Supplementary Information:

GRIL-Seq, a method for identifying direct targets of bacterial small regulatory RNA by *in vivo* proximity ligation

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Supplementary Note 1.

Identification of positively regulated genes by *in vivo* RNA ligation

A subgroup of sRNAs activates translation by base pairing with certain mRNAs and changing the translational inhibitory structures at their 5' UTRs[1, 2]. The consequence of this interaction is stabilization of the sRNA-mRNA complex and release of the sequestered ribosome-binding site. Unlike the mRNAs negatively regulated by a repressor sRNA, the 3' ends of these mRNAs seem to be more stable to the endonucleolytic cleavage, presenting ligatable 3' OH groups at the naturally-terminated 3' ends, raising the question whether the GRIL-Seq method can be used to detect sRNA targets of positively-regulated genes. Therefore, we investigated whether we could detect chimeric ligation products formed between an activating sRNA and its target mRNA.

We tested the sRNA-mRNA ligation method in *E. coli* where the sRNA RyhB controls iron homeostasis in an analogous way to PrrF1 and PrrF2 in *P. aeruginosa*[3-7]. In addition to controlling the repression of several iron-regulated genes, RyhB has also been shown to activate the expression from the self-inhibited *shiA* transcript, encoding shikimate permease, through a base-pairing mechanism[6]. This allowed us to evaluate the ability of expressed T4 RNA ligase to catalyze formation of chimeric RNA molecules between sRNAs and positively regulated mRNAs.

We constructed a strain of *E. coli* carrying plasmid pKH13-*t4rnl1* (encoding the T4 RNA ligase) and pKH6-RyhB expressing the RyhB sRNA. Based on previously published work defining the regulatory range of this sRNA[3], we first tested the *in vivo* ligation reaction in a T4 RNA ligase-expressing *E. coli* by detecting the chimeric RNAs consisting of RyhB and two of its known targets (*sodB* and *sdhD*). Following induction of expression of the T4 RNA ligase and RyhB, we analyzed the chimeric products using specific primers for the target and two non-target transcripts (*efp* and *rspT*). Notably, the ligated products were only detected with primers specific for *sodB* and *sdhD* (**Supplementary Fig. 4a**). We next examined whether RyhB could be linked to the positively regulated target *shiA* mRNA (**Supplementary Fig. 4b**). Using RyhB-binding forward primer and *shiA* binding reverse primer, the ligated products were detected. Amplicons corresponding to the chimeras were excised, cloned, and sequenced to identify the RyhB-mRNA junctions (**Supplementary Fig. 3b and 3c**). As depicted schematically in **Supplementary Fig. 4c,** ligation products between the 5' end of RyhB and three closely linked 3'-OH sites in *sodB* and *sdhD* were detected. Three different ligation products were identified as

chimeras between *shiA* and RyhB. Interestingly, they are the result of internal cleavages in both of RyhB and *shiA*: a cleavage at 60 nt of RyhB created a substrate for ligation to *shiA* in the 5' UTR (at -65) while the cleavage at position 61 of RyhB allowed for ligations at -64 and -65 relative to the start of transcription of *shiA*. From these experiments we conclude that the GRIL-Seq method is suitable for the identification of sRNA targets in different microorganisms, involving base-pairing interactions between sRNAs and positively as well as negatively regulated mRNAs.

Supplementary Note 2.

Regulatory consequences of mutations in the region of PrrF1 predicted to base-pair with *sodB* and *gloA1*.

In order to confirm the predicted base-paring between sRNA and its targets, we created mutations in PrrF1 and compensatory mutations in the base-paring region of *sodB* and *gloA1*. We the assessed the regulatory effect on target gene expression when sRNA was mutated in the predicted base-paring region. We created six mutations in PrrF1 (**Supplementary Fig. 10a and b**) and determined their effect on the *sodB-lacZ* and *gloA1-lacZ* reporter constructs as well as on the expression of the SodB-6His and GloA1-6His. We detected variable loss of regulation of target gene expression when sRNA was mutated in the predicted base-paring region. For *sodB*, substitutions M2, M2.5, M3 and to a lesser extent M1 and M1.5 resulted in an increase in the expression of the *lacZ* reporter construct and the His-tagged protein providing support for the assignment of the *sodB*-PrrF1 core base-pairing region within the RNA duplex based on the hybridization model. Mutation M4 probably does not base pair with its target, since it did not cause an alteration in the expression of *sodB* (**Supplementary Fig. 10c**). For GloA1, mutations M1, M1.5 located in the base-paring region of the two RNA molecules abolished the regulatory effect of PrrF1, as did the mutation M2.5, which is not predicted to base pair (**Supplementary Fig. 10d**). All other mutations are located outside the base-pairing region and these did not lead to alterations in the levels of *gloA1* expression. We confirmed the base-pairing for M2 and *sodB* and M1.5 and *gloA1* by engineering compensatory mutations, M2' and M1.5', respectively (**Supplementary Fig. 10e and f**). When base-pairing was restored the expression of both targets was once again subject to regulation by the corresponding PrrF1 mutant. Therefore, predictions

of base-pairing near the ligation junctions identified by GRIL-Seq can predict likely sites of regulatory interactions between sRNA and its target.

Supplementary Note 3.

Interaction of PrrF1 with a fragment the 3' end of *katA* mRNA

GRIL-Seq analysis of the PrrF1 chimeras revealed that the *katA* transcript, encoding the catalase protein, was ligated to PrrF1 near the 3' end of the mRNA with minor ligation products detected near its 5' end (**Supplementary Fig. 11a**). Interestingly, the chimera at the 3' end of *katA* mRNA is formed by the ligation of the PrrF1 fragmented at position +80 (**Supplementary Fig. 11c**). The location of the junctions within the chimera and ρ-independent terminator of *katA* mRNA shows that the *katA* derived portion is ~140 nt (**Supplementary Figs. 11b**) which is similar in size to a form of *katA* detected in *P. aeruginosa* when cultures approach stationary phase of growth (**Supplementary Fig. 11d**); we refer to this short form of the transcript as *skatA*. Previous mapping of transcription start sites[8] identified only a single promoter for the *katA* gene and no internal starts; we therefore conclude that *skatA* is a stable fragment derived from the *katA* mRNA by an endonucleolytic cleavage event. Growth of *P. aeruginosa* in media of limited iron availability (accomplished by treating the iron chelator 2,2'-dipyridyl; DIP+) leads to a strong induction of PrrF1 and PrrF2, and these conditions show minimal effects on *skatA* levels (**Supplementary Fig. 11e**). Moreover, since *skatA* can be detected even in the absence of PrrF1-F2, albeit at somewhat reduced levels, these results also indicate that *skatA* is derived from the full-length *katA* mRNA by a likely nucleolytic process not involving interaction with the sRNA. Relatively stable predicted base paring between *skatA* and PrrF1 (ΔG = -25.6 kcal/mol, IntaRNA) suggests that they interact directly with each other in an RNA duplex (**Supplementary Fig. 11f)**.

To further investigate the consequences of this atypical interaction at the 3' end of the *katA* transcript we constructed a plasmid expressing an inducible form of *skatA* (corresponding to the 147 nucleotides of the wild-type *katA* sequence) and one with a mutation in the region of base pairing, referred to as *skatA*(M), (**Supplementary Fig. 11f).** These plasmids, together with the empty vector, were introduced into *P. aeruginosa* and the effect of *skatA*, on PrrF1 regulation of *katA* was assessed following depletion of iron by addition of the chelator 2,2'-dipyridyl (DIP) (**Supplementary Fig. 11g, top panel**). Induction of PrrF1 through iron limitation for as little as

20 minutes led to significant reduction in *katA* transcript levels (**Supplementary Fig. 11g**, Vec lanes). Expression of *skatA* had a modest effect on the overall levels of *katA* mRNA, however it was more stable and showed less than 50% decay after 20 minutes (**Supplementary Fig. 11g**, *skatA* lanes). In contrast, expression of *katA*(M) with the mutation in the predicted PrrF1 (and PrrF2) base-pairing region failed to stabilize the *katA* mRNA (**Supplementary Fig. 11g**, *skatA* (M) lanes); it decayed at a rapid rate comparable to the one seen with the empty vector. These results suggest that the base-pairing interaction between PrrF1 (and very likely, PrrF2) and *skatA* influences the stability of the full-length *katA* mRNA. We have previously shown that a translational *lacZ* fusion, containing only the first 20 codons of *katA* was subject to negative regulation by PrrF1 (**Supplementary Fig. 9b**). Therefore, sequestration of PrrF1-PrrF2 by *skatA* leads to an interference with their negative regulatory function at the 5' binding site of *katA*.

Base pairing between PrrF1-PrrF2 and *skatA* should also reduce the overall availability of these sRNAs to regulate their other targets. Indeed, similar effects of the wild type *skatA* and the mutant form were observed when probing for the *sodB* transcript (**Supplemental Fig. 11g,** second panel). As was seen with *katA*, the s*odB* mRNA was stabilized by overexpression of *skatA* and this effect depended on intact base-paring sequence on *skatA*.

Results presented here provide another example of regulatory functions associated with mRNA-derived fragments. This mechanism resembles the activity of the SroC sRNA sponge, derived by processing of the *gltI* mRNA and controlling the translation/stability of amino acid biosynthesis and transporter mRNAs by sequestering the GcvB sRNA[9]. Another mRNA-derived endonucleolytic product is CpxQ, an sRNA corresponding to the 3' fragment of *cpxP* mRNA; it was shown to repress expression of a number of membrane proteins following envelope stress[10]. Although we cannot exclude the possibility that the 140 nt *skatA* also functions as a base-pairing regulatory sRNA with as yet uncharacterized mRNA targets, our data suggest that it is a sponge modulating PrrF1 (and likely, PrrF2) availability.

Supplementary Note 4.

Ligation of PrrF1 to tRNA precursors

RNA-seq analysis also showed that the levels of two aspartic acid tRNAs (tRNA-asp) were reduced in response to PrrF1 overexpression (**Table. 1** and **Supplementary Table 1**). In *P. aeruginosa*, four genes encode tRNAs with Asp anti-codon sequences: PA1804.1, PA3094.1,

PA3094.2, PA3262.1 and these will be referred to as tRNA-asp[1], tRNA-asp[2], tRNA-asp[3], tRNA-asp[4], respectively. Three tRNAs are valine specific: PA2775.1 (tRNA-val[1]), PA3094.3 (tRNA-val[2]), PA3262.2 (tRNA-val[3]). In a polycistronic arrangement, tRNA-val[2], tRNA-asp[3] and tRNA-asp[2] are transcribed as a single transcript[11], while the tRNA-val[3] is linked to tRNA-asp[4] (**Supplementary Fig. 12a**). Interestingly, our GRIL-Seq results showed extensive ligation of PrrF1 to sites at the 3' ends of tRNA-asp[3] and tRNA-asp[4]; they were observed only when the tRNA-val and tRNA-asp were in the same precursor transcript. A much smaller number of broadly distributed chimeras between PrrF1 and tRNA-val[2] and tRNA-val[3] was detected, and only in the polycistronic transcripts with tRNA-asp. Although sequences of these tRNA-asps are nearly identical (97%), the nucleotide differences adjacent to the 3' end of each tRNA-asp are unique and this allowed correct assignment of the sequencing reads to a specific tRNA-asp (**Supplementary Fig. 13a** and **b**). The ligation of PrrF1 to a few nucleotides beyond the 3'end of each tRNA indicated that the sRNA binds to the unprocessed precursor transcript. Interestingly, we found that the most stable base pairing of PrrF1 was predicted to occur to tRNA-val[2] and tRNA-val[3], but not the unlinked tRNA-val[1] (**Supplementary Fig. 12c**). This sequence (shown in **Supplementary Fig. 12d**) occurs only in tRNA-val[2], in the un-processed precursor tRNA-asp[2] and tRNA-asp[3], and in tRNA-val[3], transcribed as a polycistronic transcript with tRNA-asp[4]. Therefore, base pairing with either tRNA-val[2] or -val[3], in the precursor transcript leads to processing at the adjacent tRNA-asp[2], tRNA-asp[3] and tRNA-asp[4]. The hypothesis that the processing at the 3'end of the tRNA-asps requires a linked sequence containing the base-pairing region (tRNA-val[2] and -val[3]) for PrrF1 is supported by the observation that no chimeras were detected containing tRNA-asp[1] whose gene is not linked to any tRNA-val, in spite of its 97% identity with tRNA-asp[2], -asp[3], and -asp[4]. Moreover, tRNA-val[1], transcribed as a monocistronic transcript, was unlikely to ligate to PrrF1 and lacks the predicted PrrF1 base-pairing region (**Supplementary Fig. 12d**). These results suggest that PrrF1 appears to specifically bind to two valine tRNA (val[2] and val[3]) following transcription of the polycistronic RNA. Moreover, the most frequent ligation sites (~95%) on tRNA-asps in chimeric reads were not at the 3' end of the mature CCA sites of the tRNAs (**Supplementary Fig. 13b**). Instead, they occurred at one to four nucleotides downstream of the end of the CCA sites, implying that the precursor tRNA forms are likely favorable for this ligation before exonucleases trim these sequences. Consequently, they

cannot function as amino acid acceptors and this may explain their enhanced sRNA-facilitated turnover.

