kb fragment was cloned into the *Eco*RI site of pHY872 (Yano *et al*, 2012). This gave rise to pHY873.

The *dnaB* gene of *S. oneidensis* was PCR amplified using primers SacSDdnaBMR1F, HinddnaBMR1R, and the PCR product was cloned into pHSG398. After the sequence of the cloned *dnaB* was confirmed to be correct, the *Sac*I-*Hin*dIII region of the pHGS398 derivative was moved into the equivalent region of pHY835LAC. This gave rise to pHY1018.

2. Constructs used for biochemical assays

For biochemical assays, monomeric TrfA was used. For this purpose we introduced a mutation into each *trfA* gene on the pHSG398 derivatives constructed above by PCR amplification using primers Q279D/S292Lr and Q279D/S292Lf (Table S2), and subsequent phosphorylation and self-ligation of the PCR products. After confirmation of mutations in the pHSG398 derivatives, their respective *trfA* region was amplified using primers EcoNdeHisTrfA1F and BamHindTrfAR. The PCR products were digested with *Nde*I and *BamH*I, and then cloned into *Nde*I-*Bam*HI sites region of pET11a. This gave rise to pHY915, pHY921, pHY916, pHY917 and pHY919. The *dnaB* gene of MR-1 was

amplified with primers EcoNdeDnaBmr1F and HindDnaBmr1hisR (Table S2). The PCR products were digested, and then cloned into *Ndel-Hind*III sites of pET22b. This gave rise to pHY1032.

3. Insertion of mini-Tn7-*trfA* in the chromosome

To insert mini-Tn7 carrying *trfA* or *dnaB* into chromosomes, pHY835LAC derivatives were first introduced into *E. coli* strain BW29427 in the presence of diaminopimelic acid. Tn7 transposase expression plasmid was also introduced into BW29427. Two donor strains BW29427 (pHY835 derivatives), BW29427 (pUX-BF13) and one recipient strain, either MR-1 or BW25113 were grown overnight and one ml of each culture was pelleted and pooled in 1.5 ml tube. Cell suspensions were placed on LBA containing 100 mM diaminopimelic acids. The folowing day, mating mixtures were serially diluted in saline and plated on LBA with 30 μ g/ml Gm. The colonies were re-streaked once on the same selective agar to purify. Presence of mini-Tn7 in the specific site in the chromosome (*att*Tn7) was confirmed by PCR using primer pairs, E coli glmS and Tn7R109 (Table S2) for *E. coli* BW25113, and MR-1glmS and Tn7R109 for *S. oneidensis* MR-1.

