Supplementary Information

Evolved plasmid-host interactions reduce plasmid interference cost

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List of contents

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 *Sac*I-*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 *Sac*I-*Hin*dIII sites region within the mini-Tn*7* region of pHY835LAC. 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 *Nde*I-*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 μg/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*Tn*7*) 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

Strains or plasmids	Relevant characteristics[a]	References
Strains		
Escherichia coli		
BL21(DE3)	F- *ompT hsdS$_B$(r$_B$ m$_B$) gal dcm* (DE3)	Novagen
BW25113	F- *rrnB3 ΔlacZ4787 hsdR514 Δ (araBAD)567 Δ(rhaBAD)568 rph-1*	(Baba et al, 2006)
BW29427	*thrB1004 pro thi rpsL hsdS lacZ* ΔM15 RP4-1360 Δ*(araBAD)567 ΔdapA1341*::[*erm pir*R6K]; Erm^r, Sm^r	B .Warner (Purdue Univ.)
EC100	F- *mcrA Δ(mrr-hsdRMS-mcrBC) ϕ80dlacZ ΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara, leu)7697 galU galK λ$^-$ rpsL nupG;* Sm^r	Epicentre
EC100D *pir+*	F- *mcrA Δ(mrr-hsdRMS-mcrBC) ϕ80dlacZ ΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara, leu)7697 galU galK λ$^-$ rpsL nupG pir+ (DHFR);* Sm^r	Epicentre
HY0414	BW25113::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA1^{wt}/trfA2^b*; Gm^r	(Yano *et al*, 2012)
HY0390	BW25113::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA1*(M124L) ; Gm^r	(Yano *et al*, 2012)
HY0391	BW25113::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA2*; Gm^r	(Yano *et al*, 2012)
HY0392	BW25113::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA1*(R31P/M124L); Gm^r	This study
HY0395	BW25113::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA1*(A25T/M124L); Gm^r	This study
HY0408	BW25113::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA1*(Δ5/M124L); Gm^r	This study
HY0409	BW25113::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA1*(Δ43/M124L); Gm^r	This study
HY0475	BW25113::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA1*(Δ77/M124L); Gm^r	This study
Shewanella oneidensis		
MR-1	Gammaproteobacteria, type strain	ATCC
MR-1S	MR-1 derivative adapted to LB broth, Spontaneous Sm^r mutant; Sm^r	(Sota *et al*, 2010)
HY0759	MR-1::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac*; Gm^r, *dnaB$^-$*	This study
HY1014	MR-1::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-dnaBson*; Gm^r, *dnaB$^+$*	This study
HY0321	MR-1::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA1^{wt}/trfA2*; Gm^r	This study
HY0323	MR-1::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA1*(M124L) ; Gm^r	This study
HY0325	MR-1::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA2*; Gm^r	This study
HY0329	⌇-1::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA1*(R31P/M124L) ; Gm^r	This study
HY0333	MR-1::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA1*(A25T/M124L) ; Gm^r	This study
HY0337	MR-1::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA1*(Δ5/M124L) ; Gm^r	This study
HY0341	MR-1::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA1*(Δ43/M124L) ; Gm^r	This study
HY0345	MR-1::*att*Tn*7*::mini-Tn*7*::*aacC1-lacI^q*-P*tac-trfA1*(Δ77/M124L) ; Gm^r	This study
Pseudomonas putida		
KT2440	Gammaproteobacteria, type strain	(Bagdasarian *et al*, 1981)
Cupriavidus pinatubonensis		
JMP228rif	JMP134 derivative cured of plasmid pJP4; Rif^r	(Amy *et al*, 1985)
Plasmids		
pBBR1-MCS2	pBBR1 replicon, cloning vector; Km^r	(Kovach *et al*, 1995)
pBP136Km	IncP-1β replicon; Km^r	(Sota *et al*, 2007)
pEvoShL1I1	pBP136Km *trfA1$^-$*; Km^r	Lab collection
pMS0506	IncP-1β replicon, pBP136Δ(*trbB-traM*)::(*oriT$_{RP4}$-kan*); Km^r	(Sota *et al*, 2010)
pEvo-Sh1	pMS0506 variant adapted to strain MR-1*, trfA1*(Δ43); Km^r	(Sota *et al*, 2010)
pEvo-Sh11	pMS0506 variant adapted to strain MR-1, *trfA1*(A25T*)*; Km^r	(Sota *et al*, 2010)
pEvo-Sh5	pMS0506 variant adapted to strain MR-1, *trfA1*(R31P*)*; Km^r	(Sota *et al*, 2010)