The discovery of a regulatory role for tRNA fragments in interactions with sRNAs has been previously reported[12], where a 3' external spacer portion of the glyW-cysT-leuZ polycistronic tRNA precursor is targeted by the RyhB and RybB sRNAs. The sequestration of the sRNAs results in up-regulation of the expression from their mRNA targets. Although we detected a direct effect of PrrF1-tRNA-val interaction on the levels of the two tRNA-asps in RNA-seq analysis, we cannot exclude the possibility that this interaction also affected the availability of PrrF1 to regulate its natural mRNA targets.

Supplementary Note 5.

GRIL-Seq Protocol

A. Library Preparation for Illumina Sequencing

Reagents:

- ☐ Direct-zol™ RNA MiniPrep w/ TRI-Reagent® kit (Zymo Research, cat. no. R2053)
- ☐ Nuclease-free water (Thermo Fisher Scientific, cat. no. AM9930)
- ☐ Agilent RNA 6000 Pico Kit (Agilent Technologies, cat. no. 5067-1513)
- ☐ Agilent DNA 1000 Kit (Agilent Technologies, cat. no. 5067-1504)
- ☐ KAPA SYBR® FAST Universal One-Step qRT-PCR Kit (KAPA BIOSYSTEMS, cat no. KK4651)
- ☐ SuperScript® III First-Strand Synthesis System for RT-PCR (Thermo Fisher Scientific, cat. no. 18080051)
- ☐ 2 X GoTaq® Green Master Mix (Promega, cat. no. M7123)
- ☐ CloneJET PCR Cloning Kit (Thermo Fisher Scientific, cat. no. K1231)
- ☐ MicrobExpress kit (Ambion/Applied Biosystems, cat. no. AM1901)
- ☐ Washing Solution for Oligo MagBeads: See the procedure **6**-3-3) and **6**-3-4) for detailed use

  0.5 M GuSCN, 0.04 M Tris-Cl pH7.5, 0.008 M EDTA, 0.5% Fraction V bovine serum albumin, 0.5% Sodium Lauroryl sacosine, 0.05% Bronopol.

  - GuSCN (Sigma-Aldrich, cat. no. 50983)
  - Tris-Cl pH7.5 (Ambion/Thermo Fisher Scientific, cat. no. 15567-027)
  - EDTA (Ambion/Thermo Fisher Scientific, cat. no. AM9260G)

- Fraction V bovine serum albumin (EMD Millipore, cat. no. 2930 -100GM)
- N-Lauroylsarcosine sodium salt solution (Sigma-Aldrich, cat. no. L7414)
- 2-Bromo-2-nitro-1,3-propanediol (Bronopol) (Sigma-Aldrich, cat. no. 134708)

☐ TURBO DNase (Ambion/Thermo Fisher Scientific, cat. no. AM2239)

☐ DMSO (Sigma-Aldrich, cat. no. D8418)

☐ Actinomycin D (Sigma-Aldrich, cat. no. A1410)

☐ NEBNext® Ultra™ Directional RNA Library Prep Kit for Illumina (New England Biolab, cat. no. E7420S)

☐ NEBNext® Multiplex Oligos for Illumina® (Index Primers Set 1) (New England Biolab, cat. no. E7335S)

☐ Solid Phase Reversible Immobilization (SPRI) Beads: PCRCLEAN DX (Aline Biosciences, cat. no. C-1003-5) as an alternative as Agencourt® AMPure® XP Beads (Beckman Coulter, Inc. #A63881)

☐ UltraPure™ 1M Tris-HCI, pH 7.5 (Thermo Fisher Scientific, cat. no. 15567027)

Oligos:

☐ Primer set for qRT-PCR. See the procedure **4**-1)

The primers pairs targeting T4 RNA ligase (*t4rnl1*) mRNA, sRNA (i.e., PrrF1) and a housekeeping mRNA (i.e., *rpsL*). See the detailed sequences of each primer in **Supplementary Table 5**.

☐ P1: sRNA binding complementary sequences for the reverse transcription (RT). This anneals to the specific sRNA. See the procedure **5**-1).

☐ P2: nested primer upstream of P1 binding site for the PCR after RT. See the procedure **5**-2).

☐ P3: nested primer downstream of P1 binding site for the amplification after RT. See the procedure **5**-2).

☐ Poly dA-tailed sRNA Capture Oligonucleotide: See the procedure **6**-1) to **6**-3) for detailed design and use. 5'- AAAAAAAAAAAAAAAAAA-(sRNA binding complementary sequences) -3'

Bacterial strains:

Bacterial strain contains two compatible plasmids which are able to separately express T4 RNA ligase and sRNA. They are inducible by addition of IPTG (for T4 RNA ligase) and L-arabinose (for sRNA).

Procedure:

1. Cell culture

1) Inoculate the bacterial strain containing the two plasmids (i.e., pKH13-*t4rnl1* and pKH6-PrrF1) grown on LB agar plate with two antibiotics (carbenicillin (150 μg/mL) and gentamicin (75 μg/mL)) into 2 mL LB broth containing the same concentration of antibiotics. Cells were grown overnight (~16 h) at 37 °C with shaking at 300 rpm.
2) Dilute the overnight culture to OD_{600} = 0.01 in 30 mL LB broth containing the two antibiotics (carbenicillin (75 μg/mL) and gentamicin (37.5 μg/mL)). Continue growth of the culture. Pre-warm two flasks (50 mL) and pipettes (10 mL) at 37 °C.
3) When the OD_{600} reaches 0.5-0.6, split the culture into two pre-warmed flasks (10 mL into each). Induce the T4 RNA ligase expression for the Test culture by addition of IPTG to 1 mM. (No induction of the Control culture). Incubate each culture for an additional 1 h.
4) Induce the expression of the sRNA (i.e., PrrF1) in the Test culture by addition of L-arabinose to 0.2%, with no induction of the Control culture. Incubate for 20 min.
5) Harvest cells from 1.6 mL of each culture by centrifugation in a microcentrifuge at 13,000*g* for 1 min.
6) Discard the supernatant and immediately freeze the pellets in liquid nitrogen.
7) Proceed to RNA isolation or save pellets at -80 °C for next step.

2. RNA isolation using Direct-zol™ RNA MiniPrep kit

1) Add 700 μL of TRI Reagent® to each microcentrifuge tube (containing Test or Control sample) and immediately lyse the cells by applying vigorous vortex.
2) Remove particular matter by spinning at 13,000*g* for 1 min.
3) Add 650 μL of cell lysate into 1.5 mL microcentrifuge tubes containing 650 μL of 100% ethanol and mix thoroughly.
4) Transfer the mixture into Zymo-Spin™ IIC Column, in a Collection Tube, and centrifuge at 13,000*g* for 1 min. Discard the flow-through and the collection tube, and then transfer the column into a new collection tube.
5) Add 400 μL of RNA Direct-zol™ RNA PreWash to the column and centrifuge 13,000*g* for 1 min. Discard the flow-through.
6) For in-column DNase I treatment, prepare DNase I master mixture consisting of 8 μL DNase I (2 U/μL), 8 μL 10X Reaction Buffer and 64 μL RNA Wash Buffer, multiplied by the number of samples.

7) Add 80 µL of DNase I mixture into the column and incubate for 30 min at 37 °C.

8) Centrifuge the column at 13,000*g* for 1 min. Discard the flow-through.

9) Add 400 µL of Direct-zol™ RNA PreWash to the column and centrifuge. Discard the flow-through and repeat this step.

10) Add 700 µL of RNA Wash Buffer to the column and centrifuge for 2 minutes to ensure complete removal of the Wash Buffer. Transfer the column carefully into an RNase-free 1.5 mL microcentrifuge tube.

11) Add 40 µL of nuclease-free water and incubate for 3 minutes at room temperature. Centrifuge at 13,000*g* for 3 min and collect the total RNAs in 1.5 mL nuclease-free tube. Keep RNA samples on ice for immediate use, otherwise, storage them at -80 °C.

10) Check the concentration of RNA using Nanodrop 1000.

3. Quality check 1: To determine the RNA Integrity Number (RIN) number.

1) Prepare the samples for Bioanalyzer (Agilent RNA 6000 Pico kit). 2 ng of total RNA was used.

2) Check the RIN number. If the total RNA is showing a RIN number greater than 7, proceed to the next step.

4. Quality check 2: To test whether T4 RNA ligase and sRNA are induced in the bacteria

- One-step qRT-PCR is carried out for the Test and Control sample using KAPA SYBR FAST One-Step qRT-PCR Kits
- In addition to T4 RNA ligase and sRNA, a house-keeping gene (i.e., the ribosomal *rpsL* gene) is also tested as a control.

1) Prepare qPCR master mix: ensure all reaction components are properly thawed and mixed.

- Keep the KAPA RT Mix on ice during use, and assembled reactions on ice to avoid premature cDNA synthesis.
- Calculate the required volume of each component based on the following table:

One-step qRT-PCR mix	µL	Final conc.
RNA template (total RNA, 10 ng/µL)	2	20ng
2X KAPA SYBR FAST qPCR Master Mix	7.5	1x
Forward Primer (2 pmol/µL)	1.5	200 nM
Reverse Primer (2 pmol/µL)	1.5	200 nM
50X KAPA RT Mix	0.3	1x

Nuclease-free water	2.2	N/A
Final volume	15	

2) Program the following cycling protocol:

PCR cycles	Temp (°C)	Duration	Cycles
Reverse Transcription	42	5 min	Hold
Enzyme inactivation	95	3 min	Hold
Denaturation	95	3 sec	40
Annealing/extension/data acquisition	60	30 sec	
Dissociation (melting curve)	According to instrument guidelines		

3) Check the C_t value of each gene. Proceed to the next step if each C_t difference in the expression of T4 RNA ligase and sRNA between the Test and Control sample is more than 4.

5. Quality check 3: To test the efficiency of the *in vivo* ligation reaction.

- Under efficient ligation, some of the ligated sRNAs can be generated as double (5'-sRNA-sRNA-3') or concatameric (5'-sRNA-sRNA-…-sRNA-3') or sandwich (5'-sRNA-other RNAs-sRNA-3') ligated forms. Therefore, when RT-PCR was performed with divergent primers corresponding to near 5' and near 3' of sRNA, the ligation can be verified in Test sample by observing much brighter and higher sizes of PCR bands with some smearing compared with the Control sample (no induction of T4 RNA ligase).

1) Design of the sRNA specific binding oligomer (P1) for the reverse transcription; 19~23 nucleotides in length to give a melting temperature 50~55 °C. The sequence should be complementary to sRNA and bound to downstream of P2 oligo-binding site (i.e. for PrrF1, the P1 primer is listed in **Supplementary Table 5**: P1_R_PrrF1+45)

2) Design of the two primers (P2 and P3) for the PCR. P2 primer binds to upstream of P1 binding site with complementary sequence. The binding upstream is required for specific amplification. P3 primer binds to the downstream region of P1 oligo-binding site with sense sequences (i.e. for PrrF1, the P2 and P3 primer are listed in **Supplementary Table 5**: P2_R_PrrF1+26 and P3_F_PrrF1+62).

3) cDNA synthesis using SuperScript® III First-Strand Synthesis System: 1 μg of each total RNA isolated from Test and Control sample was used for each RT reaction.

Reverse transcription mix	μL

Total RNA (1 µg)	8
sRNA binding Primer (P1, 2 pmol/µL)	1
10 mM dNTP mix	1
Final volume	10

- Incubate the tubes at 65 °C for 5 min, then place on ice for at least 1 min.
- Prepare the following cDNA Synthesis Mix, adding each component in the indicated order:

Reverse transcription mix	µL
10X RT buffer	2
25 mM MgCl2	4
0.1 M DTT	2
RNaseOUT™ (40 U/µL)	1
SuperScript® III RT (200 U/µL)	1
Final volume	20

- Add 10 µL of cDNA Synthesis Mix to each RNA/primer mixture, Mix gently, and collect by brief centrifugation. Incubate for 50 min at 50 °C
- Terminate the reactions by incubating tubes at 85 °C for 5 min. Chill on ice.

4) PCR

PCR mix	µL
cDNA mixture (10% of reaction volume)	2
2 X GoTaq® Green Master Mix	10
F_primer (P3, 10 pmol/ µL)	1
R_primer (P2, 10 pmol/ µL)	1
Nuclease-free water	6
Final volume	20

- PCR Cycling Conditions

PCR cycles	Temp (°C)	Duration	cycles
Initial Denaturation	95	3 min	1
Denaturation	95	25 sec	30
Annealing	55	25 sec	

Extension	72	1 min	
Final Extension	72	5 min	1
Hold	4	∞	1

- Run samples on a 2% agarose gel in 1X TAE buffer.
- Excise and elute the area of the gel containing the desired chimeric DNA fragment (The amplicons from the Test sample typically show as much bright and higher size of PCR bands with some smearing compared to the Control sample).

5) TA-cloning and analysis of the PCR product

The eluted amplicons from the Test sample are cloned into CloneJET PCR Cloning Kit (ThermoFisher Scientific) as recommended by manufacturer's instructions and colony PCR is carried out with pJET1.2 Forward and Reverse primer sets. The inserts are sequenced using the pJET1.2 Reverse primer.

6) Analyze the sequencing results. Precede next step if the ligated products of sRNA are detected.

6. sRNA enrichment using a modification of the MicrobExpress kit (Ambion)

1) Design the poly dA-tailed sRNA Capture Oligonucleotide:

1-1) Generate a secondary RNA structure of the sRNA using the mfold algorithm (http://mfold.rna.albany.edu/?q=mfold/RNA-Folding-Form) and choose an unstructured 23 nucleotide DNA sequence (complementary to the sRNA), to give a melting temperature between 55 and 61 °C and a GC content of 48 to 65% using the oligo calculator (http://mcb.berkeley.edu/labs/krantz/tools/oligocalc.html).