Table S1. Strains and plasmids used in this study	
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Strains or plasmids References Strains Escherichia coli BL21(DE3) F. cmp3 blac2479 hadfb514 Å (araBAD)567 Å(haBAD)568 rph-1 (Baba et al. 2006) BW29427 thrB1004 pro thir psc. has blac2 AM15 DA(araBAD)567 (Baba et al. 2006) BW29427 thrB1004 pro thir psc. has blac2 AM15 DA(araBAD)567 (Purdue Unix) EC100 pir+ F. mcrA A(mr-hsdfMAs-mcBC) \#00diac2 AM15 DA(araZY4 recA1 Epicentre endA1 araD139 Å(ara, leu)7697 galU galK Å rpsl. hupG; Sm1 Epicentre Epicentre W10341 BW25113::atfTn7::min1-Tn7::aacC1-lacft-Ptac-trfA1(M124L); Gm1 (Yano et al. 2012) HY0391 BW25113::atfTn7::min1-Tn7::aacC1-lacft-Ptac-trfA1(AS1P/M124L); Gm1 HY0392 BW25113::atfTn7::min1-Tn7::aacC1-lacft-Ptac-trfA1(AS1P/M124L); Gm1 HY0408 BW25113::atfTn7::min1-Tn7::aacC1-lacft-Ptac-trfA1(AS1M124L); Gm1 HY0409 BW25113::atfTn7::min1-Tn7::aacC1-lacft-Ptac-trfA1(AS1M124L); Gm1 HY0408 BW25113::atfTn7::min1-Tn7::aacC1-lacft-Ptac-trfA1(AS1M124L); Gm1 HY0409 BW25113::atfTn7::min1-Tn7::aacC1-lacft-Ptac-trfA1(AS1M124L); This study HY0409 BW25113::atfTn7::min1-Tn7::aacC1-lacft-Ptac-trfA1(AS1M124L); This study HY0408	Table ST. Stra	ams and plasmids used in this study	
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EC100D pir+ F- mcrA $\Delta(mr.hsdRMS-mcrBC) is 80dlac2 AM15 AlacX4 recA1 EpicentreendA1 araD139 \Delta(ara, leu)7697 galU galK X rpsL nupG pir+ (DHFR);SmHY0414 BW25113::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1//1/trA22; Gm7 (Yano et al, 2012)HY0391 BW25113::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(M124L); Gm7 (Yano et al, 2012)HY0392 BW25113::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(R31P/M124L);Gm7 This studyGm7 thY0408 BW25113::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(A25T/M124L);Gm7 This studyGm7 thY0408 BW25113::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(A25T/M124L);Gm7 This studyHY0408 BW25113::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(A25T/M124L);Gm7 This studyGm7 thY0409 BW25113::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(A25M124L);Gm7 This studyHY0475 BW25113::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(A25M124L);Gm7 This studyGm7 thY13::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(A27T/M124L);Gm7 This studyGm4 HY0475 BW25113::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(A27T/M124L);Gm4 This studyHY0525 MR-1::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(M2T);HY0759 MR-1::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(M2T);HY0321 MR-1::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(M2T);HY0325 MR-1::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(M2T);HY0326 MR-1::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(M2T);HY0337 MR-1::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(M2T);HY0333 MR-1::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(M2T);HY0333 MR-1::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(A25TM124L);Gm4 This studyHY0334 MR-1::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(A25TM124L);Gm4 This studyHY0335 MR-1::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(A25TM124L);Gm4 This studyHY0335 MR-1::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(A25TM124L);Gm4 This studyHY0336 MR-1::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(A25TM124L);Gm4 This studyHY0337 MR-1::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-trA1(A25TM124L);Gm4 This studyHY0345 MR-1::atfTn?::mini-Tn?::aacC1-lacf1-Ptac-tr$		endA1 araD139 Δ(ara, leu)7697 galU galK λ ⁻ rpsL nupG; Sm ^r	
endA1 araD139 Δ(ara, leu)7697 galU galK λ rpsL nupG pir+ (DHFR); Sm ¹ Sm ¹ BW25113::atfTn?::mini-Tn?::aacC1-lac ^A -Ptac-trfA1 ^(m) /trfA2 ² ; Gm ¹ (Yano et al. 2012) HY0330 BW25113::atfTn?::mini-Tn?::aacC1-lac ^A -Ptac-trfA1(M1241); Gm ¹ (Yano et al. 2012) HY0392 BW25113::atfTn?::mini-Tn?::aacC1-lac ^A -Ptac-trfA1(R31P/M124L); This study HY0395 BW25113::atfTn?::mini-Tn?::aacC1-lac ^A -Ptac-trfA1(A25T/M124L); This study Gm ¹ BW25113::atfTn?::mini-Tn?::aacC1-lac ^A -Ptac-trfA1(Δ5/M124L); This study HY0408 BW25113::atfTn?::mini-Tn?::aacC1-lac ^A -Ptac-trfA1(Δ43/M124L); This study Gm ¹ BW25113::atfTn?::mini-Tn?::aacC1-lac ^A -Ptac-trfA1(Δ43/M124L); This study HY0409 BW25113::atfTn?::mini-Tn?::aacC1-lac ^A -Ptac-trfA1(Δ43/M124L); This study Gm ¹ BW25113::atfTn?::mini-Tn?::aacC1-lac ^A -Ptac-trfA1(Δ5/M124L); This study HY0475 BW25113::atfTn?::mini-Tn?::aacC1-lac ^A -Ptac-trfA1(Δ27T/M124L); This study HY0475 BW25113::atfTn?::mini-Tn?::aacC1-lac ^A -Ptac-trfA1(Δ27T/M124L); This study HY0321 MR-1:atfTn?::mini-Tn?::aacC1-lac ^A -Ptac-trfA1(Δ27T/M124L); This study HY0323 MR-1:atfTn?::mini-Tn?::aacC1-lac ^A -Ptac-trfA1(LA25T/M124	EC100D pir+	F- mcrA ∆(mrr-hsdRMS-mcrBC)	Epicentre
Sm'Sm'(Yano et al, 2012)HY0330BW25113::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(M124L); Gm'(Yano et al, 2012)HY0390BW25113::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(M124L); Gm'(Yano et al, 2012)HY0391BW25113::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(R31P/M124L);This studyHY0395BW25113::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(A25T/M124L);This studyGm'BW25113::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(Δ43/M124L);This studyHY0408BW25113::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(Δ43/M124L);This studyHY0409BW25113::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(Δ43/M124L);This studyHY0475BW25113::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(Δ43/M124L);This studyGm'BW25113::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(Δ43/M124L);This studyHY0475BW25113::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(Δ43/M124L);This studyHY0370MR-1::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(M124L);This studyHY0321MR-1::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(M124L);This studyHY0325MR-1::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(M124L);Gm'HY0333MR-1::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(A25T/M124L);Gm'HY0334MR-1::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(A25T/M124L);Gm'HY0335MR-1::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(A25T/M124L);Gm'HY0335MR-1::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(A25T/M124L);Gm'HY0335MR-1::atfTn 7::mini-Tn 7::aacC1-lacf-Ptac-trfA1(A25T/M124L); <td< td=""><td></td><td>endA1 araD139 Δ(ara, leu)7697 galU galK λ rpsL nupG pir+ (DHFR);</td><td></td></td<>		endA1 araD139 Δ (ara, leu)7697 galU galK λ rpsL nupG pir+ (DHFR);	
HY0414BW25113::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(M124L); Gm'(Yano et al. 2012)HY0390BW25113::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(M124L); Gm'(Yano et al. 2012)HY0391BW25113::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(R31P/M124L); Gm'This studyGm'Gm'This studyHY0409BW25113::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(A25T/M124L); Gm'This studyHY0409BW25113::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(\D5/M124L); Gm'This studyHY0409BW25113::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(\D5/M124L); Gm'This studyHY0475BW25113::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(\D77/M124L); Gm'This studyGm'Gm'This studyThis studyGm'BW25113::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(\D77/M124L); Gm'This studyHY0475BW25113::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(\D77/M124L); Gm'This studyHY059MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(\D77/M124L); Gm'This studyHY0321MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(M124L); Gm'This studyHY0323MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(M124L); Gm'This studyHY0333MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(D27/M124L); Gm'This studyHY0333MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(D25/M124L); Gm'This studyHY0333MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(D25/M124L); Gm'This studyHY0333MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(D25/M124L); Gm'This studyHY0341MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(D25/M124		Sm ^r	
HY0390BW25113::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA2; Gm'(Yano et al, 2012)HY0391BW25113::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(R31P/M124L); Gm'This studyHY0395BW25113::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(A25T/M124L); Gm'This studyHY0408BW25113::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(A25T/M124L); Gm'This studyHY0409BW25113::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(Δ 43/M124L); Gm'This studyHY0409BW25113::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(Δ 43/M124L); Gm'This studyHY0475BW25113::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(Δ 47/M124L); Gm'This studyHY0475BW25113::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(Δ 47/M124L); Gm'This studyHY10475BW25113::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(Δ 47/M124L); Gm'This studyHY10475BW25113::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(Δ 43/M124L); Gm'This studyHY10475MR-1::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(Δ 43/M124L); Gm'This studyHY0321MR-1::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(Δ 41/M124L); Gm'This studyHY0323MR-1::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(Δ 43/M124L); Gm'This studyHY0337MR-1::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(Δ 51/M124L); Gm'This studyHY0337MR-1::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(Δ 51/M124L); Gm'This studyHY0337MR-1::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(Δ 51/M124L); Gm'This studyHY0337MR-1::attTn7::mini-Tn7::aacC1-lacfi-Ptac-trfA1(Δ 51/M124L); Gm'	HY0414	BW25113::attTn7::mini-Tn7::aacC1-lacl ⁹ -Ptac-trfA1 ^{wt} /trfA2 ^o ; Gm ^r	(Yano <i>et al</i> , 2012)