pEvo-Sh13	pMS0506 variant adapted to strain MR-1, *trfA1*(Δ*5)*; Km^r	(Sota *et al*, 2010)
pEvo-Sh3	pMS0506 variant adapted to strain MR-1, *trfA1*(Δ77*)*; Km^r	(Sota *et al*, 2010)
pEvo-Sh15	pMS0506 variant adapted to strain MR-1, frame-shift mutation in *trfA1*; Km^r	(Sota *et al*, 2010)
pHSG399	pUC19 replicon, cloning vector; Cm^r	This study
pUX-BF13	R6K replicon, Tn7 transposase expression plasmid; Ap^r	This study
pHY1010	pBBR1-MCS2::*trfA1*(R31P/M124L) ; Km^r	This study
pHY1011	pBBR1-MCS2::*trfA1*(A25T/M124L); Km^r	This study
pHY1012	pBBR1-MCS2::*trfA1*(Δ43/M124L); Km^r	This study
pHY1014	pBBR1-MCS2::*trfA1*(Δ5/M124L); Km^r	This study
pHY1015	pBBR1-MCS2::*trfA1*(Δ77/M124L); Km^r	This study
pHY987	pBBR1-MCS2::*trfA1*(M124L); Km^r	This study
pHY988	pBBR1-MCS2:*trfA2*; Km^r	This study
pHY835LAC	R6K replicon, mini-Tn*7* vector; Ap^r, Gm^r	(Yano *et al*, 2012)
pHY884	pHY835LAC::$trfA1^{wt}$/*trfA2*; Ap^r, Gm^r	(Yano *et al*, 2012)
pHY885	pHY835LAC::*trfA1*(M124L); Ap^r, Gm^r	(Yano *et al*, 2012)
pHY886	pHY835LAC::*trfA2*; Ap^r, Gm^r	(Yano *et al*, 2012)
pHY888	pHY835LAC::*trfA1*(R31P/M124L); Ap^r, Gm^r	This study
pHY890	pHY835LAC::*trfA1*(A25T/M124L); Ap^r, Gm^r	This study
pHY892	pHY835LAC::*trfA1*(Δ5/M124L); Ap^r, Gm^r	This study
pHY897	pHY835LAC::*trfA1*(Δ43/M124L); Ap^r, Gm^r	This study
pHY895	pHY835LAC::*trfA1*(Δ77/M124L); Ap^r, Gm^r	This study
pHY1018	pHY835LAC::*dnaBson*; Ap^r, Gm^r	This study
pET11a	pMB1 replicon, protein expression vector ; Ap^r	Merck KGaA
pHY915	pET11a::his_6-*trfA1*/(M124L/Q279D/S292L); Ap^r	This study
pHY921	pET11a::his_6-*trfA2*(Q279D/S292L); Ap^r	This study
pHY916	pET11a::his_6-*trfA1*(R31P/M124L/Q279D/S292L); Ap^r	This study
pHY917	pET11a::his_6-*trfA1*(A25T/M124L/Q279D/S292L); Ap^r	This study
pHY919	pET11a::his_6-*trfA1*(Δ43/M124L/Q279D/S292L); Ap^r	This study
pHY1032	pET22b::*dnaBson*-his_6; Ap^r	This study
pHY872	R6K replicon, the $oriV_{pBP136}$ region containing eight copies of iteron; Tc^r	(Yano *et al*, 2012)
pHY873	pHY872 carrying the 1.8 kb *oriC* region of the MR-1 chromosome; Tc^r	This study
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.

