# SUPPLEMENTARY FILE

# Conjugative Plasmid-Encoded Toxin-Antitoxin System PrpT/PrpA Directly Controls Plasmid Copy Number

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- **Movie S1.** Morphologies of cells overexpressing *prpT* via pHGECm-*prpT* with 0.5 mM IPTG for 2 h were recorded by phase contrast microscopy over time.
- **Dataset S1.** The PFAM families of antitoxins associated with ParE (PF05016) toxins. 66,524 ParE-associated antitoxins, which were 70-120 aa length were analyzed.
- **Dataset S2.** Detailed information for PF03693 family antitoxins associated with ParE. The conjugative plasmids containing ParE/PF03693 pairs are highlighted in yellow and can be screened by “Plasmid.Bacteria” in Domain.

**S1 Materials and Methods**

**Bacterial strains, plasmids, and growth conditions.** The bacterial strains, plasmids and primers used in this study are listed in **Table S2** and **Table S3**. *P. rubra* was grown in Marine Broth 2216E medium (Difco) at 25°C, and the *E. coli* strains were grown in Luria broth (LB) at 37°C unless specified otherwise. DAP (2,6-diamino-pimelic acid) (50 μg/mL) was added when culturing the *E. coli* WM3064 strain. Ampicillin (100 μg/ml) was used for maintaining pUT18C-based and pMD19-T-based plasmids, kanamycin (50 μg/mL) was used for maintaining pKT25-based, pHGR01-based and pET28b-based plasmids in *E. coli*, chloramphenicol (30 μg/ml) was used for maintaining pCA24N-based plasmids, chloramphenicol (15 μg/ml) was used for maintaining pHGECm-based plasmids, Erythrocin (25 μg/ml) was used for maintaining pBBR1Ery-based plasmids and gentamicin (30 μg/ml) was used for maintaining pHGM01-based plasmids. IPTG (isopropyl-*β*-D-thiogalactopyranoside) (1 mM for protein purification, 0.5 mM for reporter activity assay) was used as an inducer.

**Analysis of the distribution of ParE/PF03693 pairs**. Using the function profile tool in the IMG/M system (http://genome.jgi.doe.gov/, Version 5.1 Sep. 2019) (1), a total of 62,457 ParE-associated antitoxins with PFAM filtered by 70-120 aa length were retrieved. The cognate antitoxins of these ParE toxins were associated with multiple PFAM families, including 20,803 PF02604 [PhdYeFM_antitox], 16,055 PF03693 [ParD_antitoxin], 9,249 PF04221 [RelB], 6,821 PF01402 [RHH_1], and 444 PF09386 [ParD] (**Dataset S1**). The distribution of the PF03693/ParE pairs in representative phyla and genera were further analyzed (**Dataset S2**), and the plasmids with "Plasmid: Bacteria" Domain was recovered from the IMG/M database (**Table S1**).

**Construction of deletion mutants in *P. rubra*.** The deletion mutants were constructed as described previously in *Pseudoalteromonas* (2). Briefly, the upstream and downstream regions of *prpT* and *prpA-prpT* operon were amplified by PCR from *P. rubra* genomic DNA with primers listed in **Table S3**. The PCR fragments were fused and ligated into pHGM01 (3) using the ClonExpress II One Step Cloning Kit (Vazyme, Nanjing, China), producing suicide plasmids pHGM01-*prpT* and pHGM01-*prpAT*. Similarly, the upstream and downstream regions of *rep* were amplified from SCSIO 6842 genomic DNA with primer pairs RepB-up-F/-R and RepB-down-F/-R and ligated into pHGM01, producing suicide plasmid pHGM01-*repB*. To monitor the plasmid pMBL6842, the Cm resistance gene cassette was amplified using primers Cm-F/Cm-R from plasmid pCA24N. The upstream and downstream regions of the targeted loci for Cm integration were amplified from SCSIO 6842 genomic DNA with primers Cm-up-F/-R, Cm-down-F/-R. The three PCR fragments were fused and ligated into pHGM01, generating suicide plasmid pHGM01-*cm.* Then, the suicide plasmids pHGM01-*prpT*, pHGM01-*prpAT*, pHGM01-*repB* and pHGM01-*cm* were respectively transferred from *E. coli* WM3064 into SCSIO 6842 by conjugation experiments using a previously described method (2). The deletion mutants were obtained via homologous recombination by sucrose resistance selection method. Finally, the mutants were confirmed by PCR and DNA sequencing.

**Construction of plasmids.** The pHGECm vector was used to express target genes in *P. rubra* and pCA24N was used to express target genes in *E. coli*. The pBBR1Ery clone vector was used to complement *prpA* or the *prpAT* operon with its own promoter in *P. rubra* Δ*prpAT* strain. The pHGR01 vector was used to construct the *lacZ* reporter plasmids. The pET28b vector was used to purify the targeted proteins or quantify the production of targeted proteins by Western blot in *E. coli.* The pKT25 and pUT18C vectors were used to test the interaction of two proteins in BACTH assays. In detail, coding region of *prpT*, *prpA* or the *prpAT* operon was amplified from *P. rubra* with primer pairs pHGECm-PrpT-F/-R, pHGECm-PrpA-F/-R or pHGECm-PrpA-F/pHGECm-PrpT-R respectively, PCR product was digested with *EcoRI/XhoI* and inserted into the corresponding sites of pHGECm using the T4 DNA Ligase, generating inducible pHGECm-*prpT*, pHGECm-*prpA* and pHGECm-*prpAT*. Coding region of *prpT*, *prpA* or the *prpAT* operon was amplified from SCSIO 6842 with primer pairs pCA24N-PrpT-F/-R, pCA24N-PrpA-F/-R, pCA24N-PrpA-F/pCA24N-PrpT-R respectively, PCR product was ligated into the *StuI* digested pCA24N vector using the ClonExpress II One Step Cloning Kit (Vazyme Biotech Co.,Ltd), generating inducible pCA24N-*prpT*, pCA24N-*prpA* and pCA24N-*prpAT*. Coding region of *prpA* or the *prpAT* operon with their own promoter was amplified from SCSIO 6842 with primer pairs pBBR1Ery-PrpA-F/-R or pBBR1Ery-PrpA-F/pBBR1Ery-PrpAT-R respectively, PCR product was

digested with *EcoRI/XhoI* and inserted into the corresponding sites of pBBR1Ery using the T4 DNA Ligase, generating complementary plasmids pBBR1Ery-*prpA* and pBBR1Ery-*prpAT*. To generate pRBS$^{prpA}$ and pRBS$^{prpT}$ reporter plasmids, promoter region of *prpAT* was amplified from SCSIO 6842 with primer pairs pRBS$^{prpA}$-F/-R, PCR product was used as template and amplified with primer pairs pRBS$^{prpA}$-F/ pRBS$^{prpT}$-R, two PCR products were digested with *Eco*RI/*Hin*dIII and inserted into the corresponding sites of pHGR01. To generating plasmid expressing Flag-Tagged PrpA or Flag-Tagged PrpT driven by T7*lac* promoter their native RBS, *prpAT* operon containing RBS$^{prpA}$ (do not containing P$_{prpA}$)was amplified and fused with Flag-tag (N-terminal of PrpA or C-terminal of PrpT) using primer pairs listed in **Table S3**, final PCR product was inserted into *XbaI* and *NcoI* digested pET28b vector, generating pET28b-RBS$^{prpA}$-N$^{FLAG}$-PrpAT and pET28b-RBS$^{prpA}$-PrpAT-C$^{FLAG}$. Other plasmids were constructed with similar method, all constructions were finally confirmed by PCR and DNA sequencing.

**Conjugation and transformation.** pHGECm-based and pBBR1Ery-based expression vectors were transferred into *P. rubra* from *E. coli* WM3064 by conjugation. pCA24N-based expression vectors and pHGR01-based promoter-reporter plasmids were electroporated into *E. coli* K-12 BW25113, pET28b-based expression vectors were electroporated into *E. coli* BL21 (DE3). The pKT25- and pUT18C-based vectors were electroporated into *E. coli* BTH101.

**Reporter activity assay.** Specific *β*-galactosidase activity of strains harboring the *prpAT* or *repB* promoter-reporter plasmids was determined by monitoring the absorbance at 420 nm using the Miller assay (4). To determine the promoter activity of *prpAT* and *repB* under overexpression of *prpA*, *prpA-prpT*, *prpA*$^{1\text{-}54}$ and *prpA*$^{55\text{-}86}$, pCA24N-*prpA*, pCA24N-*prpAT,* pCA24N-*prpA*$^{1\text{-}54}$ or pCA24N-*prpA*$^{55\text{-}86}$ was transformed into the *E. coli* host carrying the reporter. Overnight cultures were diluted 1:100 in LB with Kan and Cm, and induced with 0.5 mM IPTG at an $OD_{600}$ of 0.5. After induction for 6 h, cells were collected to determine the *β*-galactosidase activity. To compare the translation efficiency driven by RBS$^{prpA}$ and RBS$^{prpT}$, strains carrying the *lacZ* reporter plasmid pRBS$^{prpA}$ or pRBS$^{prpT}$ were diluted 1:100 in LB with Kan after overnight culture and collected at an $OD_{600}$ of 1.5, and then, *β*-galactosidase activity was determined.

**Identification pMBL6842 origin (*ori*).** A 2.4 kb PCR product was amplified with primer pairs pRepB1-F/-R and was cloned into the *E. coli* cloning vector pHGM01 which does not replicate in *P. rubra* to make pRepB1. Plasmid pRepB2 was generated by amplifying two PCR products with primer pairs pRepB1-F/pRepB2-mid-R and pRepB2-mid-F/pRepB1-R and cloning into pHGM01. The other three truncated fragments were amplified with primer pairs pRepB3-F/pRepB1-R, pRepB4-F/pRepB1-R, and pRepB1-F/pRepB5-R, and cloned into pHGM01, producing plasmids pRepB3, pRepB4 and pRepB5, respectively. These plasmids were then transferred into plasmid-free strain *P. rubra* ΔpMBL6842 from *E. coli* WM3064 by conjugation, and were selected by plating on 2216E agar plates containing gentamicin.