1-2) Add an additional sequence of 18 dAs to the 5' end of the designed sRNA-binding DNA sequence (make sure that the sequences are complementary to the sRNA). For example, 5'-AAAAAAAAAAAAAAAAAA-(sRNA binding complementary sequences)-3'.

2) Dilute the primer stock (100 pmol/μL) to 20 pmol/μL with 10 μL of nuclease-free water.

3) Follow the manufacturer's instructions in the MicrobExpress kit with the following modifications:

For the stringent washing of MagBeads, the Wash Solution is freshly made according to the previous report[13] and 300 μL is used for each wash (for a total of 3 washes).

3-1) RNA denaturation and hybridization of chimeric sRNA with the capturing oligo:

- Mix total RNAs (10 μg, 14 μL) from the Test sample and 1 μL of sRNA capture oligo (20 pmol/μL).
- Add 200 μL of Binding Buffer and vortex gently.

- Incubate the mixture at 70 °C for 15 minutes to unfold any secondary structures in the RNA and oligonucleotides. Move the mixture to a 37 °C water bath and incubate for 60 min. This will be referred to as the RNA/Capture Oligo Mix.

3-2) Preparation of oligo-dT attached Magnetic Beads (referred to as Oligo MagBeads).

- For each RNA sample, pipette 50 µL of Oligo MagBeads per sample, into a 1.5 mL microcentrifuge tube. For example, withdraw 200 µL for 4 samples (50 µL x 4 samples)
- Capture the Oligo MagBeads by placing the tube on a magnetic stand. Leave the tube on the stand until all of the Oligo MagBeads are arranged inside the tube near the magnet. This will take ~3 min.
- Carefully remove the supernatant by aspiration, leaving the beads in the tube, and discard the supernatant.
- Add nuclease-free water to the captured Oligo MagBeads; use a volume of nuclease-free water equal to the original volume of the Oligo MagBeads.
- Remove the tube from the magnetic stand, and resuspend the beads by brief, gentle vortexing.
- Recapture the Oligo MagBeads with a magnetic stand, and carefully aspirate and discard the nuclease-free water leaving the beads in the tube.
- Add Binding Buffer to the captured Oligo MagBeads; use a volume of Binding Buffer equal to the original volume of the Oligo MagBeads.
- Remove the tube from the magnetic stand, and resuspend the beads by brief, gentle vortexing.
- Recapture the Oligo MagBeads with a magnetic stand, and carefully aspirate and discard the Binding Buffer leaving the beads in the tube.
- Add fresh Binding Buffer to the captured Oligo MagBeads; use a volume of Binding Buffer equal to the original volume of Oligo MagBeads.
- Remove the tube from the magnetic stand, and resuspend the beads by gently tapping the tube or very gentle vortexing.
- Place the Oligo MagBead slurry in a 37 °C incubator, and allow the temperature to equilibrate to 37 °C before proceeding.

3-3) Capture of the chimeric sRNAs

- Gently vortex the tube of washed and equilibrated Oligo MagBeads to resuspend them, and add 50 µL of Oligo MagBeads to the RNA/Capture Oligo Mix.
- Very gently vortex or tap the tube to mix and microfuge very briefly to get the mixture to the bottom of the tube.
- Incubate 15 min at 37 °C.

- Aliquot the Wash Solution into 2 mL tube considering the required volume for each washing step (for each sample, 900 µL (300 µL x 3 time) is required) and preheat them at 37 °C, which will be used in washing step.

3-4) Wash the Oligo MagBeads with the Wash Solution

- Capture the Oligo MagBeads by placing the tube on the Magnetic Stand.
- Aspirate the supernatant and discard it. Do NOT discard the Oligo MagBeads.
- Wash the beads by adding 300 µL pre-warmed (37 °C) Wash Solution and gently pipetting beads (Do NOT vortex it).
- Place the beads on the Magnetic Stand and wait for 3 min until the solution is clear.
- Discard the supernatant, being careful not to dislodge the Oligo MagBeads.
- Repeat the wash two more times (total three times).
- Completely remove the washing solution and elute from beads using DNase.

3-5) Elution of Chimeric sRNA from MagBeads with DNase

- To elute the captured RNA, prepare a DNase mixture: prepare the following master mixture considering the number of samples and aliquot to 50 µL master mixture into each bead.

DNase Mix	µL
10X TURBO DNase Buffer	5
TURBO DNase (2 U/µL)	1.5
Nuclease-free water	43.5
Final volume	50

- Mix gently by pipetting beads (do NOT vortex) and transfer to 0.5 mL tube. Incubate at 37 °C for 25 min.
- Add 5 µL of the DNase Inactivation Reagent and incubate at room temperature for 5 min, mixing occasionally.
- Centrifuge them at 10,000*g* for 2 min and transfer 47 µL of captured RNAs into 1.5 mL collection tube (MicrobExpress kit). Add 153 µL of nuclease-free water to 200 µL.
- Precipitate by adding 4 µL of glycogen (5 mg/mL), 20 µL (1/10 volume) of 3 M sodium acetate and 500 µL of 100% ethanol. Keep it at -80 °C for more than 3 h or overnight.

7. Library preparation for Illumina sequencing with enriched chimeric RNAs

1) Recovery of enriched chimeric RNAs

- Centrifuge the precipitated samples at 21,000*g* (4 °C) for 10 min.
- Discard the supernatant and add 500 µL of 75% ethanol to wash the pellets. Centrifuge again at 4 °C for 5 min.
- Completely remove the supernatant and dry the pellet with speed vacuum.
- Add 15 µL of nuclease-free water and check the concentration with Nanodrop.

2) Quality Check: To test whether the sRNA is enriched or not

- One-step qRT-PCR is carried out for the enriched sRNA (5~20 ng is used) as shown in the procedure **4**-1).

3) Follow the protocol for use with Purified mRNA or Ribosome Depleted RNA (Chapter 3) in NEB Next® Ultra™ Directional RNA Library Prep Kit (cat. no. E7420S) with following modifications:

- As a SPRI bead, PCRCLEAN DX beads was used instead of AMPure XP beads
- In PCR Library Enrichment step, 16 cycles were carried out.

3-1) RNA Fragmentation, Priming and First Strand cDNA Synthesis

- Color dots indicate the cap color of the reagent to be added to a reaction.

RNA Fragmentation mix	µL
enriched chimeric RNAs (100 ng)	5
● NEBNext First Strand Synthesis Reaction Buffer (5X)	4
● Random Primers	1
Final volume	10

- Color dots indicate the cap color of the reagent to be added to a reaction.
- Incubate the sample at 94 °C for 15 min. Place it on ice.

3-2) First Strand cDNA Synthesis

- Prepare a concentrated stock solution of Actinomycin D (5 µg/µL) in DMSO
- Dilute the Actinomycin D stock solution to 0.1 µg/µL with nuclease-free water.
- Mix the following components

cDNA synthesis mix	µL
● Murine RNase Inhibitor	0.5
Actinomycin D (0.1 µg/µL)	5
Nuclease-free water	3.5
● ProtoScript II Reverse Transcriptase	1

Final volume	20

- Incubate the samples in a preheated thermal cycler (with the heated lid set at 105 °C) using the following protocol:

  10 minutes at 25 °C

  15 minutes at 42 °C

  15 minutes at 70 °C

  Hold at 4 °C

3-3) Second Strand cDNA Synthesis

- Add the following components (total 60 µL) to the First Strand Synthesis reaction (20 µL) and mix thoroughly by gentle pipetting.

Second Strand cDNA Synthesis mix	µL
Nuclease-free water	48
● Second Strand Synthesis Reaction Buffer (10X)	8
● Second Strand Synthesis Enzyme Mix	4
Final volume	80

- Incubate in a thermal cycler for 1 hour at 16 °C, with heated lid set at ≤ 40 °C

3-4) Purify the Double-stranded cDNA using SPRI Beads (PCRCLEAN DX)

- Vortex PCRCLEAN DX Beads to resuspend.
- In a 1.5 mL microcentrifuge tube, combine 144 µL (1.8X) of resuspended PCRCLEAN DX Beads and the second strand synthesis reaction (~80 µL). Mix well using a vortex mixer or by pipetting up and down at least 10 times.
- Incubate for 5 minutes at room temperature.
- Quickly spin the tube in a microcentrifuge to collect any sample on the sides of the tube. Place the tube on an appropriate magnetic rack to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant without disturbing the beads that contain DNA targets.
- Add 200 µL of freshly prepared 80% ethanol to the tube while on the magnetic rack. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
- Repeat once more for a total of 2 washing steps.
- Air dry the beads for 5 minutes while the tube is on the magnetic rack with lid open. Caution: Do not over dry the beads. This may result in lower recovery of DNA.

- Remove the tube from the magnet. Elute the DNA from the beads into 60 µL 10 mM Tris-HCl. Mix well by pipetting up and down. Quickly spin the tube and incubate for 2 minutes at room temperature. Place the tube in the magnetic rack until the solution is clear.
- Remove 55.5 µl of the supernatant and transfer to a clean nuclease-free PCR tube.

3-5) End Prep of cDNA Library

- Mix the following components.

Second Strand cDNA Synthesis mix	µL
Purified double-stranded cDNA	55.5
🟢 NEBNext End Repair Reaction Buffer (10X)	6.5
🟢 NEBNext End Prep Enzyme Mix	3
Final volume	65

- Incubate the sample in a thermal cycler (with the heated lid set at 75 °C) as follows:

  30 minutes at 20 °C

  30 minutes at 65 °C

  Hold at 4 °C

3-6) Adaptor Ligation

- Dilute the 🔴 (red) NEBNext Adaptor for Illumina (15 µM, cat. no. E7335) to 1.5 µM with a 10-fold dilution (1:9) with 10 mM Tris-HCl.

Adaptor Ligation	µL
End Prep Reaction	65
🔴 Blunt/TA Ligase Master Mix	15
Diluted NEBNext Adaptor	1
Nuclease-free water	2.5
Final volume	83.5

- Do not pre-mix the components to prevent formation of adaptor dimers.
- Mix by pipetting followed by a quick spin to collect all the liquid from the sides of the tube.
- Incubate for 15 minutes at 20 °C in a thermal cycler

3-7) Purify the Double-stranded cDNA using SPRI Beads (PCRCLEAN DX)

- Add nuclease-free water (16.5 µL) to the ligation reaction to bring the reaction volume to 100 µL. It is important to ensure the final volume is 100 µL prior to adding PCRCLEAN DX Beads.
- Vortex PCRCLEAN DX Beads to resuspend.

- Add 100 µL (1.0X) of the resuspended PCRCLEAN DX Beads to the ligation reaction (100 µL). Mix well on a vortex mixer or by pipetting up and down at least 10 times.
- Incubate for 5 minutes at room temperature.
- Quickly spin the tube in a microcentrifuge to collect any sample on the sides of the tube. Place the tube on an appropriate magnetic rack to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
- Add 200 µL of freshly prepared 80% ethanol to the tube while in the magnetic rack. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
- Repeat once more for a total of 2 washing steps.
- Air dry the beads for 5 minutes while the tube is on the magnetic rack with lid open. Caution: Do not over dry the beads. This may result in lower recovery of DNA target.
- Remove the tube from the magnet. Elute the DNA target from the beads with 52 µL of 10 mM Tris-HCl. Mix well on a vortex mixer or by pipetting up and down. Quickly spin the tube and incubate for 2 minutes at room temperature. Place the tube in the magnetic rack until the solution is clear.
- Transfer the 50 µL supernatant to a clean PCR tube. Discard beads.
- To the 50 µL supernatant, add 50 µL (1.0X) of the resuspended PCRCLEAN DX Beads and mix well on a vortex mixer or by pipetting up and down at least 10 times
- Incubate for 5 minutes at room temperature.
- Quickly spin the tube in a microcentrifuge and place the tube on an appropriate magnetic rack to separate beads from the supernatant.
- After the solution is clear (about 5 minutes), discard the supernatant that contains unwanted fragments (Caution: do not discard the beads).
- Add 200 µL of freshly prepared 80% ethanol to the tube while in the magnetic rack. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
- Repeat once more for a total of 2 washing steps.
- Briefly spin the tube, and put the tube back in the magnetic rack.
- Completely remove the residual ethanol, and air-dry beads for 5 minutes while the tube is on the magnetic rack with the lid open.
- Remove the tube from the magnet. Elute DNA target from the beads with 19 µL 10 mM Tris-HCl. Mix well on a vortex mixer or by pipetting up and down; incubate for 2 minutes at room temperature. Put the tube in the magnetic rack until the solution is clear.

- Without disturbing the bead pellet, transfer 17 µL of the supernatant to a clean PCR tube and proceed to PCR enrichment.