[b] *trfA* genotypes: $trfA1^{wt}$/*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 25^{th} alanine with threonine of the product; Δ5, an deletion from 93 to 107, deleting five aa of the product; Δ43, an deletion from position 97 to 225 in the *trfA1* frame, deleting 43 aa of the product; Δ77, 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 279^{th} glutamine to asparagine, and mutations from TC to CT at positions 874-875, substituting the 292^{th} 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-pBP136trfA1F	AATTGAGCTCAAGGAGGTAATACACCATGACGAACAACGAGTTCAACGA	Cloning trfA into pHSG399
SacI-SD-pBP136trfA2	AATTGAGCTCAAGGAGGTAATACACCATGGCGACCAAGAAGCGAAC	Cloning trfA into pHSG39
EcoNdeHisTrfA1F	AATTGAATTCATATGCATCACCATCACCATCACACGAACAACGAGTTCAACGAGC	Cloning trfA1 into pET11a
EcoNdeHisTrfA2F	AATTGAATTCATATGCATCACCATCACCATCACGCGACCAAGAAGCGAACGG	Cloning trfA2 into pET11a
BamHindTrfAR	AATTGGATCCAAGCTTATTACCGCTTGCAATGCACCAGGTCG	Cloning trfA into pET11a
SacSDdnaBMR1F	AATTGAGCTCAAGGAGGTATACACCATGTCACAACAAGGTGCTTTTAA	Cloning dnaB into pHY835LAC
HinddnaBMR1R	AATTAAGCTTAATCTTCTTCAAACTGAGGA	Cloning dnaB into pHY835LAC
EcoNdeDnaBmr1F	AATTGAATTCCATATGTCACAACAAGGTGCTTTTAAGC	Cloning *dnaB*son into pET22b
HindDnaBmr1hisR	AATTAAGCTTATTAATGGTGATGGTGATGGTGATCTTCTTCAAACTGAGGACCTG	Cloning *dnaB*son into pET22b
trfAM124LF	CTGGCGACCAAGAAGCGAACG	Introducing M1124L mutation to *trfA1* and tis variants
trfAM124LR	CGCACTACTCCGTTTGTCCTG	Introducing M124L mutation to *trfA1* and its variant
Q279D/S292Lf	CGGCCGGCTGGAATCGGTGCTGCTGATCCGGCGCTTCC	Introducing Q279D/S292L mutation to *trfA*
Q279D/S292Lr	ATGCGCTGGGATGAGAAGTCCATGGCCGACGCCTGGAG	Introducing Q279D/S292L mutations to *trfA*
EcoMR1oriCF	TCAGTGGAATTCAGTTGCATGCTCAAACTC	Cloning the *oriC* region of *S.oneidensis* into pHY872
EcoMR1oriCR	GCAGGAATTCCGTACTTAAAAGTGGTTCC	Cloning the *oriC* region of *S.oneidensis* into pHY872
atpF	GTTGGTTCCATACATCAGCG	qPCR, copy number of *S.oneidensis oriC* region
atpR	AACTGACGCTACAGCCCTTT	qPCR, copy number of *S.oneidensis oriC* region
tetAF	GTGAAACCCAACATACCCCT	qPCR, copy number of pHY872
tetAR	AATTGCACCAACGCATACAG	qPCR, copy number of pHY872
Ecoli_atpF	GTCGGTCCAGGTCTTCATTT	qPCR, copy number of *E.coli oriC* region
Ecoli_atpR	TGCACACGGTAATCTGGAAT	qPCR, copy number of *E.coli oriC* region
E coli glmS	CATGCACATCATCGAGATGCC	Confirmation of mini-Tn*7* insertion
MR-1glmS	GGCGGTTTAATGTATGTGTTTGC	Confirmation of mini-Tn*7* insertion
Tn7R109	CAGCATAACTGGACTGATTTCAG	Confirmation of mini-Tn*7* 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)

Table S3. Statistical evaluation of effect of DnaB overexpression on plasmid persistence dynamics[1]

Pair	Reference [Host (plasmid) /IPTG conc./Expt[2].]	Subject [Host (plasmid) /IPTG conc. /Expt.]	BIC.sep	BIC.joint	ΔBIC (BIC.sep - BIC.joint)	Welch t.test p.value
(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

1. 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".

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

Expt.[b]	Plasmid	Host	Extra *dnaB*	IPTG (uM)	Initial plasmid-free fraction (b_0) [95% c.i.]	Segregational loss rate (l) [95% c.i.]	Fitness cost (s) [95% c.i.]	quasi-extinction time (generations)[c] [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]

[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.