**Determination generation time.** Generation time ($G_t$) of cells in culture is defined as the time required for the cell number to double, accordingly, $G_t$ of *P. rubra* was measured following previously described protocols (5). In detail, three independent colonies of strain SCSIO 6842 were grown overnight in 2216E medium at 25°C. th e overnight cultures were diluted to an $OD_{600}$ of 0.1 in fresh 2216E medium, cells for 0 h, 1 h, 2 h and 3 h were collected and serially diluted in 10-fold, 10 μl was dropped on 2216E plates and incubated at 25°C for 24 h, the colony-forming units (CFUs) were recorded. The generation time was calculated by the formula: $G_t = [\log(N) - \log(N_0)] \div K$, where $N$ would equal 2 and $N_0$ would equal 1 (or N = 4, $N_0 = 2$, or any other values representing a doubling in number), $K$ representing the slope of the line during CFU increase along with culture time. The results showed that the generation time of *P. rubra* is 45.8 ± 3.6 min per generation in 2216E medium at 25°C.

**Segregational stability assay.** Strains of *P. rubra* containing plasmid pMBL6842*::cm* or its derivatives pMBL6842*::cm*Δ*prpA*, pMBL6842*::cm*Δ*prpAT* were grown in 2216E medium to determine the contribution of PrpT/PrpA TA system to plasmid stability. An 2216E preculture with Cm was used to inoculate 3 ml 2216E without antibiotic. Every 12 h of growth (~16 generations), bacterial suspensions were diluted 1000-fold in 3 ml fresh 2216E medium. The cultures were serially diluted in 10-fold dilution steps from days 1 to 14, and 10 μl was dropped on 2216E plates with or without Cm. The CFU assay was conducted every day (~32 generations) for 14 days, and the number of CFUs was determined.

Each experiment was performed in triplicate with three independent cultures.

**Quantification of plasmid copy number**. Plasmid copy number of pMBL6842 in *P. rubra* WT and TA deletion mutants were quantified by quantitative PCR (qPCR) and also by a whole-genome sequencing approach previously described (6). Overnight cultures of *P. rubra* WT and TA deletion mutants were diluted into $OD_{600}$~0.01 and cultured in 2216E medium for 24 h. Cells in exponential (~6 h) and late stationary (~24 h) phases were collected to determine the plasmid copy number of pMBL6842. Total DNA was extracted using a TIANamp Bacteria DNA kit (Tiangen, China) following manufacturer's instructions. The number of chromosomes was quantified based on the chromosomally-encoded single-copy gene *gyrB*, and the number of pMBL6842 plasmids was quantified based on the plasmid-encoded single-copy gene *repB*. Furthermore, we also employed a PCR-free, whole-genome sequencing approach, where the ratio of coverage depth between plasmid DNA and chromosomal DNA reflects the copy number of pMBL6842 in the wild-type and Δ*prpAT* strains. In the complementation study, pBBR1Ery-based plasmids expressing PrpA or the PrpT/PrpA complex under their native promoter were transferred into *P. rubra* Δ*prpAT* from *E. coli* WM3064, after 24 h immediate culture in 2216E with Ery when correct conjugants were obtained, plasmid copy number of pMBL6842 was quantified by the similar qPCR method.

**Protein purification and mass spectrometry analysis.** Protein PrpA, PrpT/PrpA complex, $PrpA^{1\text{-}54}$, $N^{FLAG}$-*prpA*-$C^{His}$ and RepB were purified from *E. coli* BL21 (DE3) containing the targeted plasmids pET28b-*prpA*-His, pET28b-*prpAT*-His, pET28b-$prpA^{1\text{-}54}$-His, pET28b-$N^{FLAG}$-*prpA*-$C^{His}$ and pET28b-*repB*-His. Briefly, the overnight cultures were diluted to an $OD_{600}$ of 0.05 in fresh LB with IPTG (1 mM) was added at $OD_{600}$ of 0.5, and one liter of the cells were collected after induction for 5 h. The subsequent steps of protein extraction from the collected pellet were performed as previously described (7). The purified proteins were desalted by passage through Sephadex G-25 pre-packed PD-10 columns, and protein concentrations were quantified using a Bi Yuntian BCA assay kit (Haimen, China). Tricine-SDS-PAGE was performed as previously described (8). For subsequent mass spectrometry analysis, protein bands were excised and analyzed by BGI Tech Solutions Co., Ltd. (Shenzhen, China). Peptide mass fingerprints were analyzed using the MASCOT search program version 2.3.01 (9) and the previously annotated *P. rubra* genome (GenBank accession numbers CP013611 to CP013613) (10) as the reference database.

**Electrophoretic mobility shift assay (EMSA).** EMSA was carried out as described previously (11) to evaluate the binding effects of purified protein on corresponding biotin-labelled DNA probe using the LightShift™ EMSA Optimization and Control Kit (Thermo, USA). The *prpAT* promoter and the *ori* were PCR amplified from the SCSIO 6842 genomic DNA using primer pairs $P_{prpAT}$-probe-F/-R and ori-probe-F/-R, mutant *ori* was amplified and fused using primer pairs listed in **Table S3**, the PCR product was gel-purified and cloned into pMD19-T, generating pMD19-T-$P_{prpAT}$, pMD19-T-*ori* and pMD19-T-*oriGC*. Biotin-labeled M13F and ordinary M13R were used to amplify the mature probe. Empty pMD19-T was used to amplify the control probe. For the binding reactions, DNA probe (0.1 pmol) was added to different amounts of purified protein in 20 μl system containing 2 μl 10×Binding buffer, 1 μl 50% Glycerol, 1 μl 1% NP40, 1 μl 1M KCl, 1 μl 100 mM $MgCl_2$ and 0.25 μl 1μg/μl Poly (dI:dC). The binding reactions were incubated at 25 °C for 2 h and loaded onto a 6% DNA retardation gel at 110 V in 0.5 ×TBE [45 mM Tris (pH 8.3), 45 mM boric acid and 1 mM EDTA] and subjected to electrophoresis at 4 °C for 60 min. DNA probe was transferred to a nylon membranes at 400 mA for 30 min and subsequently UV-crosslinked at 302 nm for 20 min. Stabilized streptavidin-HRP (50 μl) was added and the mixture was incubated at room temperature for 30 min in Blocking Buffer. After washing with 1×Wash Buffer for three times, the chemiluminescent signal was detected using Luminol/Enhancer Solution and Nucleic Acid Detection Blocking Buffer according to the manufacturer's protocol. To test the competition of PrpA and RepB for their binding effect to *ori*, one protein was first added at a fixed concentration (RepB-160 nM, PrpA-600 nM) into the reaction system and incubation for 20 minutes at 25 ℃, the other protein was then added at a gradually increased concentration (PrpA-from 0 to 600 nM, RepB-from 0 to 1800 nM ) to compete with the first one for the binding to *ori*, after incubation at 25 ℃ for another 40 min, reaction products were loaded onto 6% DNA retardation gel and followed the procedure as described ablove (**Fig. 5DE**). To assess whether adding order of PrpA and RepB has a critical effect on competition binding assay, we also performed a parallel experiment in which PrpA and

RepB were added simultaneously (**Fig. 5F**).

**Western blot analysis.** To quantify the production of PrpA or PrpT, overnight cultures of *E. coli* strains BL21/pET28b-RBS$^{prpA}$-N$^{FLAG}$-PrpAT and BL21/ pET28b-RBS$^{prpA}$-PrpAT- C$^{FLAG}$ were diluted 100-fold in fresh LB with Kan and IPTG. Cells in stationary (~12 h) were collected. To monitor the degradation of PrpA at the N and C termini, strain BL21/pET28b-N$^{FLAG}$-PrpA-C$^{His}$ was collected and used to purify proteins. Equal purified proteins were incubated with stationary-phase *P. rubra* cell lysates for varying amounts of time. Then, total protein was loaded, and tricine-SDS-PAGE was performed. The proteins were transferred to a PVDF membrane, Western blot was performed as described previously, and the antibodies were provided by the same supplier (12). Briefly, primary antibodies raised against a His-tag or Flag-tag and horseradish-peroxidase-conjugated goat anti-mouse secondary antibodies were used in the assays. The expression of anti-RNA polymerase beta antibody (RNAP) was used as a reference in all samples, and the horseradish peroxidase-conjugated goat anti-rabbit antibody was used as a secondary antibody.

**Bacterial two-hybrid (BACTH) assay.** The pKT25 and pUT18C plasmids carrying different target-cloned inserts were co-transformed into *E. coli* BTH101 (*cya−99*) competent cells. Co-transformed cells were plated on LB plates supplemented with ampicillin, kanamycin, IPTG and X-gal at final concentrations of 100 μg/ml, 50 μg/ml, 0.5 mM or 40 μg/ml, respectively. The corrected cells were cultivated at 30°C for 12-24 h. The pKT25-*zip* and pUT18C-*zip* plasmids were used as positive controls (Euromedex), and the pKT25 (without insert) and pUT18C-*zip* (fused with a leucine zipper protein) plasmids were used as negative controls (13).

**DNase I footprinting assay.** FAM-labeled probes were amplified with FAM-labeled M13F and ordinary M13R from pMD19-T-P$_{prpAT}$ and pMD19-T-*ori*. For each reaction, 5 pmol FAM-labeled probes was added to purified protein (50 times more than used in EMSA reaction) in a 50 μl system containing 5 μl 10 × Binding Buffer, 2.5 μl 50% Glycerol, 2.5 μl 1% NP40, 2.5 μl 1M KCl, 2.5 μl 100 mM $MgCl_2$ and 0.625 μl 1μg/μl Poly (dI:dC). All binding reactions were incubated at 25 °C for 2 h, 1/8 U DNase I was then added to digest the DNA probe for about 4 min, 5 μl 200 mM EDTA was used to stop the digestion and DNA probe was purified using a QIAquick® Nucleotide Removal Kit (QIAGEN, Germany). Products were subjected to STR analysis to identify protected sites by corresponding protein.