3-8) PCR Enrichment of Adaptor Ligated DNA

- To the cDNA (17 µL), add the following components and mix by gentle pipetting:

PCR Enrichment reaction mix	µL
🔵 NEBNext USER Enzyme	3
🔵NEBNext Q5 Hot Start HiFi PCR Master Mix	25
🔵Index (X) Primer/i7 Primer (10 µM), NEB #E7335	2.5
🔵Universal PCR Primer/i5 Primer, NEB #E7335	2.5
Final volume	50

- PCR Cycling Conditions

PCR cycles	Temp (°C)	Time	cycles
USER Digestion	37	15 min	1
Initial Denaturation	98	30 sec	1
Denaturation	98	10 sec	16
Annealing/ Extension	65	30 sec	
Final Extension	72	5 min	1
Hold	4	∞	1

3-9) Purify the double-stranded cDNA using SPRI Beads (PCRCLEAN DX)

- Vortex PCRCLEAN DX Beads to resuspend.
- Add 45 µL (0.9X) of resuspended PCRCLEAN DX Beads to the ligation reaction (50 µL). Mix well on a vortex mixer or by pipetting up and down at least 10 times.
- Incubate for 5 minutes at room temperature.
- Quickly spin the tube in a microcentrifuge to collect any sample on the sides of the tube. Place the tube on an appropriate magnetic rack to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the double-stranded cDNA.

- Add 200 μL of freshly prepared 80% ethanol to the tube while in the magnetic rack. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
- Repeat once more for a total of 2 washing steps.
- Air dry the beads for 5 minutes while the tube is on the magnetic rack with lid open. Caution: Do not over dry the beads. This may result in lower recovery of DNA.
- Remove the tube from the magnet. Elute the DNA from the beads into 23 μL 10 mM Tris-HCl. Mix well by pipetting up and down. Quickly spin the tube and incubate for 2 minutes at room temperature. Place the tube in the magnetic rack until the solution is clear.
- Transfer 20 μL of the supernatant to a clean PCR tube, and store at –20 °C.

3-10) Assess library quality on a Bioanalyzer (Agilent High **Sensitivity Chip).**

- Dilute 2–3 μL of the library (1:4) in 10 mM Tris-HCl.
- Run 1 μL in a DNA High Sensitivity chip.
- Check that the electropherogram shows a narrow distribution with a peak size approximately 300 bp.
- If a peak at ~ 80 bp (primers) or 128 bp (adaptor-dimer) is shown in the Bioanalyzer traces; Bring up the sample volume to 50 μl exactly with nuclease-free water and repeat the SPRI bead purification. 3).

8. Illumina RNA-Sequencing

PE150 (150bp Paired end) and Rapid HiSeq 2500 were performed.

B. Data Analysis of GRIL-Seq using CLC work bench ver.6.0

1. Import fastq.gz file

1) Select "Import" → "Illumina".
2) Select two fastq.gz files (P5 and P7 reads).
3) Select "Paired reads" and save them in a new folder.

2. Trim the adaptor sequences

1) Create the trim adaptor list: "New" → "Trim Adaptor List"→ "Add Row"
2) Select the sequences saved in **Step 1**-3: "Toolbox" → "NGS Core Tools" → "Trim Sequences".
2) Select the adaptor sequences (P5 and P7 plus index).
3) Create a new folder (i.e. Trimmed) and save them.

3. Map entire GRIL-Seq reads to sRNA genes to identify the reads containing chimeras

In this step, both chimeric and non-chimeric sRNA (intact sRNA) from the entire reads will be selected.

1) Create a reference genome composed of sRNA gene and save it as a sRNA reference genome.

- For example, open the PAO1 reference genome → find PrrF1-2 sRNA sequences → Select and save it as PrrF1-2 sRNA reference genome (Ref1_PrrF12).

2) Select the reads trimmed and saved in Step **2** and click the right button; "Toolbox" → "NGS Core Tools" → "Map reads to Reference".

3) Add the sRNA reference genome (i.e., file name: Ref1_PrrF12)

4) In Mapping options, Set up "Length fraction as 0.2" and "Similarity fraction as 0.9".

5) In Result handling, check "Create stand-alone read mapping".

6) Save them.

4. Extract the mapping reads

1) Open the mapping results.

2) Click the right button and choose "Extract Sequences".

3) Save them as in a new folder (i.e. Extracted).

5. Map the extracted sequences to <u>sRNA-deleted</u> reference genome

In this step, the extracted reads containing chimeric sRNA will be mapping to the sRNA deleted bacterial reference genome.

1) Create the <u>sRNA-deleted</u> reference genome: remove sRNA gene from PAO1 reference genome and save this modified genome as a new reference genome (i.e., file name: Ref2_PAO1_DPrrF1-2). Additional removals of genes are applicable and analyzed it again if an unsuspected mapping is detected after the final mapping of the extracted chimeric reads (Step **4**) to sRNA-deleted reference genome. For example, in case of PrrF1, two short sequences (184,326-184,346 and 3,395,294-3,395,320 in PA1061 and downstream of PA3031.1, respectively) were additionally deleted from PAO1ΔPrrF1-2 due to the observation of the sequence similarity of PrrF1-2 with these genes.

2) Generate the gene track with this reference genome: click the right button "Toolbox" → "Track tools" → "Convert to Track" → "Select the genome" → "Select 'Gene' in Annotation type" → Save them.

3) Map the extract reads generated in Step **4** to the <u>sRNA-deleted</u> reference genome as shown in Step **3**-2).

4) Save them in a new folder (i.e. Mapping to Del_sRNA PAO1).

6. Create statistics for target regions

1) Select the mapped reads generated in Step **5** and click the right button: "Toolbox" → "Resequencing Analysis" → "Create Statistics for target region" → "Select the gene track as Track of Target Regions" → Save.

2) Open the coverage generated Step **6**-1) and click “Create Track List”.

3) Click the right button on the track and select “Open this track”.

4) The target region coverage track will be shown in table view.

5) Analyze the target genes with coverage of mapping.

Supplementary Methods

Construction of LacZ translational fusion

To control the expression of mRNA, the modified IPTG inducible *tac* promoter with two *lac* operator (*lacO*) sites at both the upstream -35 element and between the -35 and -10 elements[7] was amplified with *lacIq* from the plasmid pKH4 using oligonucleotides (F_KpnI_Ptac_inf /R_Fus_Ptac_inf). The plasmid pKH4 was constructed from the plasmid pMMB67EH by introducing two *lac* operators using two primer pairs (F_MluI_lacIq/ R_Dn_lacIq and F_Up_Ptac/ R_Dn_Ptac) and SOEing PCR after linearizing the pMMB67EH with two restriction enzymes MluI and HindIII. The amplified PCR fragments containing *IacIq*, two *lacOs* and *tac* promoter sites was fused with the *lacZ* PCR fragment amplified from mini-CTX-lacZ[8] using primer pairs (F_Fus_lacZ_inf/ R_AatII_lacZ_inf). The plasmid mini-CTX-lacZ was linearized with the restriction enzymes KpnI and ActII and the fused PCR fragment was cloned into the linearized mini-CTX-lacZ to generate the plasmid pPtac-miniCTX::lacZTL. Each specific primer pairs shown in Table 1 were used for amplification of 5' UTR and 20 amino acids of each gene. The TSS (transcription start site) of mRNA identified by the previous experiment[1] was used for amplification with *P. aeruginosa* PAO1 genomic DNA as template. Each 5' UTR was cloned into EcoRI and HindIII sites of the integration vector pPtac-miniCTX::lacZTL. These constructs and the vector control were integrated into the chromosome of *P. aeruginosa* as described previously[14].

Construction of 3' 6×His Tagged translational fusion

For construction of the plasmid pPtac-miniCTX-6His, a PCR fragment 1 containing lacIq and the modified IPTG inducible *tac* promoter was amplified from plasmid pPtac-miniCTX::lacZTL by using oligonucleotides (F_KpnI_lacIq/R_pKHctx3G6His-P). To introduce multi-cloning sites and six His epitope-tags followed by stop codon, a PCR fragment 2 was amplified from plasmid pminiCTX by using oligonucelotides (F_pKHctx3G6His-P/R_speI_lacZTL_ctx_inf). The two PCR fragments 1 and 2 were ligated with Quick Ligation™ Kit (NEB) and amplified with oligonucelotides (F_KpnI_lacIq/ R_speI_lacZTL_ctx_inf), yielding the 1.7kb 'lacIq-Ptac-6His' KpnI/SpeI fragment. The plasmid pminiCTX was digested with KpnI and SpeI, and ligated with this 1.7 kb 'lacIq-Ptac-6His' fragment, resulting in the chromosomal integration vector pPtac-miniCTX::6His. Each sequence of 5' UTR and ORF (open reading frame) without a stop codon

was inserted into pPtac-miniCTX-6His by EcoRI/HindIII cloning. These construct were integrated into *P. aeruginosa* chromosome as described above.

β-Galactosidase Assays

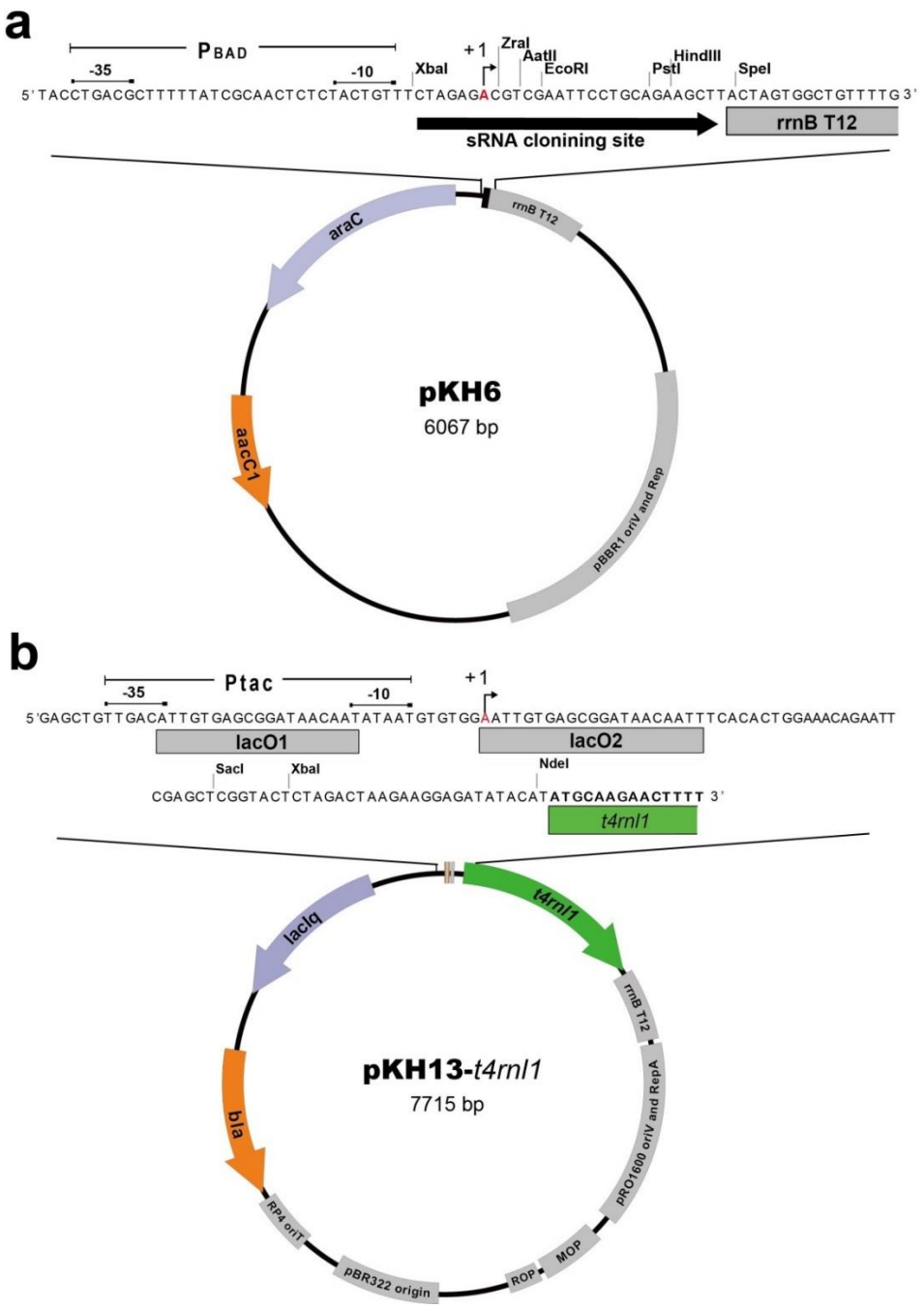
An overnight culture of *P.aeruginosa* (pPtac-miniCTX::lacZTL derivatives) was grown and contained either the empty vector (pKH6) or pKH6-PrrF1. The cells were grown in LB with 37.5 μg /mL tetracycline and 37.5 μg /mL gentamicin, and the overnight culture was diluted to an OD_{600} ~0.02 in 1 mL LB containing the same concenctration of antibiotics and then grown to OD_{600} ~1.0. To express the sRNA and mRNA, L-arabinose and IPTG were added to the final concentrations of 0.2% and 100 μM, respectively. β-galactosidase activities were measured with 50 μL of cells after 1.5 h or 3.5 h inducion with L-arabninose and IPTG. Miller Units were calculated as described previously[15].