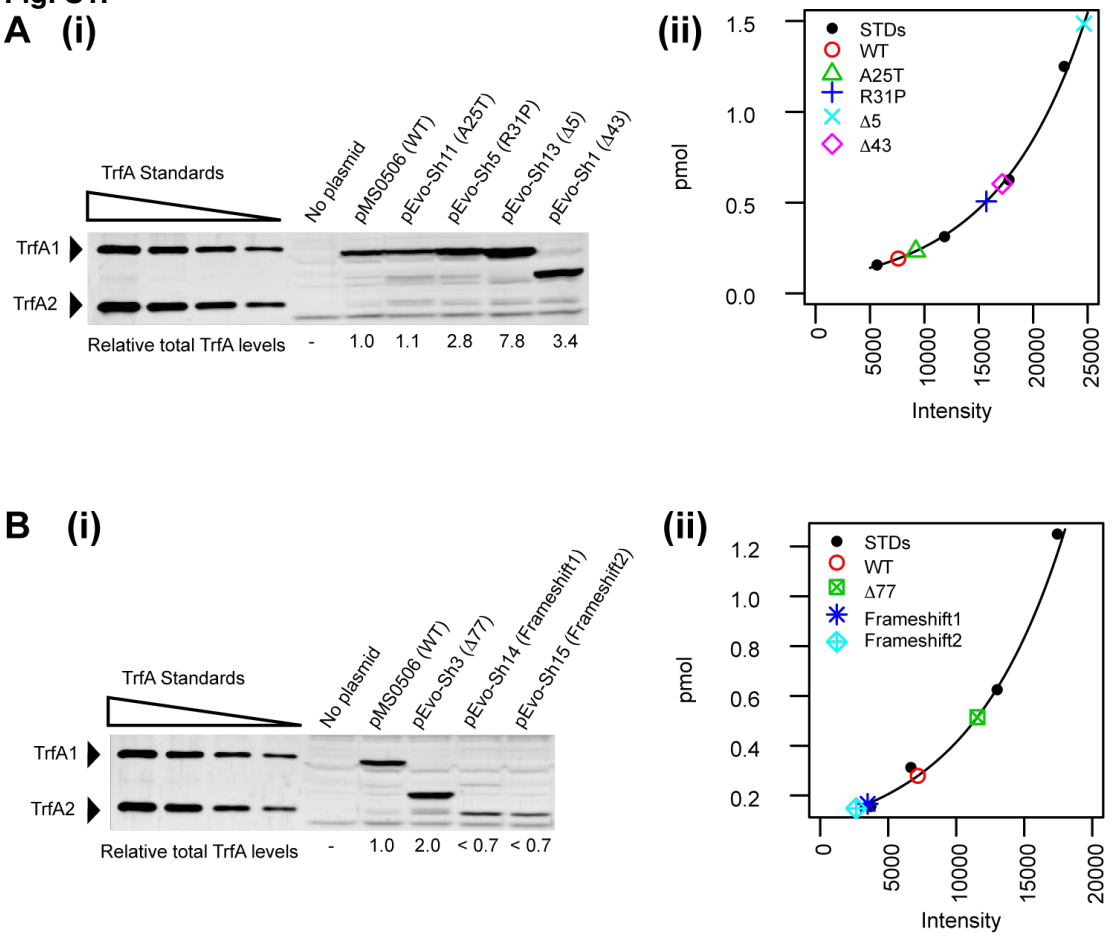

Fig. S1. TrfA expression levels in *S. oneidensis* strain MR-1 carrying pMS0506 or its evolved variants. In each lane, 125 ug of total cell extract was loaded. TrfA quantity standards (STDs; 1.25, 0.63, 0.31, 0.16 pmol for both purified proteins TrfA1 and TrfA2) are loaded in the left four lanes. TrfA 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.