**Protein degradation assay.** *P. rubra* cell lysates were prepared using early stationary *P. rubra* cells ($OD_{600}$ ~ 3.0). Cell pellets were collected by centrifugation at 10,000 g for 6 min at 4°C and washed twice with ice-cold lysis buffer (50 mM potassium phosphate buffer and 300 mM NaCl). The FastPrep-24™ homogenizer was used to homogenize cells at 20 s intervals five times, and cell debris was further removed by centrifugation at 10,000 g for 10 min at 4°C. Purified PrpA was incubated with *P. rubra* cell lysates at 25°C for varying amounts of time. Tricine-SDS-PAGE gels were first used to evaluate the degradation profiles of PrpA, and then Western blot analysis was used to determine which PrpA terminus was cleaved.

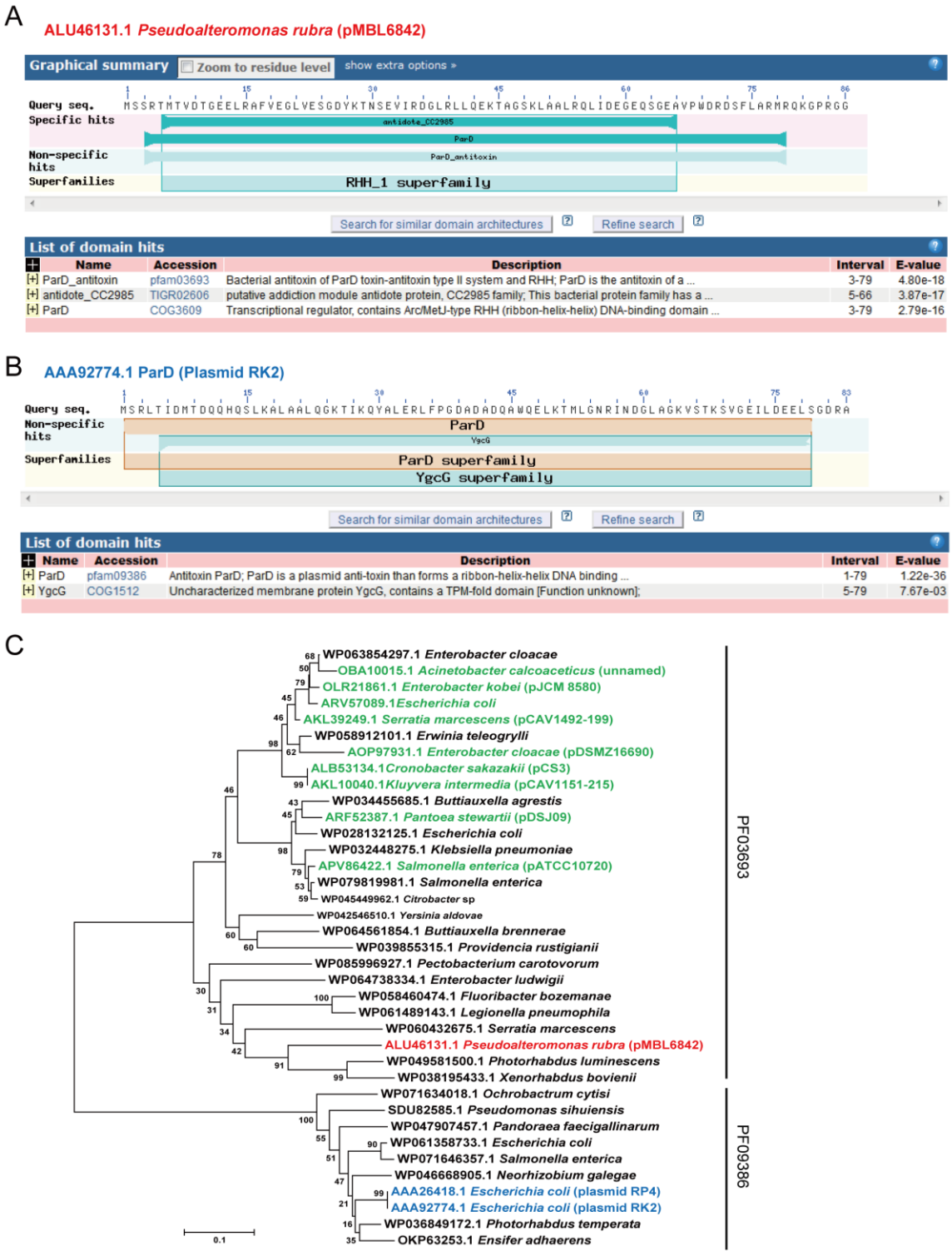


**Fig.S1. (A)** Sequence analysis of AT705_24525 by BLASTP shows that it belongs in the PF03693 PFAM family. (**B**) ParD of plasmid RK2 belongs in the PF09386 [ParD] family. (**C**) Neighbor-joining phylogenetic tree of 37 ParD antitoxin family proteins based on amino acid (aa) sequences. PF03693 in pMBL6842 is marked in red, other PF03693 homologs (listed in Table 1) from sequenced plasmids are marked in green, and PF09386 in plasmids RK2 and RP4 are marked in blue. Sequences were aligned by MUSCLE program and the dendrogram was constructed by the neighbor-joining method with the MEGA software package (1,000 bootstrap replicates). The numbers indicate the bootstrap coefficients, and the scale bar indicates 0.2 substitutions per aa position.

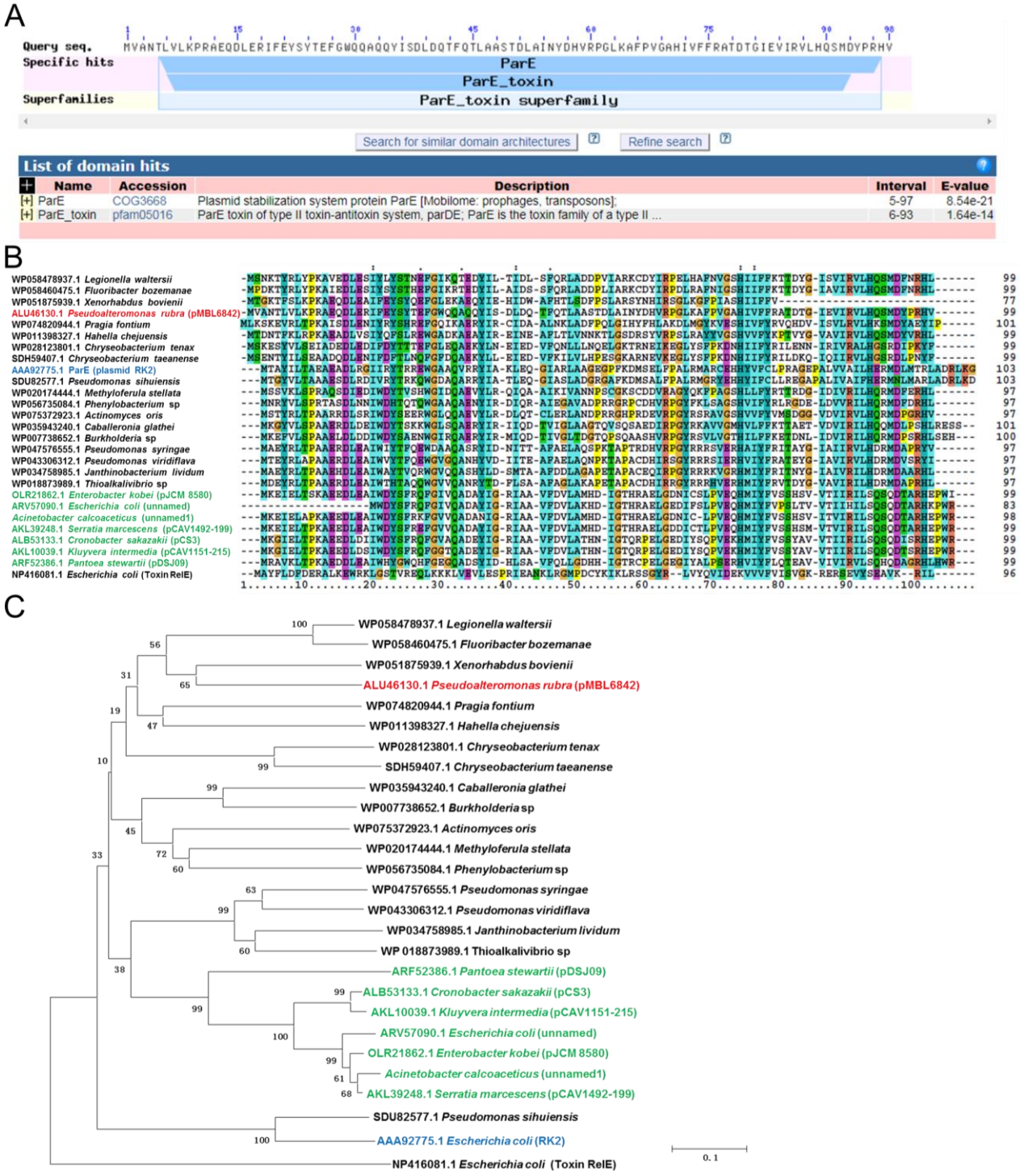


**Fig. S2. (A)** Sequence analysis of AT705_24520 by BLASTP shows that it belongs to the ParE toxin family. **(B)** Multiple sequence alignment constructed by ClustalW to compare the amino acid sequence identity of ParE toxins in the sequenced plasmids listed in **Table S1**. The numbers at the bottom refer to the amino acid position in the ParE proteins, and identical residues are shaded the same color. **(C)** Phylogenetic tree of ParE toxin proteins in (**B**) based on amino acid (aa) sequences. The RelE toxin in *E. coli* K-12 MG1655 was used as the outgroup. The dendrogram was constructed by the neighbor-joining method with the MEGA software package MUSCLE program (1,000 bootstrap replicates). The numbers indicate the bootstrap coefficients, and the scale bar indicates 0.2 substitutions per aa position.