HY0391 HY0392BW25113::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(R31P/M124L); Gm'(Yano et al. 2012)HY0395 Gm'BW25113::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(A25T/M124L); Gm'This studyHY0408 HY0409 BW25113::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(Δ5/M124L); Gm'This studyHY0409 BW25113::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(Δ43/M124L); Gm'This studyHY0409 BW25113::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(Δ43/M124L); Gm'This studyHY0475 BW25113::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(Δ77/M124L); Gm'This studyShewanella oneidensisATCC (Sola et al, 2010)MR-1 HY0579 HY0579MR-1::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(Δ77/M124L); Gm'This studyHY0321 HY0321 HY0321 HR-1::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(M124L); Gm'This studyHY0323 HY0323 HR-1::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(M124L); Gm' HY0323 MR-1::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(M124L); Gm' This studyThis studyHY0323 HY0323 HR-1::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(M27/M124L); Gm' HY0333 HR-1::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(M27/M124L); Gm' This studyThis studyHY0324 HR-1::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(L35/M124L); Gm' HY0333 HR-1::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(L35/M124L); Gm' This studyThis studyHY0334 HR-1::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(L35/M124L); Gm' HY0335This studyHY0335 HR-1::attTn 7::mini-Tn 7::aacC1-lacfl-Ptac-trfA1(L35/M124L); Gm' HY0334This studyH	HY0390	BW25113:: <i>att</i> Tn <i>7</i> ::mini-Tn <i>7</i> :: <i>aacC1-lacl</i> ⁴ -P <i>tac-trfA1</i> (M124L) ; Gm ^r	(Yano <i>et al</i> , 2012)
HY0392BW25113:::atfTn7:::mini-Tn7:::aacC1-lacfi-Ptac-trfA1(R31P/M124L); Gm'This studyHY0395BW25113:::atfTn7:::mini-Tn7:::aacC1-lacfi-Ptac-trfA1(Δ5/M124L); Gm'This studyHY0409BW25113:::atfTn7::mini-Tn7:::aacC1-lacfi-Ptac-trfA1(Δ43/M124L); Gm'This studyHY0475BW25113:::atfTn7::mini-Tn7:::aacC1-lacfi-Ptac-trfA1(Δ43/M124L); Gm'This studyHY0475BW25113:::atfTn7::mini-Tn7:::aacC1-lacfi-Ptac-trfA1(Δ47/M124L); Gm'This studyShewanella oneidensisATCCMR-1Gammaproteobacteria, type strain MR-1: atfTn7::mini-Tn7:::aacC1-lacfi-Ptac-trfA1(Δ47/M124L); Gm'ATCCNR-1Gammaproteobacteria, type strain MR-1::atfTn7::mini-Tn7:::aacC1-lacfi-Ptac-dnBSon; Gm', dnaB' HY0321This studyHY0321MR-1::atfTn7::mini-Tn7:::aacC1-lacfi-Ptac-trfA1'(M124L); Gm' HY0325This studyHY0325MR-1::atfTn7::mini-Tn7:::aacC1-lacfi-Ptac-trfA1(R31P/M124L); Gm' HY0333This studyHY0337MR-1:::atfTn7::mini-Tn7:::aacC1-lacfi-Ptac-trfA1(L25M124L); Gm' HY0337This studyHY0341MR-1:::atfTn7::mini-Tn7:::aacC1-lacfi-Ptac-trfA1(L25M124L); Gm' HY0337This studyHY0345MR-1:::atfTn7::mini-Tn7:::aacC1-lacfi-Ptac-trfA1(L25M124L); Gm' HY0337This studyPlasnid6 pBBR1- pBBR1PBBR1 PBBR1PBBR1 PBBR1Gammaproteobacteria, type strain(Bagdasarian et al, 1981)S JMP228rifJMP134 derivative cured of plasmid pJP4; Rif' MCS2(Amy et al, 1985)Plasnid6 pBBR1- pBBR1 pBBR1 pBD136KmIncP-1β replicon; Km' (Sota et al, 2007) Lab collection<	HY0391	BW25113::attTn7::mini-Tn7::aacC1-lacl ⁴ -Ptac-trfA2; Gm ^r	(Yano <i>et al</i> , 2012)
Gm' HY0395Gm' BW25113::atfTn7::mini-Tn7::aacC1-lacf ¹ -Ptac-trfA1(Δ25T/M124L); Gm'This studyHY0408BW25113::atfTn7::mini-Tn7::aacC1-lacf ¹ -Ptac-trfA1(Δ5/M124L); Gm'This studyHY0409BW25113::atfTn7::mini-Tn7::aacC1-lacf ¹ -Ptac-trfA1(Δ43/M124L); Gm'This studyHY0475BW25113::atfTn7::mini-Tn7::aacC1-lacf ¹ -Ptac-trfA1(Δ43/M124L); Gm'This studyHY0475BW25113::atfTn7::mini-Tn7::aacC1-lacf ¹ -Ptac-trfA1(Δ77/M124L); Gm'This studyShewanella oncidensisATCCMR-1Gammaproteobacteria, type strain MR-1: atfTn7::mini-Tn7::aacC1-lacf-Ptac: Gm', dnaB' HY0321This studyHY0475MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(M124L); Gm'This studyHY0321MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(M124L); Gm'This studyHY0323MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(M124L); Gm'This studyHY0325MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(M124L); Gm'This studyHY0333MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(LAST/M124L); Gm'This studyHY0333MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(LAST/M124L); Gm'This studyHY0334MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(LAST/M124L); Gm'This studyHY0345MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(LAST/M124L); Gm'This studyHY0337MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(LAST/M124L); Gm'This studyHY0345MR-1::atfTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(LAST/M124L); Gm'This studyHY0345MR-1::atfTn7::min	HY0392	BW25113::attTn7::mini-Tn7::aacC1-lacl ⁹ -Ptac-trfA1(R31P/M124L);	This study
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MH-1SMH-1 derivative adapted to LB broth, Spontaneous Sm mutant; Sm(Sola <i>et al</i> , 2010)HY0759MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac; Gm ^r , dnaB [*] This studyHY1014MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1 ^{m/} /trfA2; Gm ^r This studyHY0321MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(M124L); Gm ^r This studyHY0325MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(M124L); Gm ^r This studyHY0326MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(R31P/M124L); Gm ^r This studyHY0327MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(A25T/M124L); Gm ^r This studyHY0333MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ5/M124L); Gm ^r This studyHY0341MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ43/M124L); Gm ^r This studyHY0345MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ43/M124L); Gm ^r This studyHY0345MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ43/M124L); Gm ^r This studyPseudomonasMR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ43/M124L); Gm ^r This studyPlasmidsMR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ43/M124L); Gm ^r This studyPlasmidsMR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ277/M124L); Gm ^r This study	MR-1	Gammaproteobacteria, type strain	
HY0759MR-1::att1n7::mini-1n7::aacC1-lacf-Ptac; Gm', dnaBIns studyHY1014MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-draBson; Gm', dnaB'This studyHY0321MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1"/trfA2; Gm'This studyHY0323MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(M124L); Gm'This studyHY0325MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(M124L); Gm'This studyHY0329I-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(A25T)/M124L); Gm'This studyHY0337MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(A25T)/M124L); Gm'This studyHY0341MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(A25T)/M124L); Gm'This studyHY0345MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(Δ43/M124L); Gm'This studyHY0345MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(Δ43/M124L); Gm'This studyHY0345MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(Δ77/M124L); Gm'This studyHY0345MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(Δ77/M124L); Gm'This studyPseudomonasgadasarian et al, 1981)al, 1981)CupriavidusJMP134 derivative cured of plasmid pJP4; Rif'(Amy et al, 1985)PlasmidspBBR1pBBR1 replicon, cloning vector; Km'(Kovach et al, 1995)pBP36KmIncP-1β replicon; Km'Lab collectionpMS0506IncP-1β replicon; Km'Lab collectionpMS0506pBP136Km trfA1'; Km'Lab collectionpMS0506pMS0506 variant adapted to strain MR-1, trfA1(Δ43); Km'(Sota et al, 2010)pEvo-Sh11pMS0506 variant adapted t	MR-1S	MR-1 derivative adapted to LB broth, Spontaneous Sm' mutant; Sm'	(Sota <i>et al</i> , 2010)
HY1014MH-1::att1n7::mini-1n7::aacC1-lacf-Ptac-trfA1 ^{wf} /trfA2; GmfInis studyHY0321MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1 ^{wf} /trfA2; GmfThis studyHY0323MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(M124L); GmfThis studyHY0329I-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(R31P/M124L); GmfThis studyHY0333MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(A25T/M124L); GmfThis studyHY0337MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(A25M124L); GmfThis studyHY0337MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(A25M124L); GmfThis studyHY0341MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(Δ43/M124L); GmfThis studyHY0345MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(Δ43/M124L); GmfThis studyHY0345MR-1::attTn7::mini-Tn7::aacC1-lacf-Ptac-trfA1(Δ43/M124L); GmfThis studyPseudomonasgadasarian et al, 1981)al, 1981)PseudomonasJMP134 derivative cured of plasmid pJP4; Riff(Amy et al, 1985)PlasmidspBBR1-pBBR1 replicon, cloning vector; Kmf(Kovach et al, 1995)pBBR10pBP136KmIncP-1β replicon; Kmf(Sota et al, 2007)pK0506IncP-1β replicon, pBP136Δ(trbB-traM)::(oriT _{RP4} -kan); Kmf(Sota et al, 2010)pEvo-Sh11pMS0506 variant adapted to strain MR-1, trfA1(Δ43); Kmf(Sota et al, 2010)pEvo-Sh11pMS0506 variant adapted to strain MR-1, trfA1(242T); Kmf(Sota et al, 2010)pEvo-Sh11pMS0506 variant adapted to strain MR-1, trfA1(242T); Kmf(Sota et al, 2010)pEvo-Sh11pMS0506 varia	HY0759	MR-1::att1n/::mini-1n/::aacC1-lacl*-Ptac; Gm*, dnaB	This study