Fig. S2

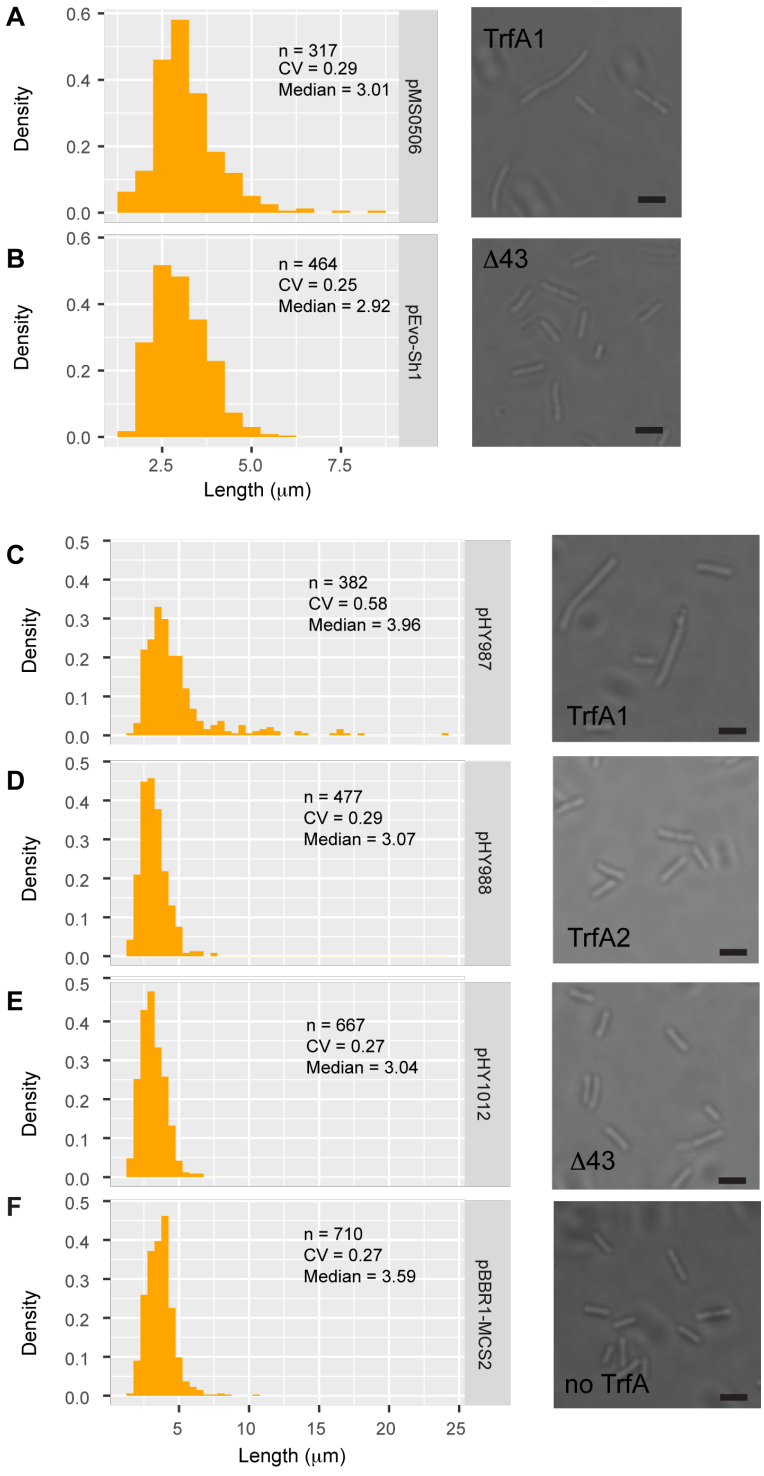

Fig. S2. Distribution of cell length of *Shewanella oneidensis* MR-1 harboring ancestral plasmid pMS0506 (**A**), its evolved variant pEvo-Sh1 (**B**), pBBR1-MCS2 (**F**), and its derivatives carrying *trfA1*(M124L) (**C**), *trfA2* (**D**), or *trfA1*(Δ43/M124L) (**E**). Examples of cell images are 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 Δ43, $P < 0.001$; TrfA1 vs pBBR1-MSC2, $P < 0.001$. Scale bar indicates 3.3 um.

Fig. S3.