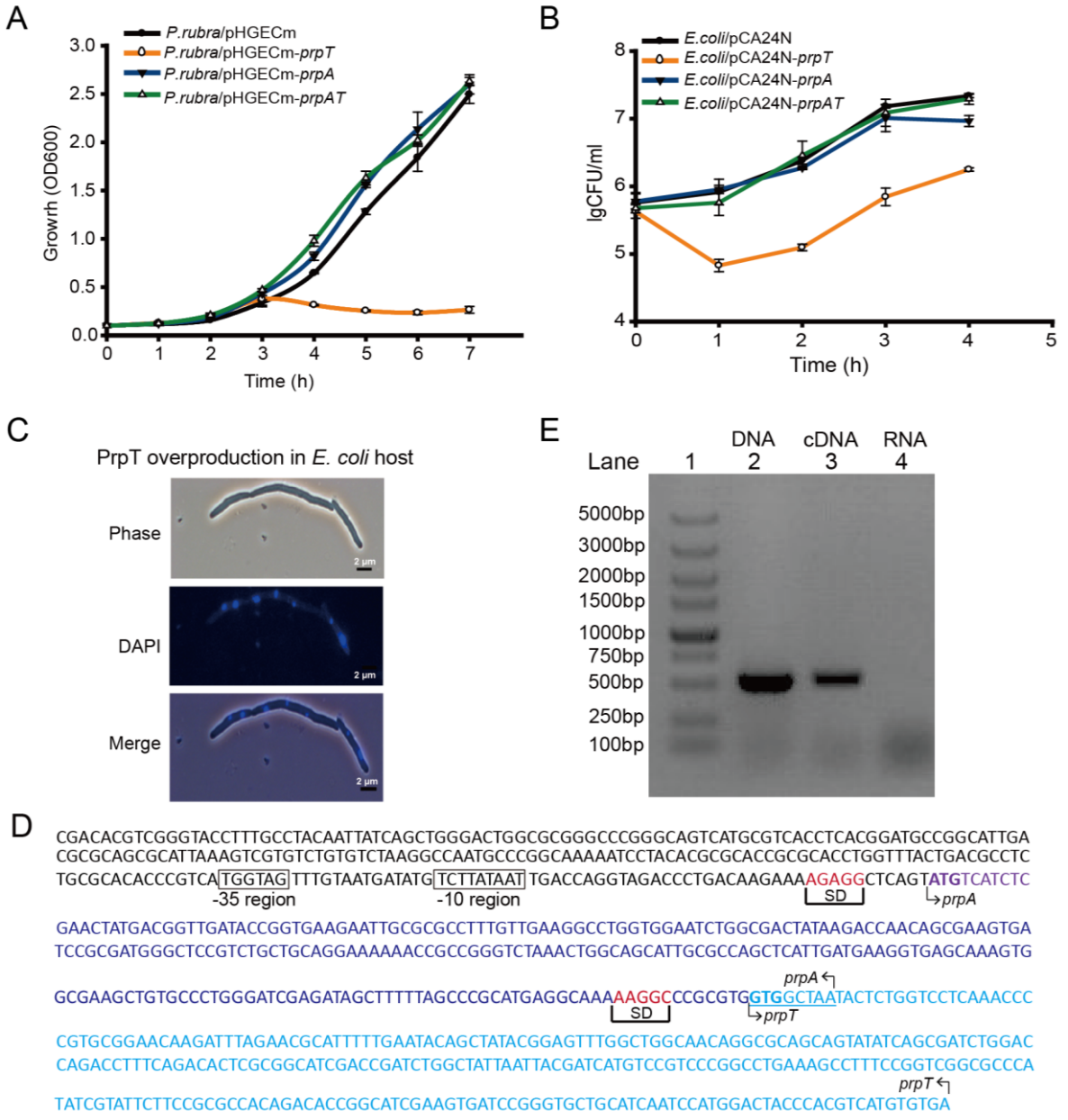


**Fig. S3.** (**A**) Growth of cells overexpressing *prpA*, *prpT* and *prpA-prpT* via pHGECm-based plasmids in *P. rubra*. (**B**) Viability of cells expressing *prpA*, *prpT* and *prpA-prpT* via pCA24N-based plasmids in *E. coli*. (**C**) Morphologies of cells overexpressing *prpT* via pCA24N-*prpT* with 0.5 mM IPTG for 2 h were examined by phase contrast microscopy (lower panel) and DAPI staining (middle panel). (**D**) Sequence analysis of *prpA-prpT* operon. The -35 and -10 boxes predicted from BPROM in Softberry were shown in box on the sequences, and the SD (Shine-Dalgano) sequences of *prpA* and *prpT* are highlighted in red. The overlapped coding region of *prpA* and *prpT* is underlined, and the start codon of PrpA and PrpT are marked. (**E**) Co-transcription of *prpA* and *prpT*. Primers were designed to amplify the whole coding region of *prpA* and *prpT*. Approximately 150 ng of cDNA reverse transcribed from *P. rubra* RNA was used as a template, the same amount of *P. rubra* genomic DNA and RNA were used as the positive and negative controls, respectively. Three independent cultures of each strain were tested in **A**-**C**, and the error bars indicate the standard errors of the means ($n$ = 3) in **A** and **B**. Only representative images are shown in **C** and **D**.

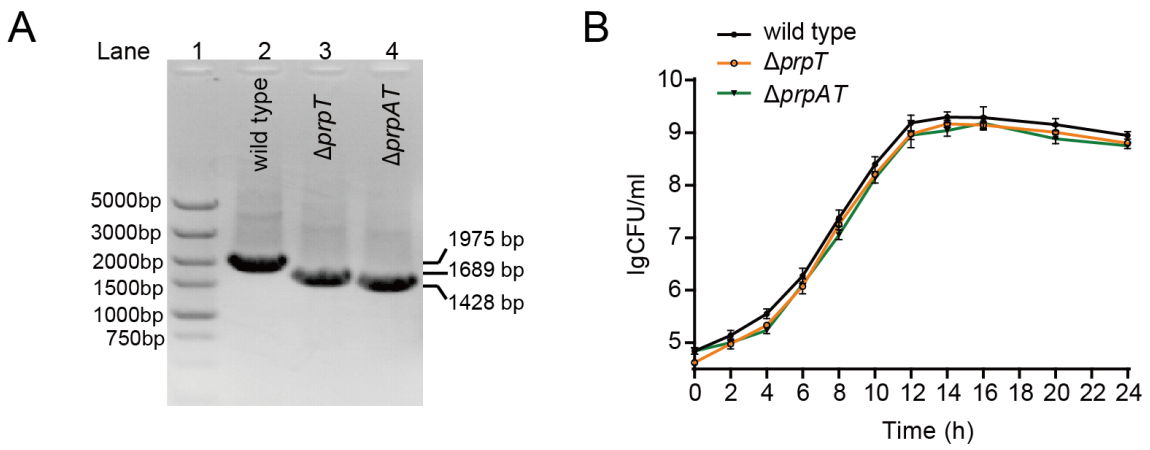


**Fig. S4.** (**A**) Verification of the *prpT* gene deletion (strain Δ*prpT*) and *prpA-prpT* deletion (strain Δ*prpAT*) in *P. rubra* by PCR. **(B)** Colony-forming unit (CFU) of the wild type, Δ*prpT* and Δ*prpAT* strains in 2216E medium ($OD_{600}$~0.01 at T=0). Cells in exponential (~6 h) and late stationary (~24 h) phases were collected for the determination of plasmid copy number, respectively.

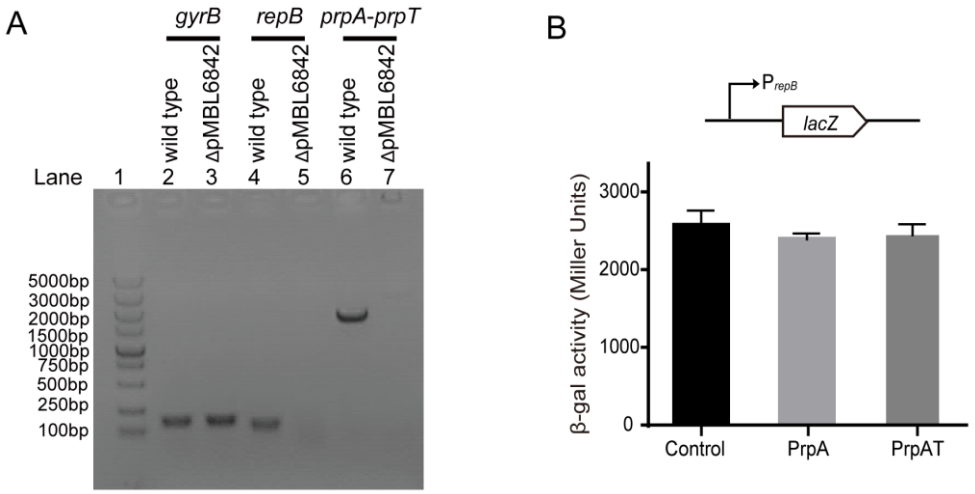


**Fig. S5. (A**) PCR verification of pMBL6842 loss when *repB* is deleted. Chromosomal gene *gyrB*, and plasmid-encoded genes *repB* and *prpA-prpT* were amplified using DNA extracted from the wild type strain and from the mutant strain ΔpMBL6842 as templates with primer pairs qgyrB-F/-R, qrep-F/-R and *prpAT*-con-F/-R. (**B**) The promoter activity of *repB* was measured when overexpressing PrpA (via pCA24N-*prpA*), PrpAT (via pCA24N-*prpAT*), and empty vector (pCA24N). β-galactosidase activity was measured 5 h after adding 0.5 mM IPTG at a turbidity of 0.8 at 600 nm.