Quantitative Western Blot analysis

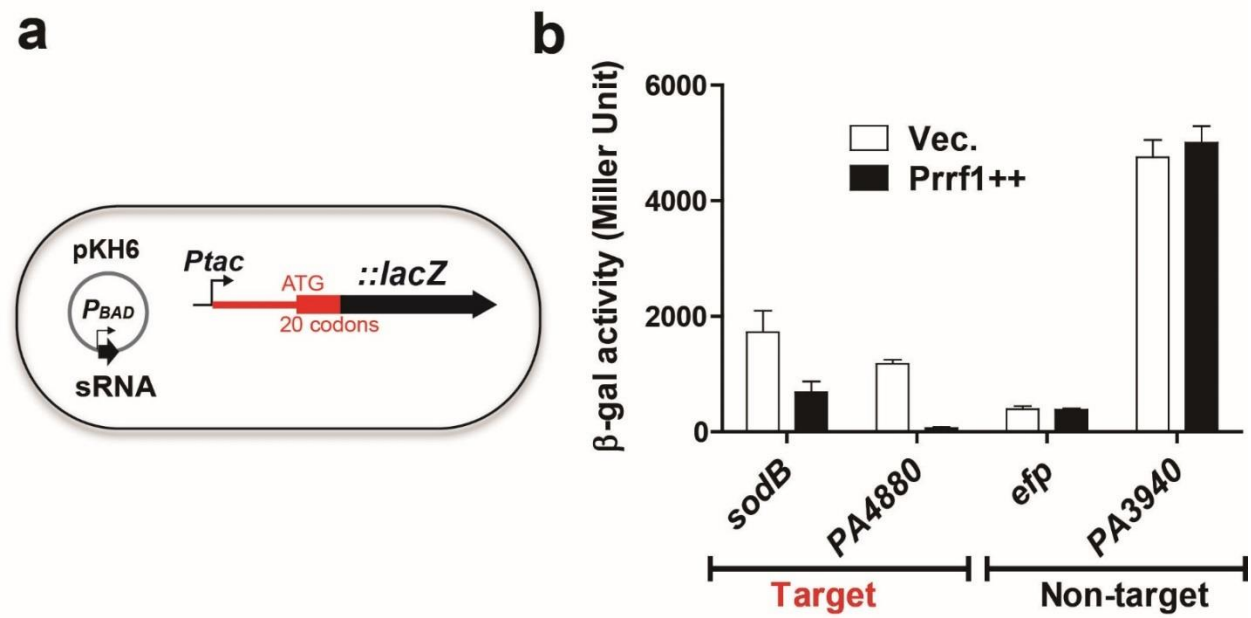
For the protein samples for Western Blot analysis, each overnight culture was diluted to an OD_{600} of 0.1 in LB containing gentamicin (37.5 μg/mL) and tetracycline (37.5 μg/mL). The cells were grown to OD600 of 0.4-0.6 and then 0.2% arabinose, with 100 μM IPTG, was added to each culture. After 4 hr, 200μL of each cell was collected by centrifugation (15000 r.c.f.; for 1 min) and optical density of each strain was measured at OD600 with the remaining culture. The pellets were suspended in ice-cold 1 x PBS to a final concentration of 0.002 OD/μL. Total proteins from equal number of cells (0.04 OD) were loaded and resolved by SDS-PAGE. The proteins were transferred to PVDF membranes and the 6His tag was detected using the monoclonal anti-His antibody (THETM Anti-His mAb, GenScript). The loading control RpoA was detected with the monoclonal anti-*E. coli* α-subunit RNA polymerase antibody (BioLegend). The signal was visualized using the Odyssey® CLx Imaging System (LI-COR) according to the manufacturer's instructions.

Northern Blot analysis for katA and skatA

Northern blot was carried out as shown in the main text (**Methods**). The DNA oligonucleotide probes used to detect *katA* mRNA and *skatA* are listed in **Supplementary Table 5.**



Supplementary Figure 1. Plasmid maps of pKH6 and pKH13-*t4nl1*. (a) pKH6 (plasmid used for sRNA cloning); arabinose inducible. (b) pKH13-*t4rnl1* (plasmid used for T4 RNA ligase); IPTG inducible.



Supplementary Figure 2. PrrF1 regulation of expression of candidate targets (*sodB* and *PA4880*) and lack of control of non-target genes (*efp* and *PA3940*). (a) Schematic of reporters used for assessing the PrrF1 dependence of gene expression. In-frame fusions between 5' UTR, the first 20 codons (60 nt) and of each gene and *lacZ* were constructed and integrated into the *P. aeruginosa* chromosome. Plasmid pKH6-PrrF1 expressing wild type PrrF1 sRNA and a control vector (pKH6-Vec) were introduced into the *P. aeruginosa* strains carrying the various fusion constructs. (b) β-galactosidase activity in cells expressing PrrF1 and the *lacZ* fusions. The *lacZ* reporter constructs were induced with 100 μM IPTG and PrrrF1 with 0.2% L-arabinose at the mid logarithmic growth phase (OD_{600} ~1.0). After 1.5 hours, the levels of the β-galactosidase activity were determined Three independent experiments were carried out and error bars indicate standard deviation of each average value.

a

Targets	# /total	5′ → 3′ Targets	sRNA (PrrF1)
sodB	1/3	+1 AUG…GCCGCCGCUGCCUUACGAAAAG +36	+1 AACUGGUCGCGAGAUCAGC…
	1/3	+1 AUG…GCCGCCGCUGCCUUACGAAAAGA +37	+1 AACUGGUCGCGAGAUCAGC…
	1/3	+1 AUG…GCCGCCGCUGCCUUACGAAAAGA +37	+1 AUAAACUGGUCGCGAGAUCAGC…
PA4880	1/3	+1 AUG…GGAAGGCGCGGUCACCGAAGCUAAA +86	+1 AACUGGUCGCGAGAUCAGC…
	1/3	+1 AUG…GGAAGGCGCGGUCACCGAAGGCUAUU +88	+1 AACUGGUCGCGAGAUCAGC…
	1/3	+1 AUG…AAGGCUAUUCGGCCGAUCGCCAGACCGUCAA +108	+1 AACUGGUCGCGAGAUCAGC…

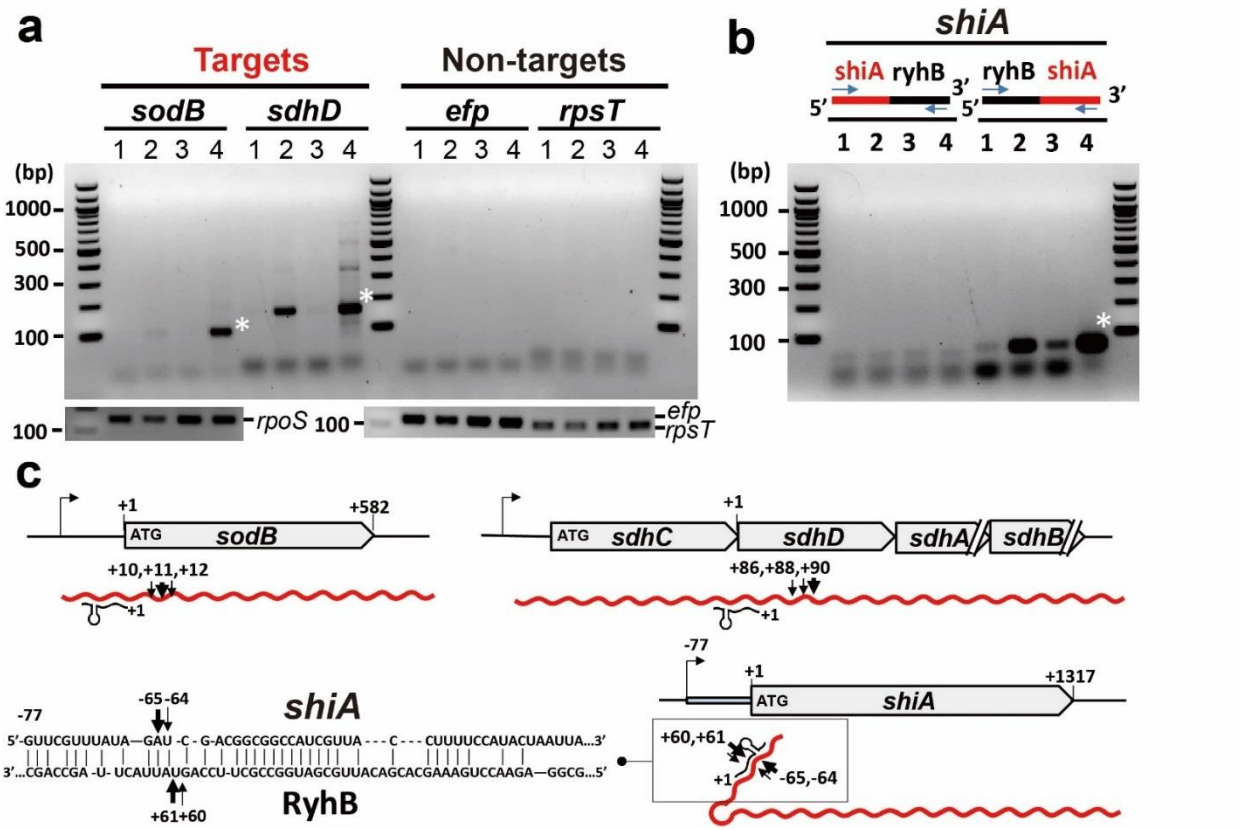
b

Targets	# /total	5′ → 3′ Targets	sRNA (Ec_RyhB)
sodB	1/5	-18 …UAAUAAAGGAGAGUAGCAAUGUCAUUCG +1 +10	+1 AUAGCGAUCAGGAAGACCCUCGC… +20
	3/5	-18 …UAAUAAAGGAGAGUAGCAAUGUCAUUCGA +1 +11	+1 AUAGCGAUCAGGAAGACCCUCGC… +20
	1/5	-18 …UAAUAAAGGAGAGUAGCAAUGUCAUUCGAAA +1 +12	+1 AUAGCGAUCAGGAAGACCCUCGC… +20
sdhD	1/5	+1 AUG…CGCUAUCGUCCUGACGCUCUACA +88	+1 AUAGCGAUCAGGAAGACCCUCGC… +20
	1/5	+1 AUG…CGCUAUCGUCCUGACGCUCUACAU +89	+1 AUAGCGAUCAGGAAGACCCUCGC… +20
	3/5	+1 AUG…CGCUAUCGUCCUGACGCUCUACAUC +90	+1 AUAGCGAUCAGGAAGACCCUCGC… +20

c

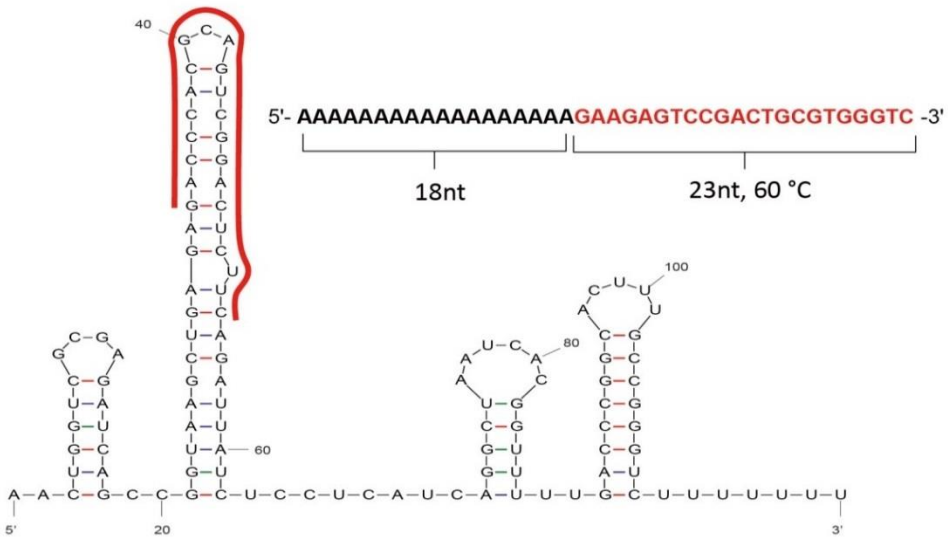
Targets	# /total	5′ → 3′ sRNA (Ec_RyhB)	Target
shiA	1/3	+32 …AAGCACGACAUUGCUCACAUUGCUUCCAG +60	-65 AUCGACGGCAAUGUGAGUUACCUUUU… -40
	1/3	+32 …AAGCACGACAUUGCUCACAUUGCUUCCAGU +61	-64 UCGACGGCAAUGUGAGUUACCUUUU… -40
	1/3	+32 …AAGCACGACAUUGCUCACAUUGCUUCCAGUAAA +61	-65 AUCGACGGCAAUGUGAGUUACCUUUU… -40

Supplementary Figure 3. Sequence analysis of the RT-PCR products created by ligation of sRNAs to their targets. For each sequence, the RT-PCR products were inserted into plasmid pJET1.2 using the CloneJET PCR Cloning kit (Thermo Fisher Scientific). Colonies were randomly selected and sequenced after amplification by colony PCR. (a) Sequencing results showing the junction sites between 5’ ends of PrrF1 and 3’ ends of two known targets (*sodB* and *PA4880*). Poly A (Blue) or AUA (Green) additions were observed at the junctions. (b) Sequencing results showing the junctions between 5’ ends of *E. coli* RyhB sRNA and 3’ ends of two down-regulated targets (*sodB* and *sdhD*). (c) Sequence junctions between 5’ ends of *E. coli* RyhB and 3’ ends of an up-regulated target (*shiA*).