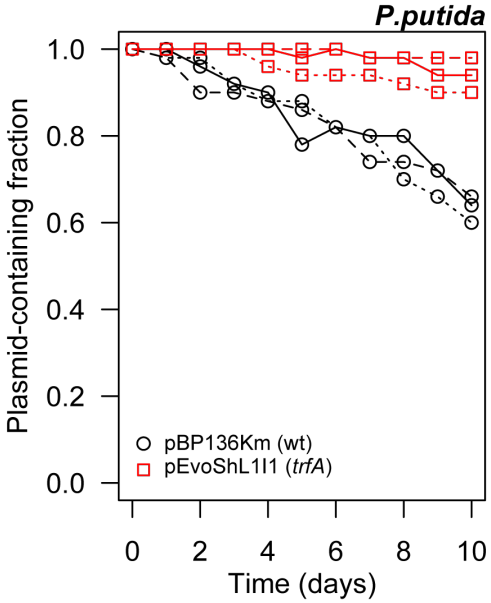

Fig. S3. Loss of TrfA1 production improves persistence of pBP136Km in *P. putida* populations. Triplicate assays were shown as distinct curves. Persistence parameter estimates were shown in Table S4.

Fig. S4.

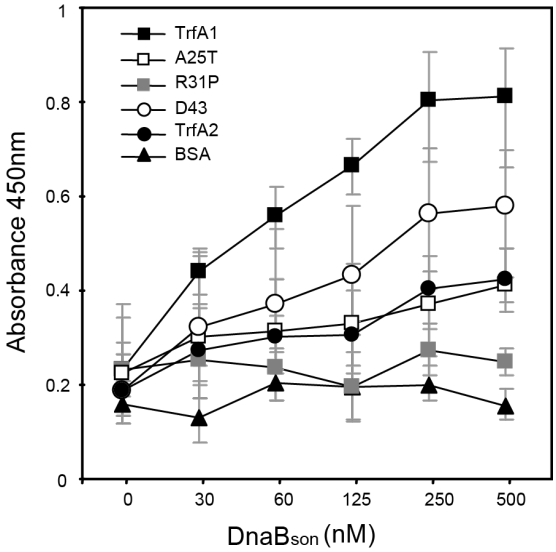

Fig. S4. ELISA showing reduced binding affinity to DnaB of TrfA1 variants. Data point indicates mean and standard deviation. M124L/Q279D/S292L were introduced into TrfA1 and its variants.

Fig. S5.