HY0321MH-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(M124L); Gm'This studyHY0323MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(M124L); Gm'This studyHY0329I-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(R31P/M124L); Gm'This studyHY0333MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(R31P/M124L); Gm'This studyHY0337MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(A25T/M124L); Gm'This studyHY0341MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ43/M124L); Gm'This studyHY0345MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ43/M124L); Gm'This studyHY0345MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ43/M124L); Gm'This studyHY0345MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ77/M124L); Gm'This studyPseudomonasMR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ77/M124L); Gm'This studyMC1240Gammaproteobacteria, type strain(Bagdasarian et al, 1981)Cupriavidus<	HY1014	MR-1::att1n/::mini-1n/::aacC1-lacl*-Ptac-dnaBson; Gm*, dnaB	This study
HY0323MH-1::att In 7::mini-In 7::aacC1-lacf'-Ptac-trfA1(M124L) ; Gm'This studyHY0325MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(R31P/M124L) ; Gm'This studyHY0329I-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(R31P/M124L) ; Gm'This studyHY0337MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(A55T/M124L) ; Gm'This studyHY0341MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ43/M124L) ; Gm'This studyHY0345MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ43/M124L) ; Gm'This studyHY0345MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ43/M124L) ; Gm'This studyHY0345MR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ43/M124L) ; Gm'This studyPseudomonasMR-1::attTn 7::mini-Tn 7::aacC1-lacf ¹ -Ptac-trfA1(Δ43/M124L) ; Gm'This studyPlasmidsGammaproteobacteria, type strain(Bagdasarian et al, 1981)CupriaviduspinatubonensiSSJMP228rifJMP134 derivative cured of plasmid pJP4; Rif ⁴ (Amy et al, 1985)PlasmidspBBR1-pBBR1 replicon, cloning vector; Km ⁷ (Kovach et al, 1985)PBBR1-pBBR1 replicon, cloning vector; Km ⁷ (Sota et al, 2007)pEvoShL111pBP136KmIncP-1β replicon, pBP136Δ(trbB-traM)::(oriT _{RP4} -kan); Km ⁷ (Sota et al, 2010)pEvo-Sh11pMS0506 variant adapted to strain MR-1, trfA1(Δ43); Km ⁷ (Sota et al, 2010)pEvo-Sh11pMS0506 variant adapted to strain MR-1, trfA1(Δ425T); Km ⁷ (Sota et al, 2010)pEvo-Sh11pMS0506 variant adapted to strain MR-1, trfA1(Δ425T); Km ⁷ <	HY0321	MR-1::att1n/::mini-1n/::aacC1-lacl*-Ptac-trfA1**/trfA2; Gm	This study
HY0325MH-1:::att In /::mini-In /::aacC1-lacf'-Ptac-trfA1(R31P/M124L); Gm'This studyHY0329I-1:::attTn 7::mini-Tn 7::aacC1-lacf'-Ptac-trfA1(R31P/M124L); Gm'This studyHY0333MR-1:::attTn 7::mini-Tn 7::aacC1-lacf'-Ptac-trfA1(A25T/M124L); Gm'This studyHY0341MR-1:::attTn 7::mini-Tn 7::aacC1-lacf'-Ptac-trfA1(Δ43/M124L); Gm'This studyHY0345MR-1:::attTn 7::mini-Tn 7::aacC1-lacf'-Ptac-trfA1(Δ43/M124L); Gm'This studyHY0345MR-1:::attTn 7::mini-Tn 7::aacC1-lacf'-Ptac-trfA1(Δ43/M124L); Gm'This studyPseudomonasMR-1:::attTn 7::mini-Tn 7::aacC1-lacf'-Ptac-trfA1(Δ77/M124L); Gm'This studyPseudomonasMR-1:::attTn 7::mini-Tn 7:::aacC1-lacf'-Ptac-trfA1(Δ77/M124L); Gm'This studyPseudomonasMR-1:::attTn 7::mini-Tn 7:::aacC1-lacf'-Ptac-trfA1(Δ77/M124L); Gm'This studyMC2140Gammaproteobacteria, type strain(Bagdasarian et al, 1981)CupriavidusJMP134 derivative cured of plasmid pJP4; Rif'(Army et al, 1985)PlasmidspBBR1-pBBR1 replicon, cloning vector; Km'(Sota et al, 2007)pEvoShL111pBP13	HY0323	MR-1::att1n/::mini-1n/::aacC1-lacl*-Ptac-trfA1(M124L); Gm*	This study
HY0329I-1::att1n7::mini-1n7::aacC1-lacf ² -Ptac-trfA1(R31P/M124L); Gm'Ihis studyHY0333MR-1::attTn7::mini-Tn7::aacC1-lacf ² -Ptac-trfA1(A25T/M124L); Gm'This studyHY0337MR-1::attTn7::mini-Tn7::aacC1-lacf ² -Ptac-trfA1(Δ5/M124L); Gm'This studyHY0341MR-1::attTn7::mini-Tn7::aacC1-lacf ² -Ptac-trfA1(Δ3/M124L); Gm'This studyHY0345MR-1::attTn7::mini-Tn7::aacC1-lacf ² -Ptac-trfA1(Δ43/M124L); Gm'This studyPseudomonasMR-1::attTn7::mini-Tn7::aacC1-lacf ² -Ptac-trfA1(Δ77/M124L); Gm'KmSupposeJMP134 derivative cured of plasmid pJP4; Rif'(Amy et al, 1985)PlasmidspBR1	HY0325	MR-1::att1n/::mini-1n/::aacC1-lacl [*] -Ptac-trfA2; Gm [*]	This study
HY0333MR-1::att1 n 7::mini-Tn7::aacC1-lacf ¹ -Ptac-trfA1(A251/M124L); Gm ¹ This studyHY0337MR-1::attTn7::mini-Tn7::aacC1-lacf ¹ -Ptac-trfA1(Δ 5/M124L); Gm ¹ This studyHY0341MR-1::attTn7::mini-Tn7::aacC1-lacf ¹ -Ptac-trfA1(Δ 43/M124L); Gm ¹ This studyHY0345MR-1::attTn7::mini-Tn7::aacC1-lacf ¹ -Ptac-trfA1(Δ 43/M124L); Gm ¹ This studyHY0345MR-1::attTn7::mini-Tn7::aacC1-lacf ¹ -Ptac-trfA1(Δ 77/M124L); Gm ¹ This studyPseudomonasResult of the studyThis studyputidaKT2440Gammaproteobacteria, type strain(Bagdasarian et al, 1981)CupriavidusJMP134 derivative cured of plasmid pJP4; Rif ⁴ (Amy et al, 1985)PlasmidspBBR1-pBBR1 replicon, cloning vector; Km ¹ (Kovach et al, 1995)pBBR10IncP-1β replicon; Km ¹ (Sota et al, 2007)pEvoShL111pBP136Km trfA1; Km ¹ Lab collectionpMS0506IncP-1β replicon, pBP136Δ(trbB-traM)::(oriT _{RP4} -kan); Km ¹ (Sota et al, 2010)pEvo-Sh11pMS0506 variant adapted to strain MR-1, trfA1(Δ 43); Km ¹ (Sota et al, 2010)pEvo-Sh11pMS0506 variant adapted to strain MR-1, trfA1(Δ 43); Km ¹ (Sota et al, 2010)pEvo-Sh11pMS0506 variant adapted to strain MR-1, trfA1(Δ 42); Km ¹ (Sota et al, 2010)pEvo-Sh11pMS0506 variant adapted to strain MR-1, trfA1(Δ 251; Km ¹ (Sota et al, 2010)	HY0329	I-1:: <i>att</i> Tn7::mini-Tn7:: <i>aacC1-lacP</i> -P <i>tac-trfA1</i> (R31P/M124L); Gm	This study
HY0337MR-1::attTn 7::mni-Tn 7::aacC1-lach-Ptac-trfA1(Δ 5/M124L); Gm'This studyHY0341MR-1::attTn 7::mini-Tn 7::aacC1-lach-Ptac-trfA1(Δ 43/M124L); Gm'This studyHY0345MR-1::attTn 7::mini-Tn 7::aacC1-lach-Ptac-trfA1(Δ 77/M124L); Gm'This studyPseudomonasMR-1::attTn 7::mini-Tn 7::aacC1-lach-Ptac-trfA1(Δ 77/M124L); Gm'This studyPseudomonasMR-1::attTn 7::mini-Tn 7::aacC1-lach-Ptac-trfA1(Δ 77/M124L); Gm'This studyPseudomonasGammaproteobacteria, type strain(Bagdasarian et al, 1981)CupriavidusJMP28rifJMP134 derivative cured of plasmid pJP4; Rif'(Amy et al, 1985)PlasmidspBBR1- pBBR1 replicon, cloning vector; Km'(Kovach et al, 1995)pBP136KmIncP-1β replicon; Km' pBP136Km trfA1; Km'(Sota et al, 2007) Lab collectionpEvoShL111 pMS0506pBP136Δ(trbB-traM)::(oriT _{RP4} -kan); Km' (Sota et al, 2010) pEvo-Sh11(Sota et al, 2010) pMS0506 variant adapted to strain MR-1, trfA1(Δ 43); Km' (Sota et al, 2010)pEvo-Sh11 pMS0506 variant adapted to strain MR-1, trfA1(Δ 43); Km'(Sota et al, 2010) (Sota et al, 2010)	HY0333	MR-1:: <i>att</i> Tn7::mini-Tn7:: <i>aacC1-lacl</i> ⁺ -P <i>tac-trfA1</i> (A25T/M124L) ; Gm	This study
HY0341 HY0345MR-1::attl In 7::mini- In 7::aacC1-lacht-Ptac-trfA1(Δ43/M124L); Gm' MR-1::attTn 7::mini-Tn 7::aacC1-lacht-Ptac-trfA1(Δ77/M124L); Gm'This studyHY0345 Pseudomonas putida KT2440Gammaproteobacteria, type strain(Bagdasarian et al, 1981)Cupriavidus pinatubonensi s JMP228rifJMP134 derivative cured of plasmid pJP4; Rift(Amy et al, 1985)Plasmids pBBR1- pBBR1 replicon, cloning vector; Kmt pEvoShL111(Kovach et al, 1995)pBP136Km pEvoShL111IncP-1β replicon; Kmt pMS0506 pEvo-Sh1(Sota et al, 2007) Lab collection pMS0506 variant adapted to strain MR-1, trfA1(Δ43); Kmt pMS0506 variant adapted to strain MR-1, trfA1(Δ425T); Kmt(Sota et al, 2010) (Sota et al, 2010)	HY0337	MR-1:: <i>att</i> Tn7::mini-Tn7:: <i>aacC1-lacl</i> ⁻ -P <i>tac-trfA1</i> (Δ5/M124L) ; Gm'	This study
HY0345MR-1::att1n7::mini-1n7::aacC1-lacf'-Ptac-trfA1(Δ77/M124L); Gm'This studyPseudomonasFileGammaproteobacteria, type strain(Bagdasarian et al, 1981)CupriavidusGammaproteobacteria, type strain(Bagdasarian et al, 1981)pinatubonensiJMP134 derivative cured of plasmid pJP4; Rif ^r (Amy et al, 1985)PlasmidsJMP134 derivative cured of plasmid pJP4; Rif ^r (Kovach et al, 1985)pBBR1-pBBR1 replicon, cloning vector; Km ^r (Kovach et al, 1995)pBP136KmIncP-1β replicon; Km ^r (Sota et al, 2007)pEvoShL111pBP136Km trfA1; Km ^r Lab collectionpEvo-Sh1pMS0506 variant adapted to strain MR-1, trfA1(Δ43); Km ^r (Sota et al, 2010)pEvo-Sh111pMS0506 variant adapted to strain MR-1, trfA1(Δ42); Km ^r (Sota et al, 2010)pEvo-Sh11pMS0506 variant adapted to strain MR-1, trfA1(Δ42); Km ^r (Sota et al, 2010)pEvo-Sh11pMS0506 variant adapted to strain MR-1, trfA1(Δ42); Km ^r (Sota et al, 2010)pEvo-Sh11pMS0506 variant adapted to strain MR-1, trfA1(Δ42); Km ^r (Sota et al, 2010)	HY0341	MR-1:: <i>att</i> Tn7::mini-Tn7:: <i>aacC1-lacI</i> ^r -P <i>tac-trfA1</i> (Δ43/M124L) ; Gm ^r	This study
Pseudomonas putida KT2440Gammaproteobacteria, type strain(Bagdasarian et al, 1981)Cupriavidus pinatubonensi sJMP134 derivative cured of plasmid pJP4; Rif(Amy et al, 1985)Plasmids pBBR1- MCS2pBBR1 replicon, cloning vector; Km ^r (Kovach et al, 1995)pBP136KmIncP-1 β replicon; Km ^r (Sota et al, 2007) Lab collectionpEvoShL111 pMS0506pBP136Km trfA1; Km ^r Lab collection (Sota et al, 2010) pEvo-Sh1pEvo-Sh11 pEvo-Sh11pMS0506 variant adapted to strain MR-1, trfA1(A25T); Km ^r (Sota et al, 2010) (Sota et al, 2010) pEvo-Sh11pEvo-Sh11 pC0-Sh11pMS0506 variant adapted to strain MR-1, trfA1(A25T); Km ^r (Sota et al, 2010) (Sota et al, 2010)	HY0345	MR-1:: <i>att</i> Tn <i>7</i> ::mini-Tn <i>7</i> :: <i>aacC1-lacl^e-Ptac-trfA1</i> (Δ77/M124L) ; Gm'	This study
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pEvo-Sh11 pMS0506 variant adapted to strain MR-1, <i>trfA1</i> (A25T); Km ^r (Sota <i>et al</i> , 2010)	pEvo-Sh1	pMS0506 variant adapted to strain MR-1, <i>trfA1</i> (Δ 43); Km ^r	(Sota <i>et al</i> , 2010)
$P_{\rm res} = P_{\rm res} = P_{\rm$	pEvo-Sh11	pMS0506 variant adapted to strain MR-1, <i>trfA1</i> (A25T); Km ^r	(Sota <i>et al</i> , 2010)
$p \equiv v_0 - S_{115}$ production variant adapted to strain MR-1, <i>trtA</i> (R31P); Km ⁻¹ (Sota <i>et al</i> , 2010)	pEvo-Sh5	pMS0506 variant adapted to strain MR-1, <i>trfA1</i> (R31P); Km ^r	(Sota <i>et al</i> , 2010)