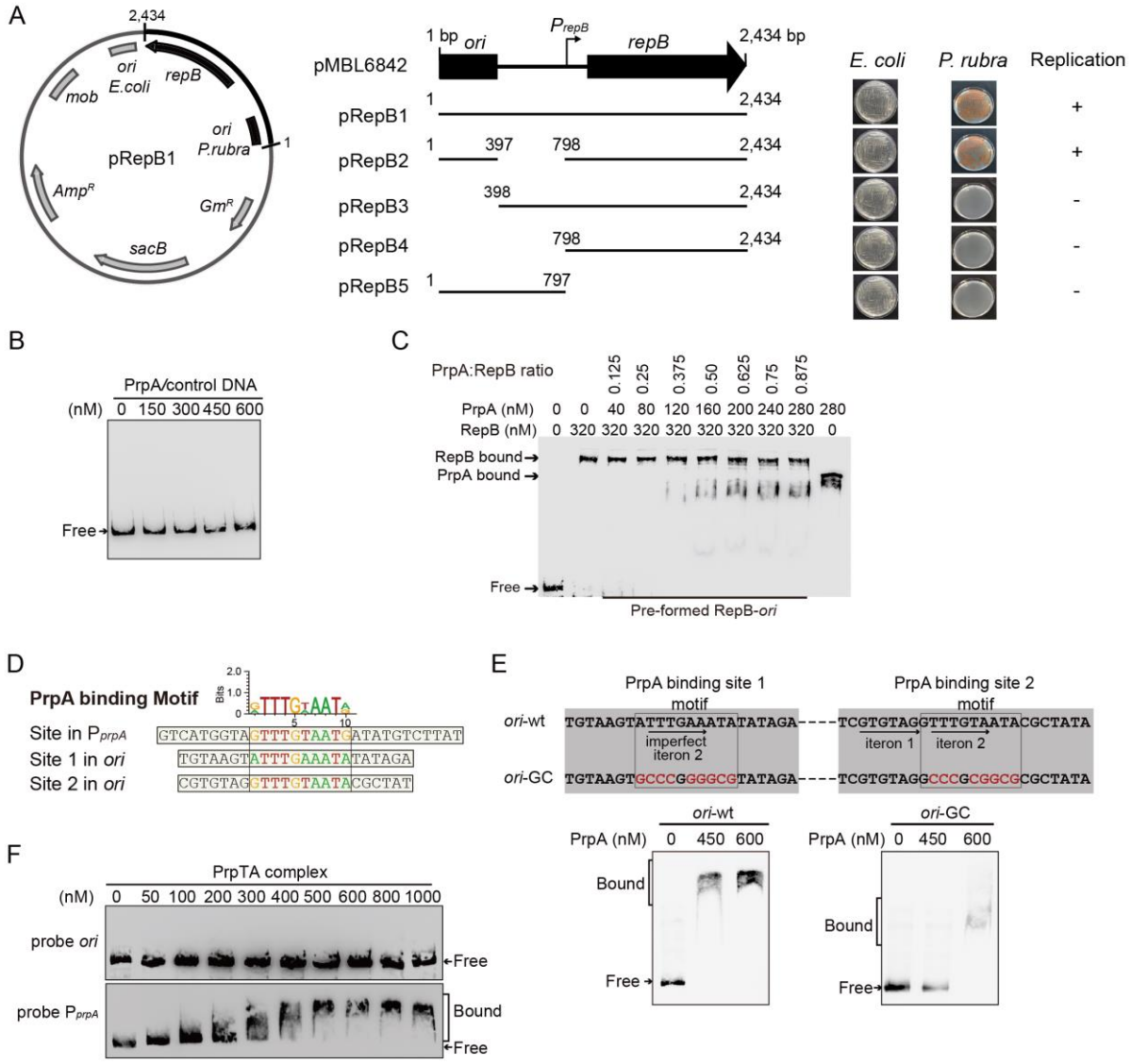


**Fig. S6. (A)** Determination of the pMBL6842 origin (*ori*). Deletion analysis of the pMBL6842 origin (*ori*). Plasmid pRepB1-B5 contained the whole 2.4 kb *ori* fragment or partial *ori* fragments. All the plasmids can replicate in *E. coli* WM3064 host. The "+" or "-" shows the replication ability of each plasmid in *P. rubra* ΔpMBL6842. (**B**) EMSA results show that PrpA did not bind and shift the control DNA. (**C**) EMSA results show that PrpA was unable to dislodge RepB bound to *ori* DNA if less than 1:1 ratio (**D**) Generating the PrpA binding motif by comparing the binding sites of PrpA in the *prpAT* promoter and pMBL6842 *ori*. (**E**) EMSA results show mutating the motif greatly reduces PrpA binding to *ori*. (**F)** EMSA results show that the PrpT/PrpA complex does not bind or shift *ori* compared with the *prpAT* promoter.

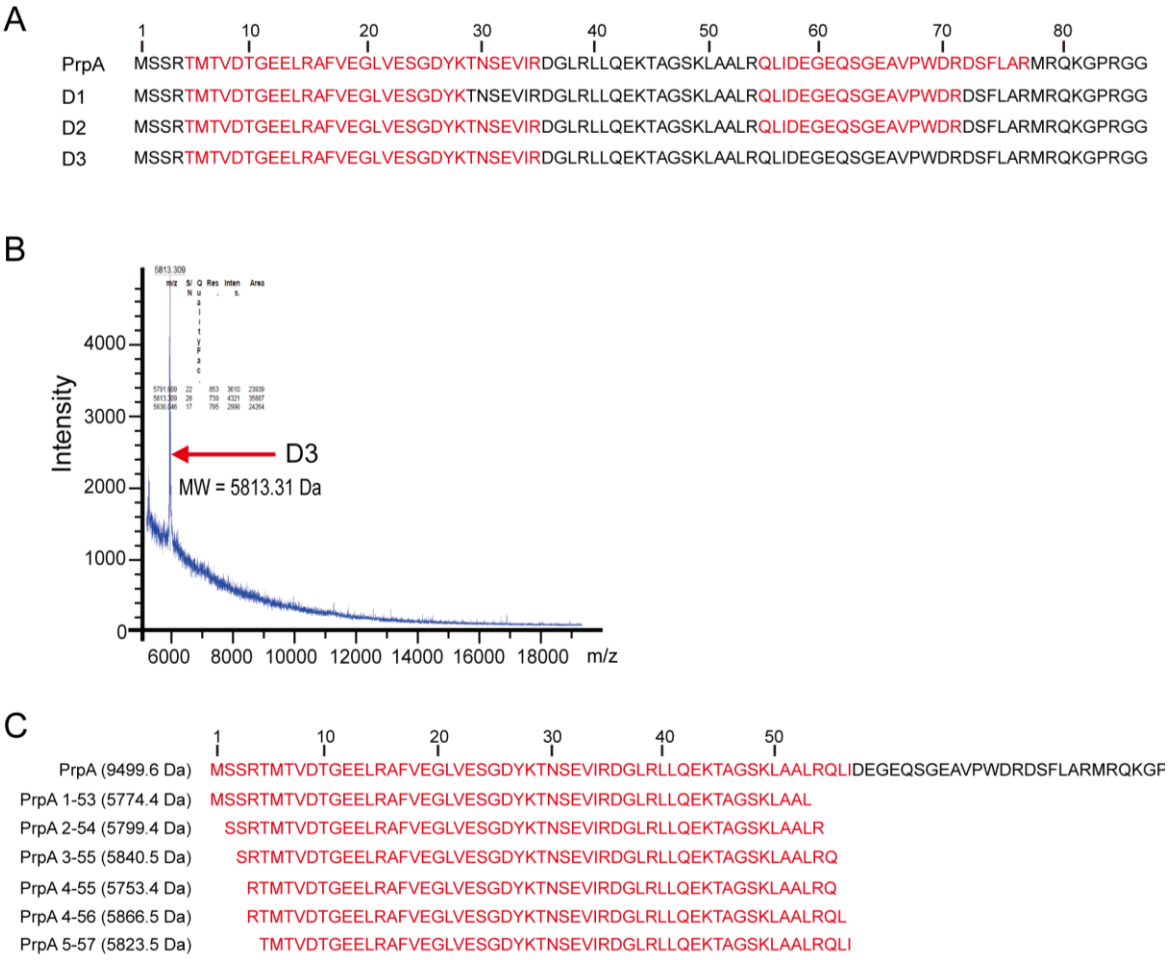


**Fig. S7.** (**A**) Mass spectrometry analysis of D1, D2 and D3 for partially degraded antitoxin PrpAs as shown in **Fig. 6A**; the full-length PrpA was used as a positive control. (**B**) The molecular weight of degraded PrpA D3 was analyzed using MALDI-TOF MS. (**C**) The end of the C-terminus of D3 was between the 53-57 aa of PrpA (all close to 5.8 kDa for 1-53 aa, 2-54 aa, 3-55 aa, 4-55 aa, 4-56 aa or 5-57 aa).