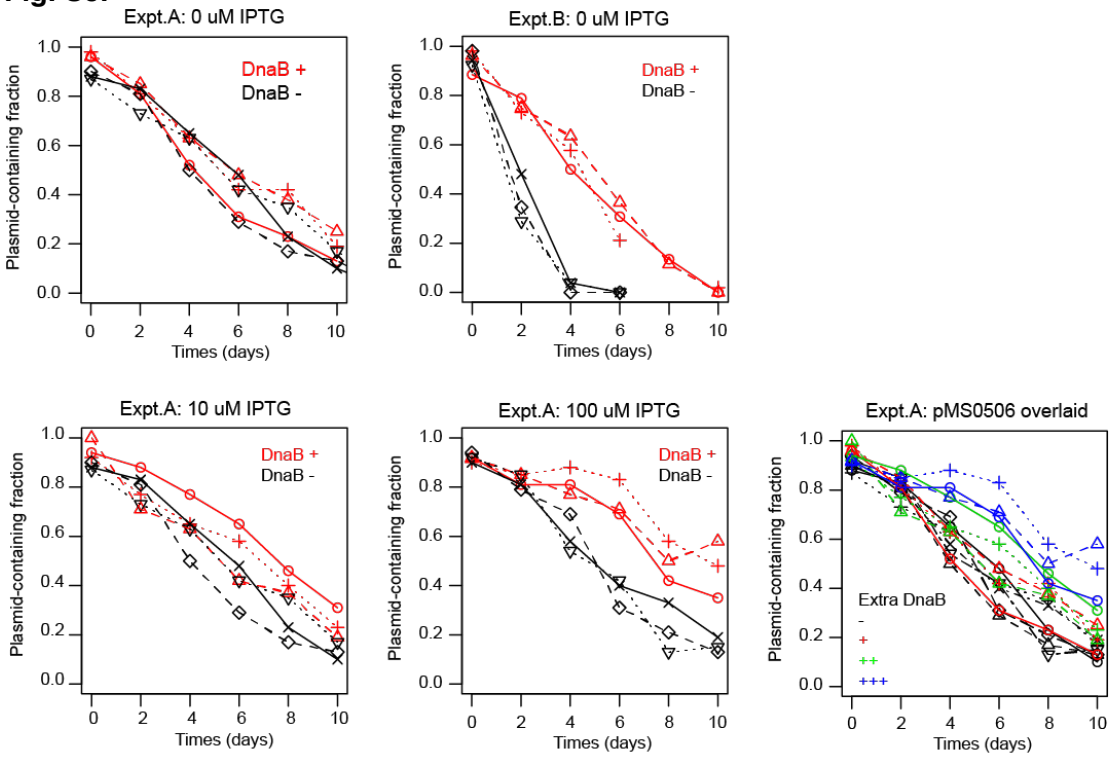

Fig. S5. **Effect of DnaB overproduction on the persistence of ancestral plasmid pMS0506.** Three replicate data sets obtained at the same time are separately shown within the same plot. The hosts used were *S. oneidensis* HY0759 for "DnaB$^-$", and HY1041 for "DnaB$^+$" condition, respectively. In the 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

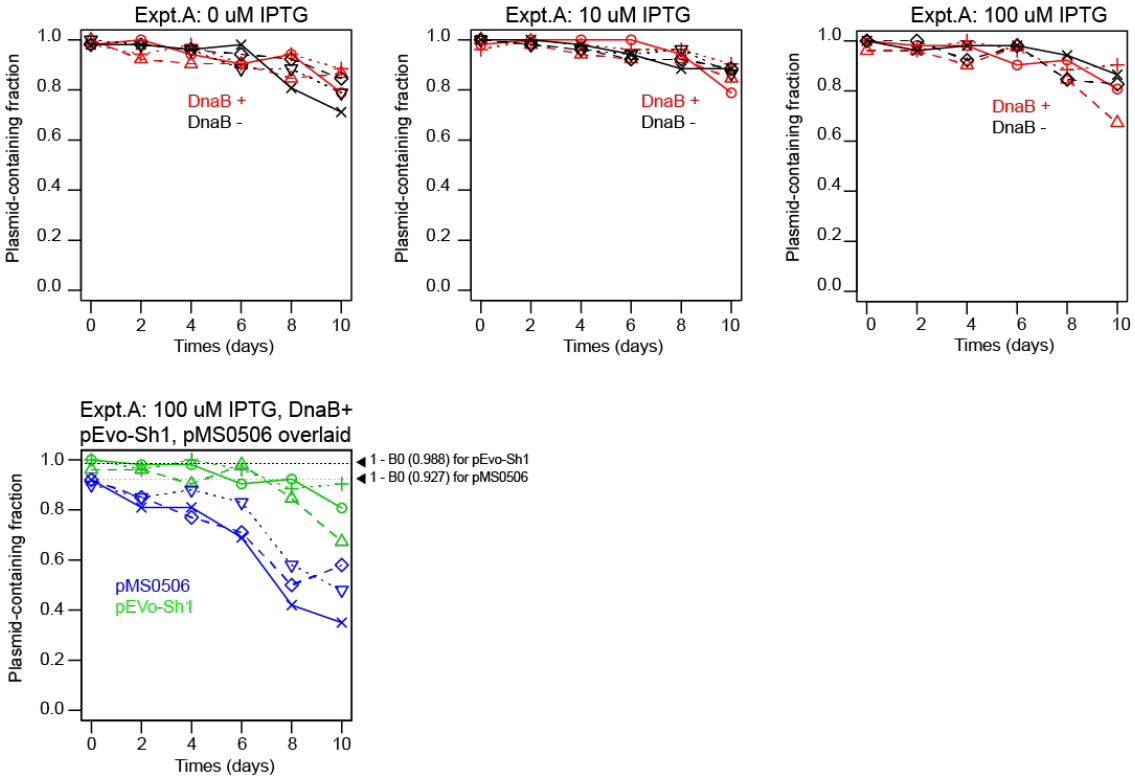

Fig. S6. Effect of DnaB overproduction on the persistence of pEvo-Sh1. Upper panels: Three replicate data obtained at the same time are separately shown within the same plot (only two for "DnaB$^-$" at 100 uM IPTG). The hosts used were the same as Fig.S5. Lower panels, left: Data of pMS0506 and pEvo-Sh1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