pEvo-Sh13	pMS0506 variant adapted to strain MR-1, <i>trfA1</i> ($\Delta 5$); Km ^r	(Sota <i>et al</i> , 2010)
pEvo-Sh3	pMS0506 variant adapted to strain MR-1, trfA1(Δ 77); Km ^r	(Sota <i>et al</i> , 2010)
pEvo-Sh15	pMS0506 variant adapted to strain MR-1, frame-shift mutation in	(Sota <i>et al</i> , 2010)
	trfA1; Km ^r	
pHSG399	pUC19 replicon, cloning vector; Cm ^r	This study
pUX-BF13	R6K replicon, Tn7 transposase expression plasmid; Apr	This study
pHY1010	pBBR1-MCS2:: <i>trfA1</i> (R31P/M124L) ; Km ^r	This study
pHY1011	pBBR1-MCS2::trfA1(A25T/M124L); Km ^r	This study
pHY1012	pBBR1-MCS2:: <i>trfA1</i> (Δ43/M124L); Km ^r	This study
pHY1014	pBBR1-MCS2:: <i>trfA1</i> (Δ5/M124L); Km ^r	This study
pHY1015	pBBR1-MCS2:: <i>trfA1</i> (Δ77/M124L); Km ^r	This study
pHY987	pBBR1-MCS2:: <i>trfA1</i> (M124L); Km ^r	This study
pHY988	pBBR1-MCS2: <i>trfA2</i> ; Km ^r	This study
pHY835LAC	R6K replicon, mini-Tn7 vector; Ap ^r , Gm ^r	(Yano <i>et al</i> , 2012)
pHY884	pHY835LAC:: <i>trfA1^{wt}/trfA2</i> ; Ap ^r , Gm ^r	(Yano <i>et al</i> , 2012)
pHY885	pHY835LAC:: <i>trfA1</i> (M124L); Ap ^r , Gm ^r	(Yano <i>et al</i> , 2012)
pHY886	pHY835LAC:: <i>trfA2</i> ; Ap ^r , Gm ^r	(Yano <i>et al</i> , 2012)
pHY888	pHY835LAC:: <i>trfA1</i> (R31P/M124L);	This study
pHY890	pHY835LAC:: <i>trfA1</i> (A25T/M124L);	This study
pHY892	pHY835LAC:: <i>trfA1</i> (Δ5/M124L); Ap ^r , Gm ^r	This study
pHY897	pHY835LAC:: <i>trfA1</i> (Δ43/M124L); Ap ^r , Gm ^r	This study
pHY895	pHY835LAC:: <i>trfA1</i> (Δ77/M124L); Ap ^r , Gm ^r	This study
pHY1018	pHY835LAC:: <i>dnaBson</i> ; Ap ^r , Gm ^r	This study
pET11a	pMB1 replicon, protein expression vector ; Ap ^r	Merck KGaA
pHY915	pET11a::his ₆ - <i>trfA1</i> /(M124L/Q279D/S292L);	This study
pHY921	pET11a::his ₆ - <i>trfA2</i> (Q279D/S292L);	This study
pHY916	pET11a::his ₆ - <i>trfA1</i> (R31P/M124L/Q279D/S292L);	This study
pHY917	pET11a::his ₆ - <i>trfA1</i> (A25T/M124L/Q279D/S292L);	This study
pHY919	pET11a::his ₆ - <i>trfA1</i> (Δ43/M124L/Q279D/S292L);	This study
pHY1032	pET22b:: <i>dnaBson</i> -his ₆ ; Ap ^r	This study
pHY872	R6K replicon, the $oriV_{pBP136}$ region containing eight copies of iteron;	(Yano <i>et al</i> , 2012)
	Tc ^r	
pHY873	pHY872 carrying the 1.8 kb <i>oriC</i> region of the MR-1 chromosome; Tc ^r	This study
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pHY924 pHY872 carrying the 2.5 kb *oriC* region of the *E.coli* chromosome; Tc^r (Yano *et al*, 2012) ^a Antibiotic resistance: Ap, ampicillin; Km, kanamycin; Gm, gentamycin; Cm, chloramphenicol, Sm, streptomycin. Rif, rifampicin. Tc, tetracycline. *dnaB*⁺ and *dnaB*⁻ indicate presence or absence of additional *dnaB* copy in the chromosome.