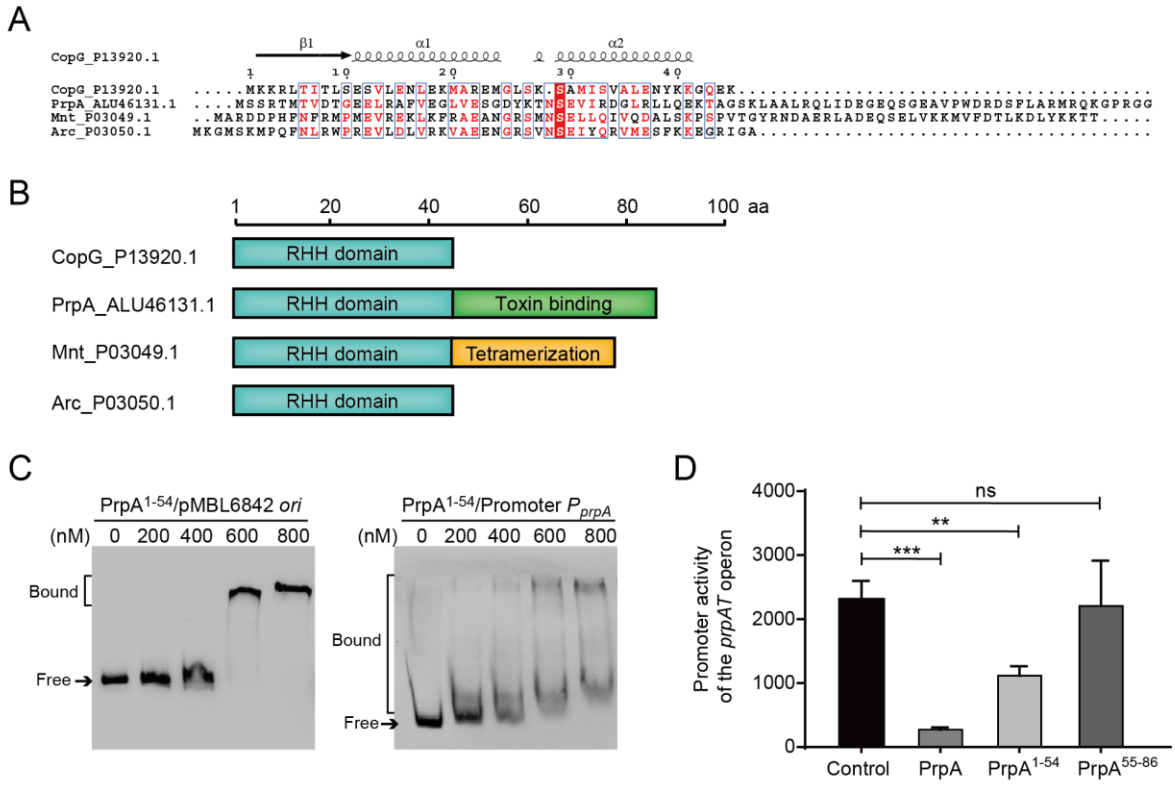


**Fig. S8.** (**A**) Secondary structure of PrpA and three previously-characterized RHH regulatory proteins. The numbers at the top refer to the aa position in the CopA protein. Conserved residues are boxed, the highly-conserved turn connecting two helices is shaded in black, and non-conserved residues are marked in red. CopG is from the *Streptococcus agalactiae* pMV158 plasmid (P13920.1), Arc is from *Enterobacteria* phage P22 (P03050.1) and Mnt is from *Enterobacteria* phage P22 (P03049.1). (**B**) Schematic of the modular organization of PrpA compared with three previously-characterized RHH regulatory proteins. (**C**) EMSA results showed the C-terminal-truncated PrpA (PrpA$^{1-54}$) bound and shifted the pMBL6842 *ori* and the *prpA-prpT* promoter. (**D**) The promoter activity of *prpA* was measured when overexpressing full length PrpA, truncated PrpA$^{1-54}$, PrpA$^{55-86}$, and empty vector. β-galactosidase activity was measured 6 h after adding 0.5 mM IPTG at $OD_{600}$ of 0.5.

**Table S1.** ParE/PF03693 (PrpT/PrpA) TA pairs and closely-related TA pairs in large, sequenced plasmids (> 50 kb).

| Host strain | Plasmid | GenBank ID | Plasmid size (bp) | PF03693 length /similarity | ParE length /similarity | Conjugation gene cluster | Description |
|---|---|---|---|---|---|---|---|
| *Salmonella enterica* | pATCC10720 | CP019182.1 | 124,201 | 88 aa, 80% | 95 aa, 54% | + | Abortive phage infection |
| *Pantoea stewartii* | pDSJ09 | CP017590.1 | 132,938 | 88 aa, 77% | 98 aa, 50% | + | - |
| *Enterobacter kobei* | pJCM_8580 | MKXD01000001.1 | 122,368 | 86 aa, 78% | 98 aa, 57% | + | Copper resistance |
| *Escherichia coli* | Plasmid unnamed | CP019561.1 | 148,529 | 86 aa, 78% | 82 aa, 53% | + | Copper resistance, TetA |
| *Serratia marcescens* | pCAV1492-199 | CP011641.1 | 199,444 | 86 aa, 78% | 98 aa, 57% | + | Heavy metal resistance |
| *Cronobacter sakazakii* | pCS3 | CP012256.1 | 53,383 | 86 aa, 80% | 98 aa, 58% | - | Copper resistance |
| *Kluyvera intermedia* | pCAV1151-215 | CP011600.1 | 215,092 | 86 aa, 80% | 98 aa, 58% | + | Heavy metal resistance |
| *Enterobacter cloacae* | pDSMZ16690 | CP017185.1 | 151,583 | 86 aa, 76% | 98 aa, 76% | + | Carbapenem resistance (14) |
| *Pseudoalteromonas rubra* | pMBL6842 | CP013613.1 | 69,868 | 86 aa, 100% | 98 aa, 100% | + | H-NS |
| *Yersinia enterocolitica* | pYVa127/90 | NC_004564.1 | 66,591 | 80 aa, 64% | 100 aa, 51% | + | T3SS, virulence (15) |
| *Yersinia enterocolitica* | pYVe8081 | NC_005017.1 | 67,720 | 80 aa, 64% | 100 aa, 51% | + | T3SS, virulence (16) |
| Uncultured bacterium | pTP6 | NC_007680.1 | 54,344 | 89 aa, 67% | 104 aa, 40% | + | Mercury resistance (17) |
| *Pseudomonas* sp | pADP-1 | NC_004956.1 | 108,845 | 93 aa, 67% | 104 aa, 40% | + | - |
| *Ensifer meliloti* | pSmeSM11b | NC_010865.1 | 181,251 | 94 aa, 60% | 99 aa, 45% | + | Nitrogen-fixing (18) |
| *Yersinia pestis* | pCD1 | CP000722.1 | 70,305 | 91 aa, 51% | 99 aa, 49% | + | T3SS, virulence |
| *Enterobacter aerogenes* | R751 | NC_001735.4 | 53,423 | 89 aa, 67% | 104 aa, 40% | + | - |
| - | pB8 | NC_007502.1 | 57,198 | 89 aa, 67% | 104 aa, 40% | + | Multi-drug resistance (19) |

**-:** not available or not found.

**Table S2. Strains and plasmids used in this study.**

| Bacterial strains/plasmids | Description[a] | Source |
|---|---|---|
| ***E. coli* strains** | | |
| K-12 BW25113 | *lacI*$^{q}$ *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$ | (20) |
| BL21(DE3) | F$^{-}$*ompT hsdSB*(rB$^{-}$ mB$^{-}$) gal dcm λ(DE3) Ω PtacUV5::T7 polymerase | Novagen |
| WM3064 | *thrB1004 pro thi rpsL hsdS lacZ*Δ*M15* RP4-1360) Δ(*araBAD*)567Δ*dapA*1341::[*erm* pir(wt)] | (21) |
| DH5α | F$^{-}$Φ80*lacZ*Δ*M15* Δ(*lacZYA-argF*) U169 *recA1 endA1 hsdR17* (rK$^{-}$,mK$^{+}$) *phoA supE44* λ$^{-}$ *thi*$^{-1}$ *gyrA96 relA1* | Invitrogen |
| BTH101 | F$^{-}$, *cya-99*, *araD139*, *galE15*, *galK16*, *rpsL1* (Str$^{R}$), *hsdR2*, *mcrA1*, *mcrB1* | (22) |
| ***P. rubra* strains** | | |
| SCSIO 6842 | SCSIO 6842 wild type, containing conjugative plasmid pMBL6842 | (10) |
| ΔpMBL6842 | Plasmid pMBL6842 cured mutant constructed by deleting *repB* gene | This study |
| **Plasmids** | | |
| pMBL6842 | 69.9 kb native plasmid in strain *P. rubra* SCSIO 6842 | (10) |
| Δ*prpT* | Deletion *prpT* in plasmid pMBL6842 | This study |
| Δ*prpAT* | Deletion *prpA-prpT* operon in plasmid pMBL6842 | This study |
| pMBL6842::*cm* | Integration a Cm resistance gene into the intergenic region of AT705_RS24695 and AT705_RS24700 of plasmid pMBL6842 | This study |
| pMBL6842::*cm*Δ*prpT* | Deletion *prpT* in plasmid pMBL6842::*cm* | This study |
| pMBL6842::*cm*Δ*prpAT* | Deletion *prpAT* in plasmid pMBL6842::*cm* | This study |
| pCA24N | Cm$^{R}$; *lacI*$^{q}$, IPTG inducible expression vector in *E. coli* | (23) |
| pCA24N-*prpT* | Cm$^{R}$; *lacI*$^{q}$, P$_{T5\text{-}lac}$::*parE* | This study |
| pCA24N-*prpA* | Cm$^{R}$; *lacI*$^{q}$, P$_{T5\text{-}lac}$::*prpA* | This study |
| pCA24N-*prpAT* | Cm$^{R}$; *lacI*$^{q}$, P$_{T5\text{-}lac}$:: *prpA-prpT* | This study |
| pCA24N-*prpA*$^{1\text{-}54}$ | Cm$^{R}$; *lacI*$^{q}$, P$_{T5\text{-}lac}$::*prpA*$^{1\text{-}54}$ | This study |
| pCA24N-*prpA*$^{55\text{-}86}$ | Cm$^{R}$; *lacI*$^{q}$, P$_{T5\text{-}lac}$::*prpA*$^{55\text{-}86}$ | This study |
| pHGECm | pHGE-P$_{tac}$, Cm$^{R}$, IPTG inducible expression vector in *P. rubra* | (24) |
| pHGECm-*prpT* | Cm$^{R}$; over-expression vector for *prpT* | This study |
| pHGECm-*prpA* | Cm$^{R}$; over-expression vector for *prpA* | This study |
| pHGECm-*prpAT* | Cm$^{R}$; over-expression vector for *prpA-prpT* | This study |