Fig. S7

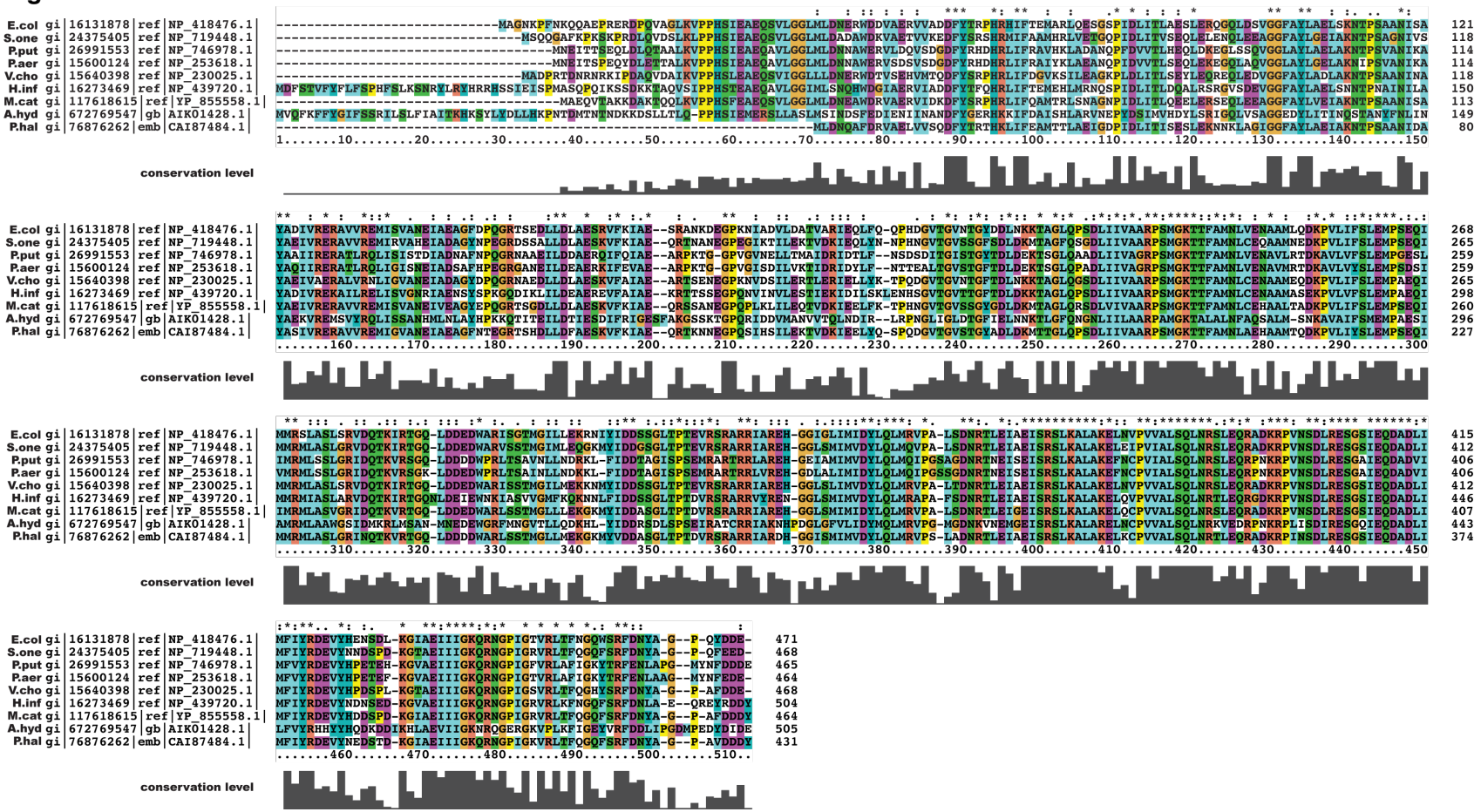

Fig. S7. Alignment of DnaB proteins of representative gammaproteobacterial species. E.col, *Escherichia coli* MG1655; S.one, *Shewanella oneidensis* MR-1; P.put, *Pseudomonas putida* KT2440; P.aer, *Pseudomonas aeruginosa* PAO1; V.col, *Vibrio cholerae* O1 biovar El Tor str. N16961; H.inf, *Heamophilus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

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