dnaB copy in the chromosome. ^b *trfA* genotypes: *trfA*1^{wt}/*trfA2*, wild-type *trfA* allele encoding both TrfA1 and TrfA2; M124L, a point mutation from ATG to CTC in the 124th codon, substituting methionine to leucine of the product and eliminating translation of TrfA2; R31P, a point mutation from G to C at position 92 of the *trfA1* frame, substituting the 31th arginine with proline of the product; A25T, a point mutation from G to A at position 73 of the *trfA1* frame, substituting the 25th alanine with threonine of the product; Δ 5, an deletion from 93 to 107, deleting five aa of the product; Δ 43, an deletion from position 97 to 225 in the *trfA1* frame, deleting 43 aa of the product; Δ 77, an deletion from position 24 to 254 in the *trfA1* frame, deleting 77 aa of the product; Q279D/S292L, a mutation from C to G at position 835, substituting 279th glutamine to asparagine, and mutations from TC to CT at positions 874-875, substituting the 292th serine to leucine of TrfA1; these mutations make TrfA1 monomeric. The same nomenclature was applied to the equivalent mutations in TrfA2.

Table S2. Oligonucleotides used in this study.

Name	Sequences	Purpose of use
SacI-SD-	AATTGAGCTCAAGGAGGTAATACACCATGACGAA	Cloning trfA into pHSG399
pBP136trfA1F	CAACGAGTTCAACGA	
SacI-SD-	AATTGAGCTCAAGGAGGTAATACACCATGGCGAC	Cloning trfA into pHSG39
pBP136trfA2	CAAGAAGCGAAC	
EcoNdeHisTrfA1F	AATTGAATTCATATGCATCACCATCACCATCACAC GAACAACGAGTTCAACGAGC	Cloning trfA1 into pET11a
EcoNdeHisTrfA2F	AATTGAATTCATATGCATCACCATCACCATCACGC GACCAAGAAGCGAACGG	Cloning trfA2 into pET11a
BamHindTrfAR	AATTGGATCCAAGCTTATTACCGCTTGCAATGCAC CAGGTCG	Cloning trfA into pET11a
SacSDdnaBMR1F	AATTGAGCTCAAGGAGGTATACACCATGTCACAA CAAGGTGCTTTTAA	Cloning dnaB into pHY835LAC
HinddnaBMR1R	AATTAAGCTTAATCTTCTTCAAACTGAGGA	Cloning dnaB into pHY835LAC
EcoNdeDnaBmr1F	AATTGAATTCCATATGTCACAACAAGGTGCTTTTA AGC	Cloning <i>dnaB</i> son into pET22b
HindDnaBmr1hisR	AATTAAGCTTATTAATGGTGATGGTGATGGTGATC TTCTTCAAACTGAGGACCTG	Cloning <i>dnaB</i> son into pET22b
trfAM124LF	CTGGCGACCAAGAAGCGAACG	Introducing M1124L mutation to trfA1 and tis variants
trfAM124LR	CGCACTACTCCGTTTGTCCTG	Introducing M124L mutation to <i>trfA1</i> and its variant
Q279D/S292Lf	CGGCCGGCTGGAATCGGTGCTGCTGATCCGGCG CTTCC	Introducing Q279D/S292L mutation to <i>trfA</i>
Q279D/S292Lr	ATGCGCTGGGATGAGAAGTCCATGGCCGACGCC TGGAG	Introducing Q279D/S292L mutations to <i>trfA</i>
EcoMR1oriCF	TCAGTGGAATTCAGTTGCATGCTCAAACTC	Cloning the <i>oriC</i> region of S.oneidensis into pHY872
EcoMR1oriCR	GCAGGAATTCCGTACTTAAAAGTGGTTCC	Cloning the <i>oriC</i> region of <i>S.oneidensis</i> into pHY872
atpF	GTTGGTTCCATACATCAGCG	qPCR, copy number of <i>S.oneidensis</i> oriC region
atpR	AACTGACGCTACAGCCCTTT	qPCR, copy number of <i>S.oneidensis</i> oriC region
tetAF	GTGAAACCCAACATACCCCT	qPCR, copy number of pHY872
tetAR	AATTGCACCAACGCATACAG	qPCR, copy number of pHY872
Ecoli_atpF	GTCGGTCCAGGTCTTCATTT	qPCR, copy number of <i>E.coli oriC</i>
Ecoli_atpR	TGCACACGGTAATCTGGAAT	qPCR, copy number of <i>E.coli oriC</i> region
E coli glmS	CATGCACATCATCGAGATGCC	Confirmation of mini-Tn7 insertion
MR-1glmS	GGCGGTTTAATGTATGTGTTTGC	Confirmation of mini-Tn7 insertion
Tn7R109	CAGCATAACTGGACTGATTTCAG	Confirmation of mini-Tn7 insertion
S.o.dnaB_F	GATACCCCAGTCACACCGTT	qPCR, dnaB
S.o.dnaB_R	GCGCTTTACTCGATTTGGCC	qPCR, dnaB
S.o.gapA_F	TGCTCCCGTGAAAGAAGACG	qPCR, gapA (internal control)
S.o.gapA_R	CGTGGTCATCGATCCGTGTT	qPCR, gapA (internal control)
- · -		