| | | |
|---|---|---|
| pBBR1Ery | $Ery^R$, $Kan^R$; Broad-host-range mobilizable plasmid | (2) |
| pBBR1Ery-*prpA* | $Ery^R$, $Kan^R$; pBBR1Ery containing *prpA* and its own promoter | This study |
| pBBR1Ery-*prpAT* | $Ery^R$, $Kan^R$; pBBR1Ery containing *prpA-prpT* operon and its own promoter | This study |
| pET28b | $Kan^R$, *lacI*$^q$, expression vector | Novagen |
| pET28b-*prpA*-His | $Kan^R$, *lacI*$^q$, pET28b $P_{T7\text{-}lac}$::*prpA* with C-terminal His-tagged | This study |
| pET28b-*prpAT*-His | $Kan^R$, *lacI*$^q$, pET28b $P_{T7\text{-}lac}$::*prpA-prpT* with C-terminal His-tagged for *prpT* | This study |
| pET28b-$N^{FLAG}$-*prpA*-$C^{His}$ | $Kan^R$, *lacI*$^q$, pET28b $P_{T7\text{-}lac}$::*prpA* with C-terminal FLAG-tagged and C-terminal His-tagged | This study |
| pET28b-*prpA*$^{1\text{-}54}$-His | $Kan^R$, *lacI*$^q$, pET28b $P_{T7\text{-}lac}$::*prpA*$^{1\text{-}54}$ with C-terminal His-tagged | This study |
| pET28b-*repB*-His | $Kan^R$, *lacI*$^q$, pET28b $P_{T7\text{-}lac}$::*repB* with C-terminal His-tagged | This study |
| pET28b-$RBS^{prpA}$-$N^{FLAG}$-PrpAT | $Kan^R$, *lacI*$^q$, pET28b $P_{T7\text{-}lac}$::*prpAT* with the RBS of PrpA and a N-terminal FLAG-tagged PrpA | This study |
| pET28b-$RBS^{prpA}$-PrpAT- $C^{FLAG}$ | $Kan^R$, *lacI*$^q$, pET28b $P_{T7\text{-}lac}$::*prpAT* with the RBS of PrpA and C-terminal FLAG-tagged PrpT | This study |
| pHGM01 | $Gm^R$, $Amp^R$, *sacB*, *ori*-R6K, sucide plasmid for generating in-frame deletions | (3) |
| pHGM01-*prpT* | $Gm^R$, pHGM01 containing the homologous arms of *prpA* | This study |
| pHGM01-*prpAT* | $Gm^R$, pHGM01 containing the homologous arms of *prpA-prpT* operon | This study |
| pHGM01-*cm* | $Gm^R$, $Cm^R$, pHGM01 containing a Cm resistance gene with the intergenic region between AT705_RS24695 and AT705_RS24700 as the homologous arms | This study |
| pHGM01-*repB* | $Gm^R$, pHGM01 containing the homologous arms of *repB* | This study |
| pMD19-T | $Amp^R$, *E.coli* cloning vector | Invitrogen |
| pMD19-T-$P_{prpAT}$ | $Amp^R$, pMD19-T containing the promoter region of *prpAT* | This study |
| pMD19-T-$P_{repB}$ | $Amp^R$, pMD19-T containing the promoter region of *repB* | This study |
| pMD19-T-*ori* | $Amp^R$, pMD19-T containing 397 bp *ori* region | This study |
| pMD19-T-*ori*-GC | $Amp^R$, pMD19-T containing 397 *ori* where A/T was mutated to G/C in PrpA binding motif | This study |
| | | |
| pKT25 | $Kan^R$, encoding T25 fragment | (13) |
| pUT18C | $Amp^R$ encoding T18 fragment | (13) |
| pKT25-*prpT* | *prpT* was fused to the C termini of adenylate cyclase in pKT25 | This study |
| pKT25-*prpA* | *prpA* was fused to the C termini of adenylate cyclase in pKT25 | This study |
| pUT18C-*prpA* | *prpA* was fused to the C termini of adenylate cyclase in pUT18C | This study |
| pUT18C-*prpA*$^{6\text{-}86}$ | 6-86 aa of *prpA* was fused to the C termini of adenylate cyclase in pUT18C | This study |

| | | |
|---|---|---|
| pUT18C-*prpA*$^{1\text{-}54}$ | 1-54 aa of *prpA* was fused to the C termini of adenylate cyclase in pUT18C | This study |
| pUT18C-*prpA*$^{55\text{-}68}$ | 55-86 aa of *prpA* was fused to the C termini of adenylate cyclase in pUT18C | This study |
| pHGR01 | Kan$^{R}$, R6K *ori*, promoterless-*lacZ* reporter vector | (26) |
| pRBS$^{prpA}$ | Fuse *prpAT* promoter with *lacZ* in pHGR01 | This study |
| pRBS$^{prpT}$ | Replace the RBS$^{prpA}$ with RBS$^{prpT}$ in pRBS$^{prpA}$ | This study |
| pHGR01-P$_{repB}$ | Fuse *repB* promoter with *lacZ* in pHGR01 | This study |

[a]Gm$^{R}$, Cm$^{R}$, Kan$^{R}$, Ery$^{R}$ and Amp$^{R}$ indicate gentamicin, chloramphenicol, kanamycin, erythrocin and ampicillin resistance, respectively.

**Table S3.** Oligonucleotides used for the construction of plasmids, for mutant strains, and for DNA sequencing. F indicates the forward primer and R indicates the reverse primer. Restriction enzyme sites and vector sequence for one-step recombination are underlined.

| Purpose/Primer name | Sequence (5'-3') | Purpose |
|---|---|---|
| | **Primers used for TA characterization** | |
| pHGECm-PrpA-F | CCCGGAATTCATGTCATCTCGAACTATGACGGTT | pHGECm-*prpA*, pHGECm-*prpT* or pHGECm-*prpAT* |
| pHGECm-PrpA-R | CCGCTCGAGTTAGCCACCACGCGGGCCTTTTTG | |
| pHGECm-PrpT-F | CCGGAATTCGTGGTGGCTAATACTCTGGTCCTCA | |
| pHGECm-PrpT-R | CCGCTCGAGTCACACATGACGTGGGTAGTCCATG | |
| pBBR1Ery-PrpA-F | CCGGAATTCAACGTGCTCATTGTGTTAGGGCTG | pBBR1Ery-*prpA* or pBBR1Ery-*prpAT* |
| pBBR1Ery-PrpA-R | CCGCTCGAGTTAGTGATGGTGATGGTGGTGGCCACCACGCGGGCCTT | |
| pBBR1Ery-PrpAT-R | CCGCTCGAGTCAGTGATGGTGATGGTGGTGCACATGACGTGGGTAGT | |
| pCA24N-PrpA-F | ATACGGATCCGGCCCTGAGGATGTCATCTCGAACTATGACG | pCA24N-*prpA*, pCA24N-*prpT* and pCA24N-*prpAT* |
| pCA24N-PrpA-R | ACCCTTAGCGGCCGCATAGGTTAGCCACCACGCGGGCCTTTTTGC | |
| pCA24N-PrpT-F | ATACGGATCCGGCCCTGAGGGTGGTGGCTAATACTCTGGTCCTCA | |
| pCA24N-PrpT-R | ACCCTTAGCGGCCGCATAGGTCACACATGACGTGGGTAGTC | |
| | **Primers used for promoter activity assays** | |
| pRBS$^{prpA}$-F | CCGGAATTCCCATATTACATATCTCACCACG | pRBS$^{prpA}$ or pRBS$^{prpT}$ |
| pRBS$^{prpA}$-R | CCCAAGCTTGTAATCATGGTCATACTGAGCCTCTTTTCTTGTCA | |
| pRBS$^{prpT}$-R | CCCAAGCTTGTAATCATGGTCACCACGCGGGCCTTTTTGCCTCATACTGAGCCTCTTTTCTTGTCA | |
| pHGR01-P$_{repB}$-F | CCGGTAGTCAATAAACCGGTGCACTGAGGTGAAAAATATGCG | pHGR01-P$_{repB}$ |
| pHGR01-P$_{repB}$-R | AGTGCCAAGCTTGTCGACGGATCAATCATGGTCATTTTACTACACAATATCCATTG | |
| pCA24N-PrpA-F | ATACGGATCCGGCCCTGAGGATGTCATCTCGAACTATGACG | pCA24N-*prpA*$^{1-54}$ |
| pCA24N-PrpA$^{1-54}$-R | ACCCTTAGCGGCCGCATAGGTTAGCGCAATGCTGCCAGTTTAGAC | |
| pCA24N-PrpA$^{55-86}$-F | ATACGGATCCGGCCCTGAGGATGCAGCTCATTGATGAAGGTGAG | pCA24N-*prpA*$^{55-86}$ |