Supplementary Figure 4. Detection of RyhB targets in chimeric RNAs following T4 RNA ligase expression in *E. coli*. (a) Detection of specific amplicons by RT-PCR (white stars) indicating ligation between RyhB and its two repressed targets (*sodB* and *sdhD*) following expression of RyhB and T4 RNA ligase. The number indicates a different combination of RyhB and T4RNA ligase expression conditions in *E. coli*. 1: no IPTG, no L-arabinose, 2: no IPTG, with L-arabinose, 3: with IPTG and no L-arabinose, 4: with both IPTG and L-arabinose. PCR amplification of cDNA for *rpoS* and two non-targets (*efp* and *PA3940*) was carried out to ensure the presence of equal amount of target cDNA and cDNA of two non-target genes in all samples, respectively. Results are representative of duplicate experiments. (b) Detection of specific amplicons (white stars) of the ligated RyhB and its up-regulated target using the RyhB binding forward primer and *shiA* binding reverse primer. Results are representative of duplicate experiments. (c) Mapping the junction sites between RyhB and targets, based on the Sanger sequencing results of RT-PCR products. Bent arrows indicate transcription start sites. Arrows indicate the nucleotide positions of the sites in RyhB and target transcripts, joined by T4 RNA ligase.

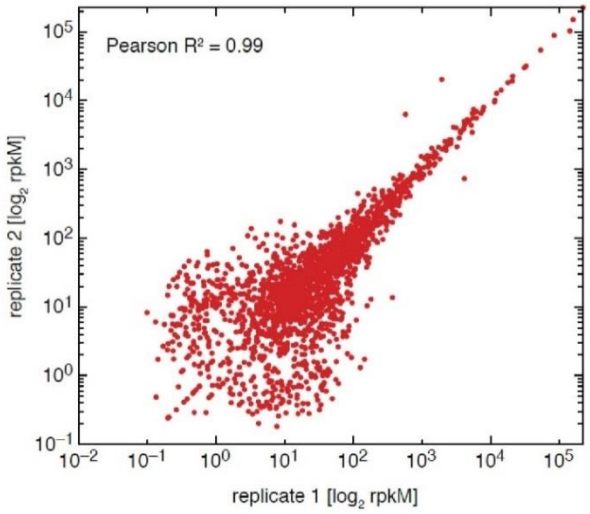
a



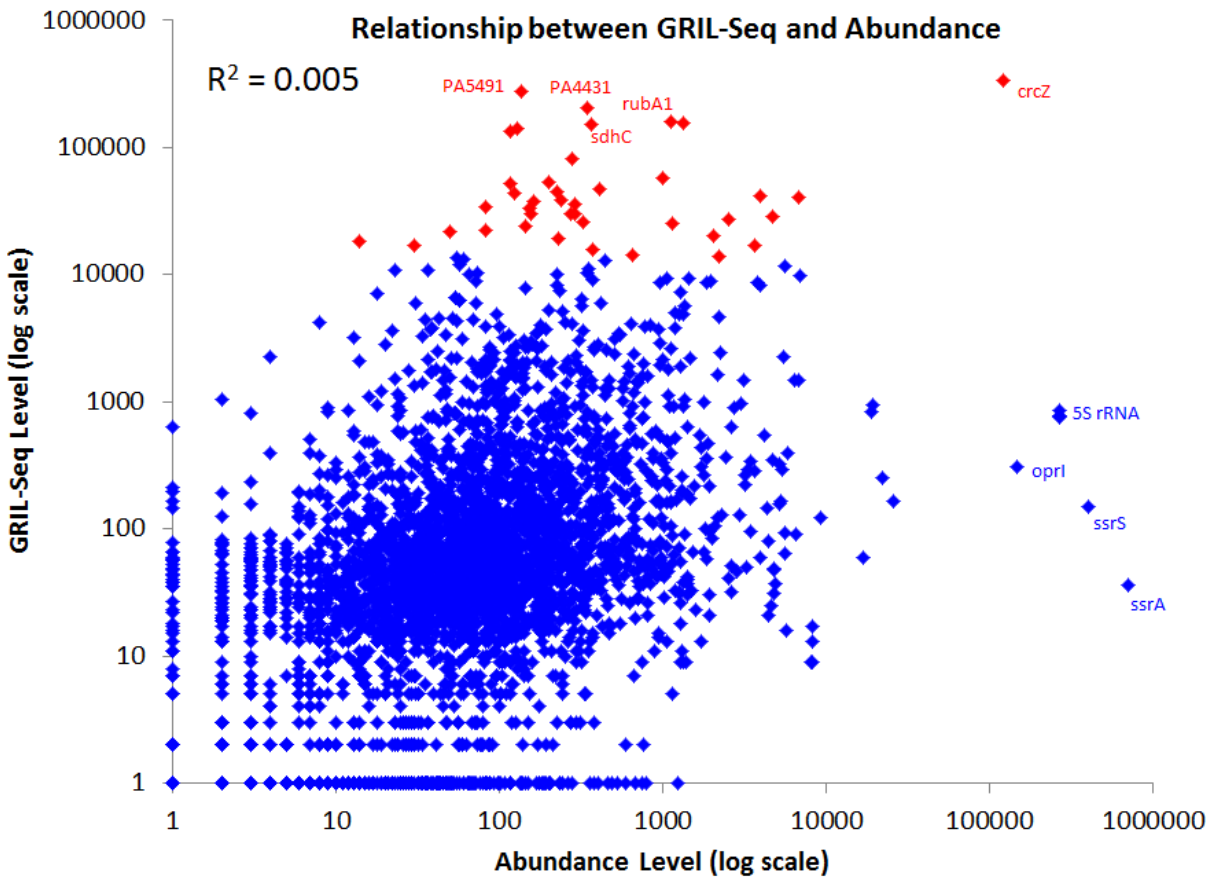
b

Lib.	Total Reads	% mapping to rRNAs	# corresponding to PrrF chimeras	%corresponding to PrrF chimeras
L1	47,247,379	53%	6,811,939	14%
L2	36,319,510	48%	6,473,670	18%

c



Supplementary Figure 5. Design of the capture oligonucleotide used for the enrichment of PrrF1 containing chimeras for GRIL-Seq and comparison of the duplicates in the GRIL-Seq experiment. (a) The PrrF1-chimeras capturing oligo. A DNA oligonucleotide containing 23 base complementary to PrrF1 (shown in red) an 18 poly(A) oligonucleotide sequence. (b) Results of GRIL-Seq. Two libraries from biological replicates were constructed and sequenced using Illumina Rapid HiSeq 2500 (150 PE). (c) The RPKM of the two biological replicates were plotted against each other displaying a high level correlation between the datasets with a Pearson correlation value of 0.99.



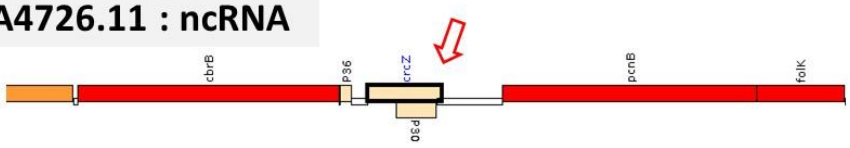
Supplementary Figure 6. Relationship between GRIL-Seq and transcript abundance. The scatter plot illustrates the relationship between abundance levels and GRIL-Seq levels for each gene. Abundance levels are determined by RNA-seq as detailed in Supplementary Table 1. GRIL-Seq levels are provided in Supplementary Table 2. Points corresponding to genes among the top 40 GRIL-Seq levels are shown in red. A few points with high GRIL-Seq levels or high abundance levels are labelled with their gene names to provide context. The R^2 value of the points is 0.005, indicating little to no significant correspondence between GRIL-Seq levels and abundance levels.

Supplementary Figure 7. Detailed analysis of the top 40 targets of PrrF1 identified by GRIL-Seq. For each target shown are: (a) the locus in the *P. aeruginosa* PAO1 genome is indicated from www.pseudomonas.com (b) the location of the transcription start site of the target determined by PA14 transcriptome mapping[8] (c) The genomic position of enriched PrrF1-target chimeric RNA (red peak) in PAO1 determined by GRIL-Seq. The number on the right side indicates the value for the maximum coverage (height of the peak) (d) The sequences of target region most likely involved in base-paring with PrrF1. The sequences that were used in *in silico* hybridization prediction are shown in red. The predicted PrrF1 base-paring region in the target transcript is in blue. Translation start and stop codon are shown in a box and bold italics, respectively. Where included, the inverted repeat sequence of the translational terminator is also indicated by opposing arrows (e) *In silico* base-pairing predictions using IntaRNA[16].

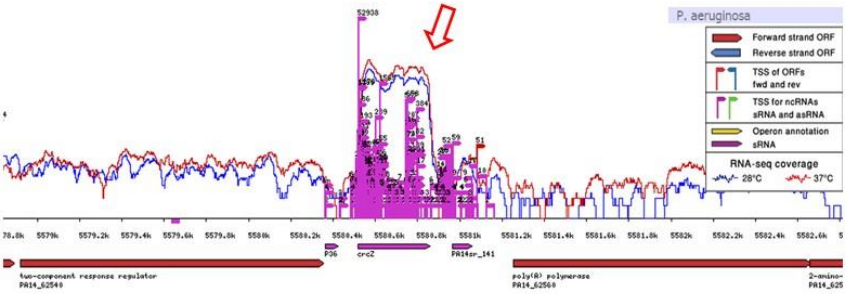
Supplementary Figure 7 - #1

#1 CrcZ, PA4726.11 : ncRNA

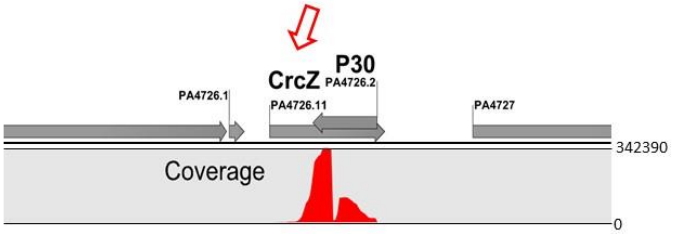
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



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AGCACAACAAAAACAAAATCGCGGAGGCGCAGCTAACTGATTCTTTTGGAGAGGAGTTGCTGTCGGGACCCGTCCCG

CAGCCAGTCGGAAGAAGAATAAAACTGCCTCGAGGCAGCGCACAGACTGGTTGGATCGCTCGACGATCATGGCAGCA

+217 +221 +227 +242 +249 +280

TCAGCGACCAAAGCAATCCGTTTGCTATTGAACTCCCAGCCTGGGAGATATCCCTGAAGCGACTGGCTCAAGGGACG

GGTCGACAAACAAAAACAACAAGCCCGAAATCATAATAAAAACAAAGCACGCACCTACTTGGGGGGGAGCTTCGGCT

+406

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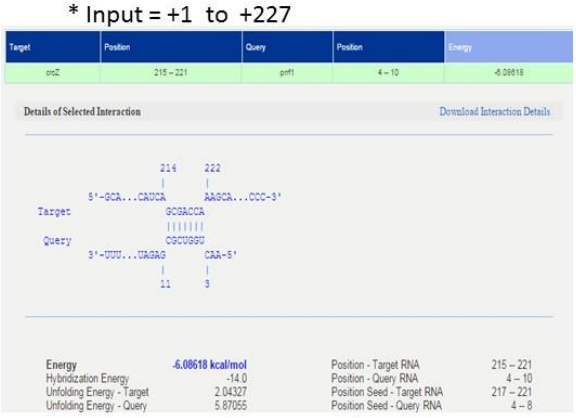
e

* Input = +1 to +227



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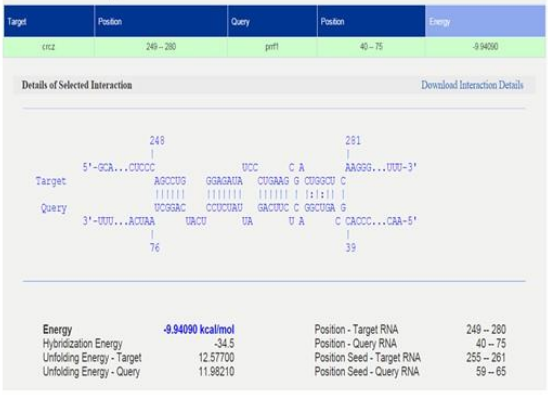
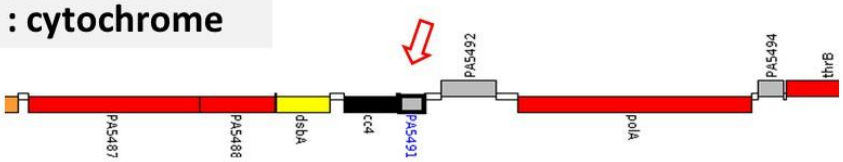


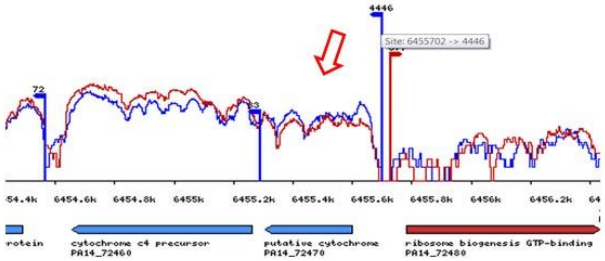
Supplementary Figure 7 - #2

#2 PA5491 : cytochrome

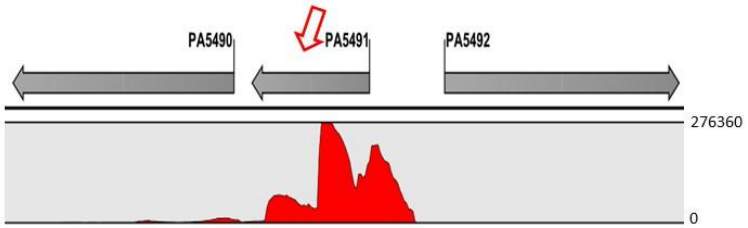
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