Pair	Reference [Host (plasmid) /IPTG	Subject [Host (plasmid) /IPTG conc.	BIC.sep	BIC.joint	ΔBIC (BIC.sep -	Welch t.test p.value
	conc./Expt ² .]	/Expt.]			BIC.joint)	
(i)	HY0759(pMS0506)/0 uM IPTG/B	HY1014(pMS0506)/0 uM IPTG/B	136.9	377.7	-240.8*	0.011*
(ii)	HY0759(pMS0506)/10 uM IPTG/A	HY1014(pMS0506)/0 uM IPTG/A	205.0	212.4	-7.3*	0.123
(iii)	HY0759(pMS0506)/10 uM IPTG/A	HY1014(pMS0506)/10 uM IPTG/A	227.4	252.3	-24.8*	0.034*
(iv)	HY0759(pMS0506)/100 uM IPTG/A	HY1014(pMS0506)/100 uM IPTG/A	236.0	355.9	-119.9*	0.018*
(v)	HY1014(pMS0506)/0 uM IPTG/A	HY1014(pMS0506)/10 uM IPTG/A	215.7	212.8	2.8	0.171
(vi)	HY1014(pMS0506)/10 uM IPTG/A	HY1014(pMS0506)/100 uM IPTG/A	245.6	274.6	-28.9*	0.028*
(vii)	HY1014(pMS0506)/100 uM IPTG/A	HY1014(pEvo-Sh1)/100 uM IPTG/A	237.2	466.3	-229.0*	0.013*
(viii)	HY0759(pMS0506)/10 uM IPTG/A	HY0759(pMS0506)/100 uM IPTG/A	217.7	208.7	8.9	0.218

Table S3. Statistical evaluation of effect of DnaB overexpression on plasmid persistence dynamics¹

BIC.sep: the BIC of the model that assumes that two given plasmid persistence data sets are governed by different dynamics; BIC.joint: the "null" model that the stability dynamics in both data sets are the same. More negative ΔBIC values are indicative of larger differences between two plasmid persistence dynamics. *: The difference is significant. We also conducted a Welch t.test on the plasmid-containing fractions on Day 10 or Day 4 (for pair (i)).

2. Strains HY0759 and HY1014 have a mini-Tn7 inserted in their chromosome, respectively without and with an extra copy of *dnaB*. Experiments performed in parallel are indicated by the same letter. Data from experiment A are shown in Fig. 3. We could not obtain data for pMS0506 in strain HY0759 with 0 uM IPTG condition due to a technical mistake in experiment A. An additional experiment B was conducted on different days and by a different person. Data set "HY0759(pMS0506)/10 uM IPTG/B" was used as control to evaluate data set "HY1014(pMS0506)/0 uM IPTG/B".