| | | |
|---|---|---|
| pCA24N-PrpA-R | ACCCTTAGCGGCCGCATAGGTTAGCCACCACGCGGGCCTTTTTGC | |
| | **Primers for protein expression and purification** | |
| pET28b-PrpA-F | TAACTTTAAGAAGGAGATATACATGTCATCTCGAACTATGACGGT | pET28b-*prpA*-His, pET28b-*prpA*$^{1\text{-}54}$-His and pET28b-*prpAT*-His, pET28b-N$^{FLAG}$-*prpA*-C$^{His}$ |
| pET28b-PrpA-His-R | GATGATGGCTGCTGCCCATGTTAGTGATGGTGATGGTGGTGGCCACCACGCGGGCCTTT | |
| pET28b-PrpA$^{1\text{-}54}$-His-R | GATGATGGCTGCTGCCCATGTTAGTGATGGTGATGGTGGTGGCGCAATGCTGCCAGTTT | |
| pET28b-PrpT-His-R | GATGATGGCTGCTGCCCATGTCAGTGATGGTGATGGTGGTGCACATGACGTGGGTAGTC | |
| pET28b-FLAG-*prpA*-F | TTAAGAAGGAGATATACATGGATTACAAGGATGACGACGATAAGTCATCTCGAACTATG ACGGT | |
| pET28b-RepB-F | TTAAGAAGGAGATATACATGATAAGAAAAACTGTGACTC | pET28b-*repB*-His |
| pET28b-RepB-R | GATGATGGCTGCTGCCCATGTCAGTGATGGTGATGGTGGTGCTTCTTAACCAGCTGTTC | |
| pET28b-RBS$^{prpA}$-N$^{FLAG}$-PrpAT-F1 | ATGGATTACAAGGATGACGACGATAAGTCATCTCGAACTATGACGGTTG | pET28b-RBS$^{prpA}$-N$^{FLAG}$-PrpAT |
| pET28b-RBS$^{prpA}$-N$^{FLAG}$-PrpAT-F2 | TGACAAGAAAAGAGGCTCAGTATGGATTACAAGGATGACGAC | |
| pET28b-RBS$^{prpA}$-N$^{FLAG}$-PrpAT-F3 | GTGAGCGGATAACAATTCCCCTTGACAAGAAAAGAGGCTCAGT | |
| pET28b-RBS$^{prpA}$-N$^{FLAG}$-PrpAT-R | GATGATGATGGCTGCTGCCCATGTCACACATGACGTGGGTAGTCCATG | |
| pET28b-RBS$^{prpA}$-PrpAT-C$^{FLAG}$-F1 | TGACAAGAAAAGAGGCTCAGTATGTCATCTCGAACTATGACG | pET28b-RBS$^{prpA}$-PrpAT-C$^{FLAG}$ |
| pET28b-RBS$^{prpA}$-PrpAT-C$^{FLAG}$-F2 | GTGAGCGGATAACAATTCCCCTTGACAAGAAAAGAGGCTCAGT | |
| pET28b-RBS$^{prpA}$-PrpAT-C$^{FLAG}$-R1 | TCACTTATCGTCGTCATCCTTGTAATCCACATGACGTGGGTAGTCCATG | |
| pET28b-RBS$^{prpA}$-PrpAT- | GATGATGATGGCTGCTGCCCATGTCACTTATCGTCGTCATCCTTG | |

| | | |
|---|---|---|
| $C^{FLAG}$-R2 | | |
| **Primers for bacterial two-hybrid (BACTH) assays** | | |
| pKT25-PrpT-F | CGGGGTACCTGCTAATACTCTGGTCCTCAAACCCC | pKT25-*prpT* |
| pKT25-PrpT-R | CGGGGTACCTCACACATGACGTGGGTAGTCCATG | |
| pKT25-PrpA-F | CGGGGTACCTTCATCTCGAACTATGACGGTTGAT | pKT25-*prpA* |
| pKT25-PrpA-R | CGGGGTACCTTAGCCACCACGCGGGCCTTTTTG | |
| pUT18C-PrpA-F | CGGGGTACCGTCATCTCGAACTATGACGGTTGAT | pUT18C-*prpA*, pUT18C-$prpA^{6-86}$, pUT18C-$prpA^{1-54}$ or pUT18C-$prpA^{55-86}$ |
| pUT18C-PrpA-R | CCGGAATTCTTAGCCACCACGCGGGCCTTTTTG | |
| pUT18C-$PrpA^{6-86}$-F | CGGGGTACCGACGGTTGATACCGGTGAAGAATTGC | |
| pUT18C-$PrpA^{1-54}$-R | CCGGAATTCTTAGCGCAATGCTGCCAGTTTAG | |
| pUT18C-$PrpA^{55-86}$-F | CGGGTACCGCGCCAGCTCATTGATGAAGGT | |
| **Primers for identification the replication origin of pMBL6842** | | |
| pRepB1-F | GGGGACAAGTTTGTACAAAAAAGCAGGCTAAAATAGGGGGTCGTACTTACTTTG | pRepB1, pRepB2, pRepB3, pRepB4 or pRepB5 |
| pRepB1-R | GGGACCACTTTGTACAAGAAAGCTGGGTTCACTTCTTAACCAGCTGTTCTTC | |
| pRepB2-mid-R | CACAAATACAGAGGGACAACACCTAAGGTGTAGTGTTGTACGAAT | |
| pRepB2-mid-F | ATTCGTACAACACTACACCTTAGGTGTTGTCCCTCTGTATTTGTG | |
| pRepB3-F | GGGGACAAGTTTGTACAAAAAAGCAGGCTTGGGGTATTTCCTACACCCAGAG | |
| pRepB4-F | GGGGACAAGTTTGTACAAAAAAGCAGGCTGTGTTGTCCCTCTGTATTTGTG | |
| pRepB5-R | GGGACCACTTTGTACAAGAAAGCTGGGTGTGTTCAGGATCAGCACGCTCC | |
| **Primers for probe amplification of EMSA and DNase I footprinting assays** | | |
| *ori*-probe-F | AAAATAGGGGGTCGTACTTAC | Used in Figure 5C-G or Fig 6C |
| *ori*-probe-R | CTAAGGTGTAGTGTTGTACGA | |
| $P_{prpA}$-probe-F | CTTTTCAACCCGTTGTTCATTA | Used in Figure 3D-F or Fig 6C |
| $P_{prpA}$-probe-R | ACTGAGCCTCTTTTCTTGTCAG | |

| | | |
|---|---|---|
| *oriGC*-up-R1 | TATACGCCCCGGGCACTTACAATTTATAGCACTAGATCGATCTG | Mutate A/T to G/C in PrpA binding sites |
| *oriGC*-up-R2 | TACACCATAGAATTATTATATCTATACGCCCCGGGCACTTA | |
| *oriGC*-up-R3 | CCTACACGATGCCGTACAAAGCTACACCATAGAATTATTATATC | |
| *oriGC*-up-F1 | CCCGCGGCGCGCTATAGTGTAGCTTTGTACGGTTTGC | |
| *oriGC*-up-F2 | GCTTTGTACGGCATCGTGTAGGCCCGCGGCGCGCTATAG | |
| **Primers for the construction of deletion mutants in SCSIO 6842** | | |
| prpAT-up-F | GGGGACAAGTTTGTACAAAAAAGCAGGCTCACAAGGCACCCTGATACTGGAC | pHGM01-*prpA* or pHGM01-*prpAT* |
| prpAT-up-R | GGTCCGGGTTCGCTATCTATACTGAGCCTCTTTTCTTGTCAG | |
| prpAT-down-F | ATAGATAGCGAACCCGGACCCGTCCCCCGACTGCGGGCCCTA | |
| prpAT-down-R | GGGACCACTTTGTACAAGAAAGCTGGGTGATCTCCTCGTTGCCTCTGTCC | |
| prpT-down-F | GGTCCGGGTTCGCTATCTATTTAGCCACCACGCGGGCCTTTTTG | |
| Cm-up-F | GGGGACAAGTTTGTACAAAAAAGCAGGCTCAGTTTCACAGTTATGTGTTG | pHGM01-*cm* |
| Cm-up-R | GTGATCTTCCGTCACAGGTAGTCAATTCATTCAACCGTTG | |
| Cm-F | CAACGGTTGAATGAATTGACTACCTGTGACGGAAGATCAC | |
| Cm-R | TTAATTAAATCAAACGCTTATTACGCCCCGCCCTGCCACT | |
| Cm-down-F | AGTGGCAGGGCGGGGCGTAATAAGCGTTTGATTTAATTAA | |
| Cm-down-R | GGGACCACTTTGTACAAGAAAGCTGGGTGAGACACCTTTAATATCCTG | |
| RepB-up-F | GCTCTAGATGGCATCGAGGTGGACTT | pHGM01-*repB* |
| RepB-up-R | CCGGAATTCGCAACGGGTTACGGAGGT | |
| RepB-down-F | CCGGAATTCACAAATACAGAGGGACAAC | |
| RepB-down-R | CCCAAGCTTTTTCATATCCGTAAGCAG | |
| **Primers for verification of the constructed plasmids and deletion mutant strains** | | |
| pHGECm-F | CACCTCGCTAACGGATTCACC | |
| pHGECm-R | ACACTACCATCGGCGCTACG | |
| pCA24N-F | GATAACAATTTCACACAGAATT | |

| | |
|---|---|
| pCA24N-R | GTCAGAGGTTTTCACCGTCATCA |
| pHGR01-F | CGTCAATTATTACCTCCACG |
| pHGR01-R | GTGCTGCAAGGCGATTAAG |
| pET28b-F | TAATACGACTCACTATAGGG |
| pET28b-R | TATGCTAGTTATTGCTCAG |
| pKT25-F | CGCATCTGTCCAACTTCCGC |
| pKT25-R | CGCCAGGGTTTTCCCAGTCA |
| M13F | CGCCAGGGTTTTCCCAGTCACGAC |
| M13R | AGCGGATAACAATTTCACACAGGA |
| pUT18C-F | GCGAGGGCTATGTCTTCTACG |
| pUT18C-R | GGGCTGGCTTAACTATGCGG |
| *prpAT*-con-F | CTGCAAGCCTGTTCAACGCCAC |
| *prpAT*-con-R | GCCATATTCTCTGTGCTATTGG |
| Cm-con-F | CTATGGATAAACCGTTCGAC |
| Cm-con-R | GTCCGAGGTGATGCGATTTG |
| **Primers used for qPCR** | |
| qgyrB-F | CAAGCAACGCAACAGGTGAT |
| qgyrB-R | CACCCGGGTTTTCCAGTAGA |
| qrep-F | CTGTCAACCCAGCACCATGT |
| qrep-R | AATGGCTTTTTTGCGGTTGT |

**S1 References**


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