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TTCCGCGCGGTAGAACGCGGGCGTTCCC**C**CTGATGAGGACGATCATGAAGAAACTGCTGC**T**CGCAGCGACAGTCGTT

GCGGTGGCCGTTACGGCGCAGGCGGCGCAGGACCCGGAAGCAGTGTTCAACCGGACCTGTGGCGCGTGTCACGACG

GCCAGCTGCCGATGGCGCCGAAAAAGGGCGACCATGCCGCCTGGGAGCCGCGGCTGGCCAAGGGCATGCCGACCCT

GGTCCAGCATGTGACCAACGGATTCAACGCCATGCCGCCCCGCGGCCTGTGCACCGACTGCAGTGCCGAAGACTACCA

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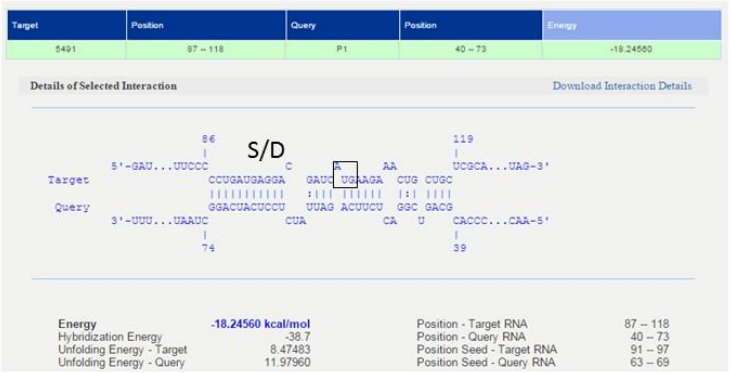
+1 +87 S/D +103 +118 +396

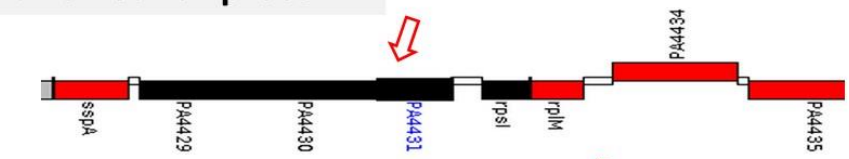
e



Supplementary Figure 7 - #3

#3 PA4431 : iron-sulfur protein

a PAO1



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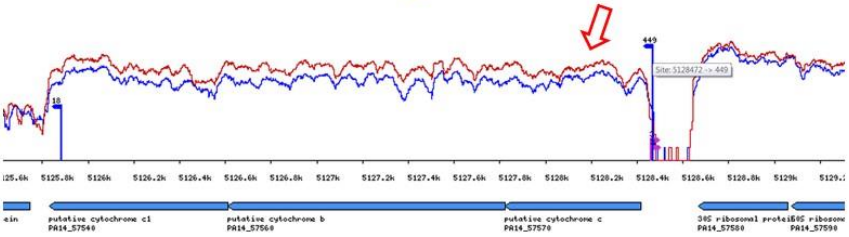
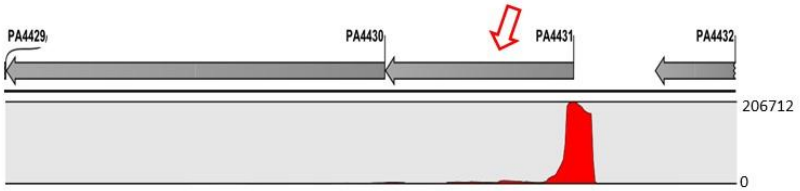


c PAO1 (GRIL-seq)



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S/D +63 +73

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+155

CTCTGTGGTTGGCGCGGCGGGAGCGGTGGGTGCTGCGGTCCCGTTCGTAGGGTCATGGTTCCCCAGTGCCAAAGCCA

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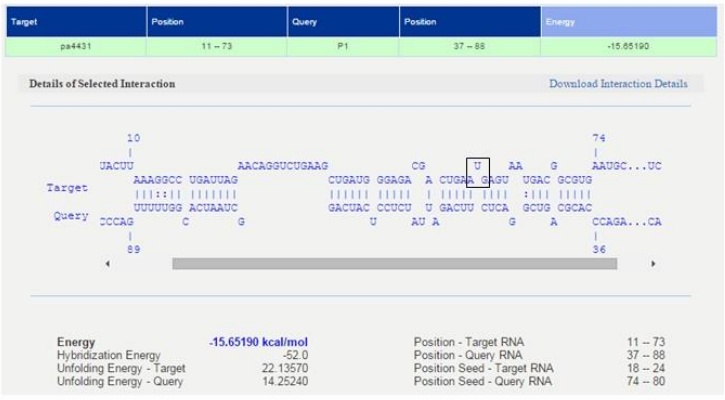
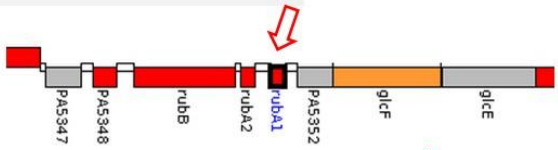
e



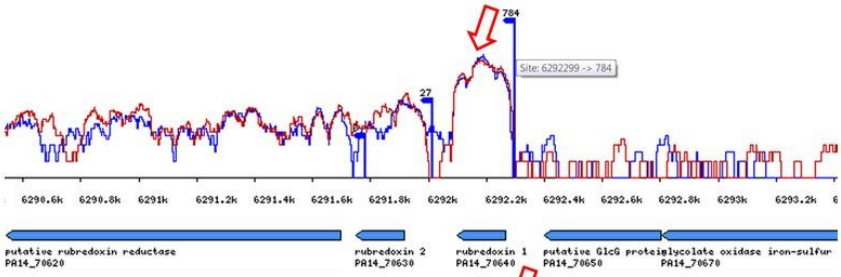
Supplementary Figure 7 - #4

#4 rubA1, PA5351 : rubredoxin

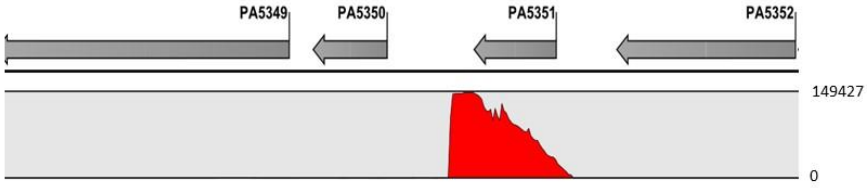
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



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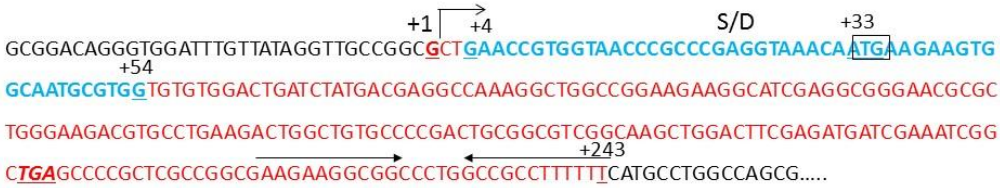

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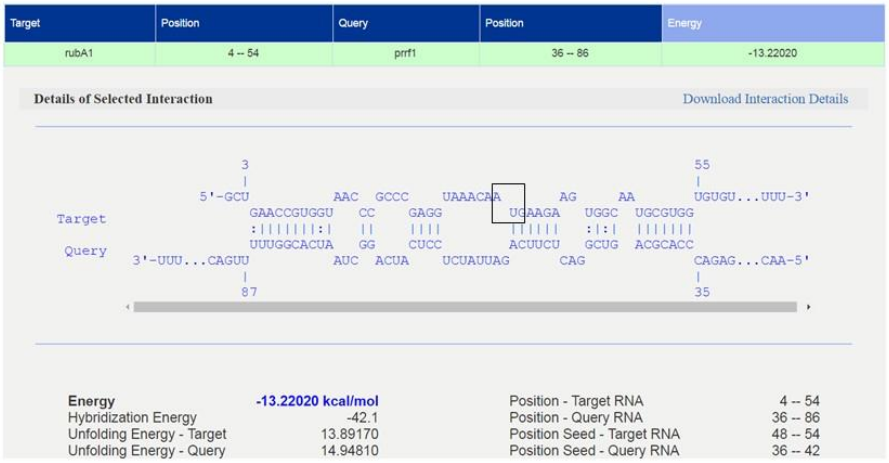
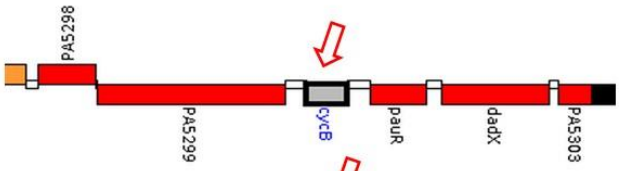


Supplementary Figure 7 - #5

#5 cycB, PA5300 : cytochrome C5

a PAO1



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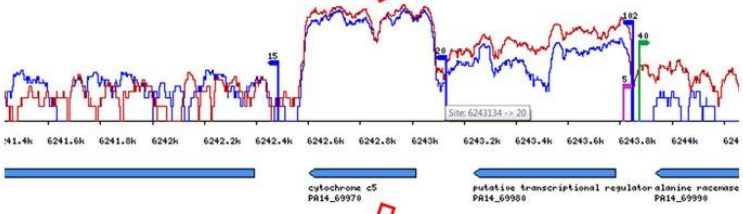

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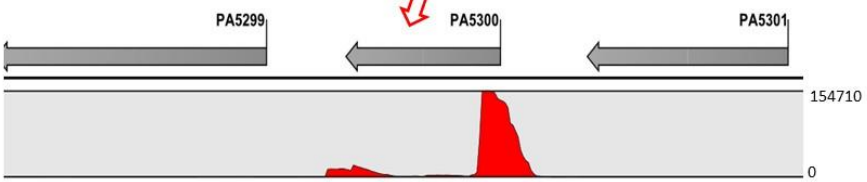

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+74 +95 S/D +121

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e

Target	Position	Query	Position	Energy
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Details of Selected Interaction — Download Interaction Details

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                    |                   |
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Target                    GUGAU AGCC  GAUGAGGG
                          ||||| ||||  |||||||:
Query                     CACUA UCGG  CUACUCCU
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                    |                   |
                    82                  62
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Unfolding Energy - Target	7.77884	Position Seed - Target RNA	89 -- 95
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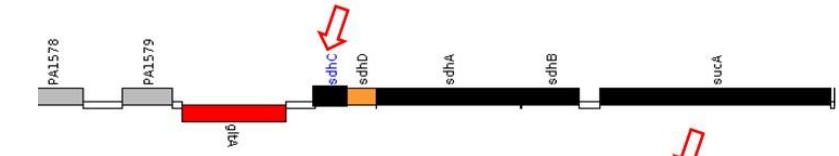
Supplementary Figure 7 - #6

#6 PA1581 : succinate dehydrogenase subunit C

a PAO1



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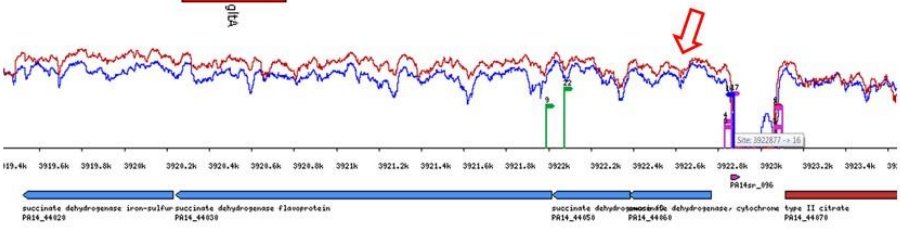

c PAO1 (GRIL-seq)

d

+1

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S/D sdhC→

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+118 +133

AAAAGCCGTGAATAGCAAACGACCTGTAAACCTAGACCTAAGGACCATCAAACTCCCTGTCACCGCTTACACGTCCATT

+212

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+413

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S/D sdhD→

CGTGGCTCCAAAATCGTCATCGCCATCGCGGTGGTGCTGATCGTTCTGGCGGGGGTATGGGTATGGCTAACTAACGTCAC

+567 +576 +587

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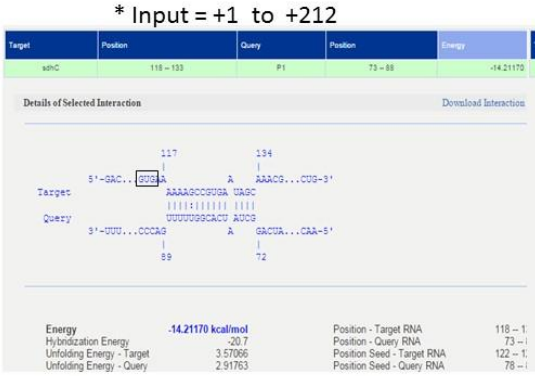
e