								quasi-extinction
								time (generations)
			Extra	IPTG	Initial plasmid-free	Segregational loss rate (I)	Fitness cost (s)	С
Expt. ^b	Plasmid	Host	dnaB	(uM)	fraction (b ₀) [95% c.i.]	[95% c.i.]	[95% c.i.]	[95% c.i.]
B (Fig. S4)	pMS0506	S. oneidensis HY0759	-	0	0.051 [0.019 - 0.081]	2.69E-03 [1.04E-10 - 1.77E-02]	0.226 [0.146 - 0.287]	45 [29 - 84]
B (Fig. S4)	pMS0506	S. oneidensis HY1014	+	0	0.068 [0.038 - 0.096]	2.55E-04 [7.85E-11 - 4.04E-03]	0.085 [0.065 - 0.094]	120 [92 -173]
A (Fig. 3A, Fig. S4)	pMS0506	S. oneidensis HY1014	+	0	0.030 [0.007 - 0.061]	7.94E-03 [4.33E-03 - 1.16E-02]	0.025 [0.011 - 0.043]	224 [136 - 467]
A (Fig. S4)	pMS0506	S. oneidensis HY0759	-	10	0.110 [0.058 - 0.139]	2.38E-08 [8.68E-11 - 4.11E-03]	0.059 [0.044 - 0.065]	163 [122 - 242]
A (Fig. 3A, Fig. S4)	pMS0506	S. oneidensis HY1014	+	10	0.055 [0.029 - 0.091]	3.64E-03 [8.68E-11 - 4.11E-03]	0.036 [0.023 - 0.051]	219 [156 - 508]
A (Fig. 3A, Fig. S4)	pMS0506	S. oneidensis HY0759	-	100	0.072 [0.035 - 0.113]	3.51E-03 [8.16E-04 - 6.38E-03]	0.048 [0.035 - 0.062]	172 [120 - 290]
A (Fig. 3A, Fig. S4)	pMS0506	S. oneidensis HY1014	+	100	0.073 [0.047 - 0.090]	9.18E-10 [5.46E-10 - 7.02E-03]	0.043 [0.028 - 0.047]	239 [148 - 391]
A (Fig. 3B, Fig. S5)	pEvo-Sh1	S. oneidensis HY1014	+	100	0.012 [0.000 - 0.021]	2.55E-10 [5.80E-11 - 2.47E-03]	0.043 [0.014 - 0.057]	302 [169 - >1000]
A (Fig. 3B, Fig. S5)	pEvo-Sh1	S. oneidensis HY0759	-	100	0.000 [0.000 – 0.004]	5.24E-04 [6.42E-05 - 9.88E-04]	0.029 [0.010 - 0.054]	401 [211 - >1000]
A (Fig. 3A rep. 1)	pMS0506	S. oneidensis HY1014	+	100	0.070 [0.020 - 0.091]	5.78E-10 [3.01E-11 - 4.29E-03]	0.050 [0.030 - 0.058]	207 [140 - 408]
A (Fig. 3A rep. 2)	pMS0506	S. oneidensis HY1014	+	100	0.087 [0.018 - 0.136]	2.27E-08 [3.83E-11 - 5.62E-03]	0.038 [0.008 - 0.047]	263 [151 - >1000]
A (Fig. 3A rep. 3)	pMS0506	S. oneidensis HY1014	+	100	0.060 [0.007 - 0.082]	1.34E-10 [2.01E-11 - 4.08E-03]	0.042 [0.013 - 0.053]	252 [153 - >1000]
A (Fig. 3B rep. 1)	pEvo-Sh1	S. oneidensis HY1014	+	100	0.000 [0.000 - 0.010]	9.16E-04 [5.89E-05 - 1.60E-03]	0.012 [0.000 - 0.045]	748 [226 - >1000
A (Fig. 3B rep. 2)	pEvo-Sh1	S. oneidensis HY1014	+	100	0.027 [0.000 - 0.053]	1.16E-09 [1.00E-11 - 2.02E-03]	0.035 [0.032 - 0.045]	337 [203 - >1000]
A (Fig. 3B rep. 3)	pEvo-Sh1	S. oneidensis HY1014	+	100	0.003 [0.000 - 0.006]	2.38E-10 [2.38E-12 - 4.62E-04]	0.065 [0.041 - 0.088]	230 [145 - 978]
C (Fig. S2)	pBP136Km	P. putida KT2440	-	-	0.000 [0.000 - 0.001]	2.09E-03 [1.37E-03 - 2.99E-03]	0.022 [0.006 - 0.033]	387 [261 - >1000]
C (Fig. S2)	pEvo-ShL1I1	P. putida KT2440	-	-	0.000 [0.000 - 0.003]	1.41E-04 [9.75E-12 - 2.53E-04]	0.039 [0.020 - 0.066]	363 [203 - >1000]

Table S4. Maximum likelihood estimates (MLEs) of plasmid persistence parameters^a

^{a.} The model used is the following segregation-selection (SS) model (Ponciano *et al*, 2007): $X_t = [2(1 - n_{t-1}) + 2^{1+s}n_{t-1}]/[n_{t-1}2^{1+s} + 2(1 - n_{t-1})]$, where Xt is a fraction of plasmid-free cell at generation time *t*, n_t is the plasmid-containing fraction at generation time *t*. The number of plasmid-free colonies among 52 colonies over time was used to estimate parameters. MLEs were obtained after 100 times bootstrapping. We used the SS model for pMS0506 and pEvo-Sh1 since these plasmids are not transmissible. We also used the SS model and not the HT (horizontal transfer) model to estimate the parameters for experiment C because it was a better fit for the data. c.i.: Credibilty interval.

^{b.} A, B, C: data sets with the same letter were generated at the same time. Rows with rep1, rep2, rep3 show parameter estimates for each of triplicate assays. Note that high initial plasmid free fraction in pMS0506-carrying population is reproducible. Other rows are the estimates for the pooled data of the triplicate assays.

^c The time (in generations) it takes for the plasmid to be lost from 99% of the population given the average estimated parameters shown in the previous columns.



Fig. S1. TrfA expression levels in *S. oneidensis* strain MR-1 carrying pMS0506 or its **evolved variants.** In each lane, 125 ug of total cell extract was loaded. TrfA quantity standards (STDs; 1.25, 0.63, 0.31, 0.16 pmol for both purified proteins TrfA1 and TrfA2) are loaded in the left four lanes. TrfA proteins were detected as previously described (Yano *et al*, 2012). A. (i) Example of blot for pMS0506 and four evolved plasmids pEvo-Sh11, pEvo-Sh5, pEvo-Sh13, pEVo-Sh1. Quantitation of the blot is shown in panel A (i). B (i) Example of blot for pEvo-Sh3, pEvo-Sh14, pEvo-Sh15. (ii) Quantitation for blot shown in panel B (i). The relative TrfA levels below the blots are means from four replicate quantitative Western blotting experiments.

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Fig. S2
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Fig. S2. Distribution of cell length of *Shewanella oneidensis* MR-1 harboring ancestral plasmid pMS0506 (**A**), its evolved variant pEvo-Sh1 (**B**), pBBR1-MCS2 (**F**), and its derivatives carrying *trfA1*(M124L) (**C**), *trfA2* (**D**), or *trfA1*(Δ 43/M124L) (**E**). Examples of cell images are shown next to the corresponding histogram. Note that the coefficient of variance (CV) of TrfA1-producing cells was consistently higher than that of strains without TrfA1. The difference in distribution was compared using one-tailed two-sample Kolmogorov-Smirnov test: pMS0506 vs pEvo-Sh1, *P* = 0.034; TrfA1 vs TrfA2, *P* < 0.001; TrfA1 vs Δ 43, *P* < 0.001; TrfA1 vs pBBR1-MSC2, *P* < 0.001. Scale bar indicates 3.3 um.

Fig. S3.



Fig. S3. Loss of TrfA1 production improves persistence of pBP136Km in *P. putida* populations. Triplicate assays were shown as distinct curves. Persistence parameter estimates were shown in Table S4.

Fig. S4.



Fig. S4. ELISA showing reduced binding affinity to DnaB of TrfA1 variants. Data point indicates mean and standard deviation. M124L/Q279D/S292L were introduced into TrfA1 and its variants.



Fig. S5. Effect of DnaB overproduction on the persistence of ancestral plasmid pMS0506. Three replicate data sets obtained at the same time are separately shown within the same plot. The hosts used were *S. oneidensis* HY0759 for "DnaB", and HY1041 for "DnaB⁺" condition, respectively. In the experiment A: 0 uM IPTG condition (Top left), we could not obtain data for "DnaB" due to a technical mistake; instead, the "DnaB" condition at 10 uM IPTG condition was shown in the plot as reference.



Fig. S6. Effect of DnaB overproduction on the persistence of pEvo-Sh1. Upper panels: Three replicate data obtained at the same time are separately shown within the same plot (only two for "DnaB" at 100 uM IPTG). The hosts used were the same as Fig.S5. Lower panels, left: Data of pMS0506 and pEvo-Sh1 obtained under the same conditions are shown within the same plot for comparison.





Fig. S7. Alignment of DnaB proteins of representative gammaproteobacterial species. E.col, *Escherichia coli* MG1655; S.one, *Shewanella oneidensis* MR-1; P.put, *Pseudomonas putida* KT2440; P.aer, *Pseudomonas aeruginosa* PAO1; V.col, *Vibrio cholerae* O1 biovar El Tor str. N16961; H.inf, *Heamophilus influenzae* Rd KW20; M.cat, *Moraxella catarrhalis*; A.hyd, *Aeromonas hydrophila* subsp. hydrophila ATCC 7966; P.hal, *Pseudoalteromonas haloplanktis* TAC125.

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