* Input = +1 to +212



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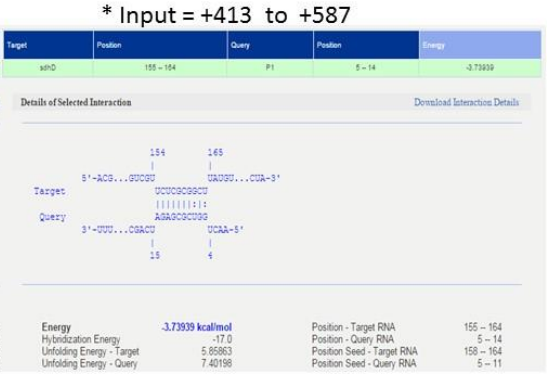
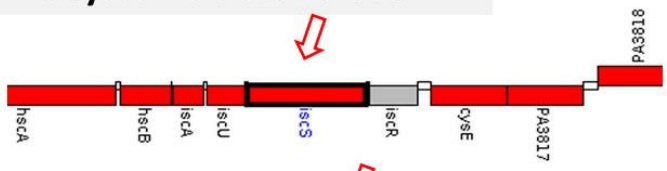


Supplementary Figure 7 - #7

#7 iscS, PA3814 : cysteine desulfurase

a PAO1



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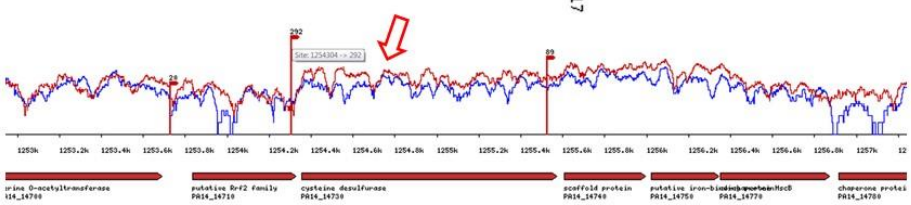
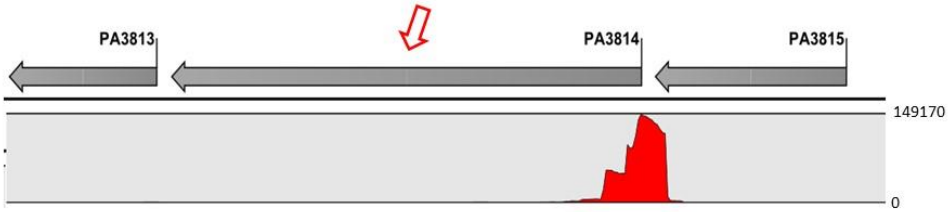


c PAO1 (GRIL-seq)



d

iscR→ +1

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+35 S/D +57 iscS→

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TCGCCCAGAAAATGAGCGAATGCCTGCTGATGGACGGCAACTTCGGCAACCCGGCTTCGCGTTCCCACGTGTTCGGCT

+187

GGAAAGCCGAAGAAGCCGTGGAGAACGCCCGTCGCCAGGTGGCCGAGCTGGTCAACGCCGATCCCCG

e

Target	Position	Query	Position	Energy
iscS	35 -- 57	prrf1	66 -- 85	-9.36563

Details of Selected Interaction Download Interaction Details

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                    |                   |
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Target                AGCC    G  GUUA CCUGAUGA
                      |:||    |  :||| ||||||||
 Query                UUGG    C  UAAU GGACUACU
         3'-UUU...AGUUU         AC    C       CCUCU...CAA-5'
                    |                         |
                    86                        65
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Hybridization Energy	-16.7	Position - Query RNA	66 -- 85
Unfolding Energy - Target	4.34500	Position Seed - Target RNA	51 -- 57
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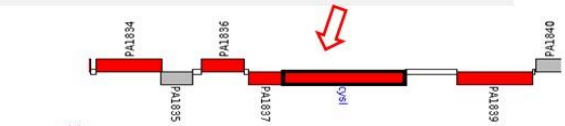
Supplementary Figure 7 - #8

#8 cysI, PA1838 : sulfite reductase

a PAO1



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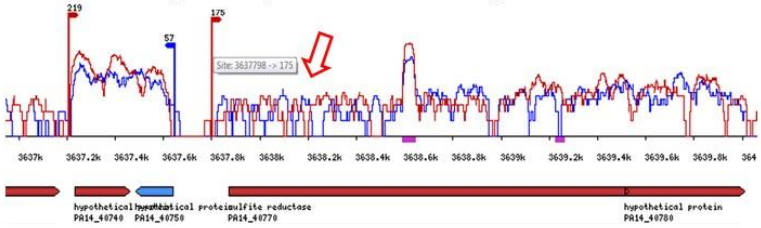
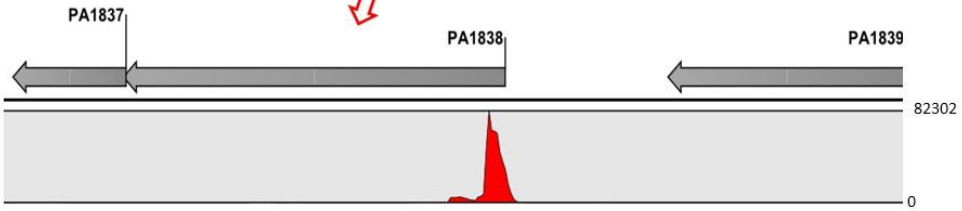


c PAO1 (GRIL-seq)



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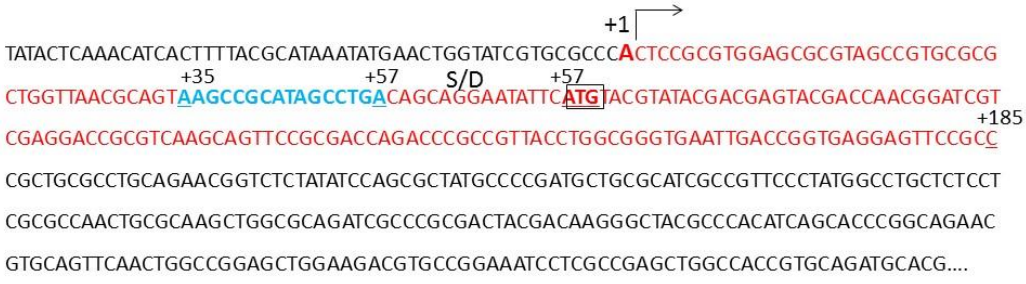

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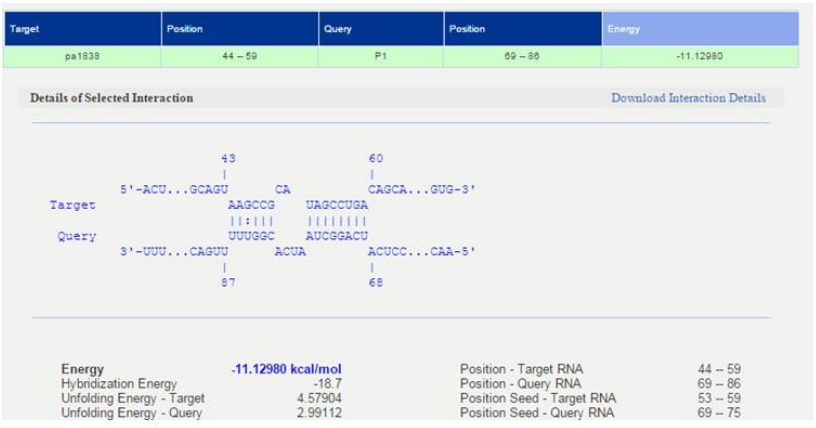
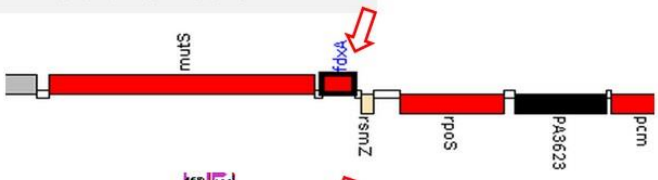


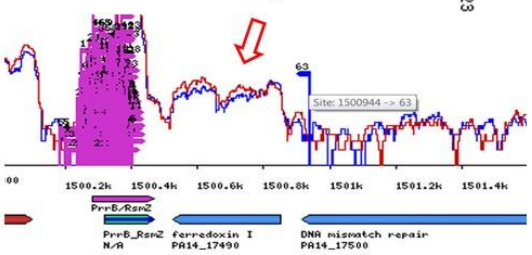
Supplementary Figure 7 - #9

#9 fdxA, PA4236 : ferredoxin I

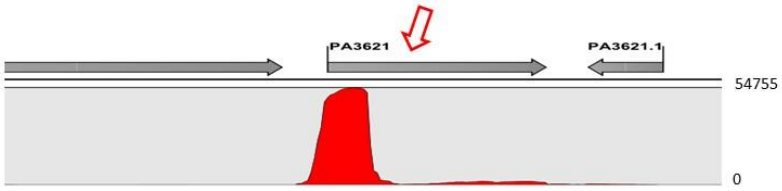
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

+1

ATCGATGAATTGTCGAGGATCAATCCCGACGATATCAGCCCGCGGCAAGCTCTCGATCT**G**TTATATGCATGGAAGATGCG

S/D +95

GGTCTGACGGACTACCGCACAAGCTGCTAGAATCGCGCGCGCTTAGCCGCCCGCCTGAGGAGAGAACTAGAAA**ATGA**

+137 +150

CCTTCGTCGTCACCGACAACTGCATCAAGTGCAAATACA**CCGACTGCGTGG**AAGTCTGCCCGGTAGACTGCTTCTACGA

AGGACCCAACTTCCTGGTCATCCATCCGGACGAGTGCATCGACTGCGCCCTGTGCGAGCCCGAATGTCCGGCCCAGGC

GATTTTCTCCGAAGACGAAGTGCCGGAGAACATGCAGGAATTCATCGAGCTGAACAGCGAGCTGGCGGAAGTCTGGC

CGAACATCACCGAGAAGAAGGATGCCCTGCCCGACGCTGAGGAATGGGACGGCGTCGCCGGCAAGCTCCAGCATCTG

+583

GAGCGC***TGA***

e

Target	Position	Query	Position	Energy
fdxA	138 -- 149	prrf1	36 -- 47	-17.51520

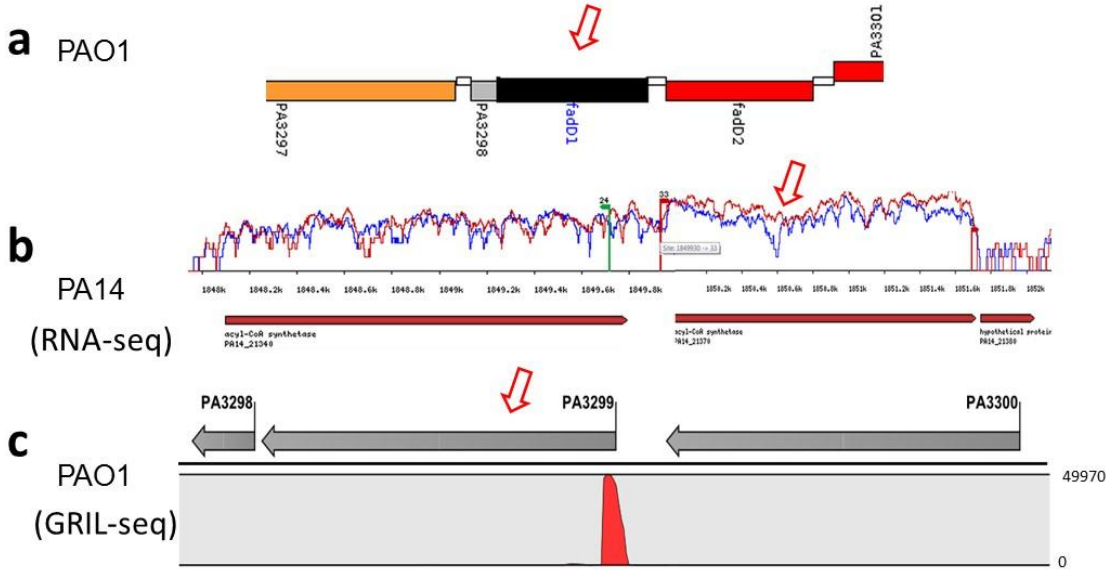
Details of Selected Interaction Download Interaction Details

```
                    137          150
                     |            |
          5'-GUU...AUACA          AAGUC...UGA-3'
Target                 CCGACUGCGUGG
                       ||||||||||||
Query                  GGCUGACGCACC
          3'-UUU...UCUCA          CAGAG...CAA-5'
                     |            |
                    48           35
```

Energy	**-17.51520 kcal/mol**	Position - Target RNA	138 -- 149
Hybridization Energy	-26.5	Position - Query RNA	36 -- 47
Unfolding Energy - Target	4.86753	Position Seed - Target RNA	143 -- 149
Unfolding Energy - Query	4.11729	Position Seed - Query RNA	36 -- 42

Supplementary Figure 7 - #10

#10 fadD1, PA3299 : long-chain-fatty-acid--CoA ligase



d

```
                                                                +1
GGTCATGTTTGGTGCTGGTCTGCCTCTGGCAAAGCTGATAATCTCGGCGCGCCTAATAGCCCTGGGTCCAGGGTTAGCG
                                                    +85  S/D        +95
AGCAGATCACAACAAGATGCCGCCATGAGCGGTGAAGATTAGCTGTTGCTTAGGAGTGGGCTTCCATGATCGAAAACT
                +66
TCTGGAAGGACAAGTACCCAGCGGGTATTGCTGCCGAGATCAATCCCGACCAGTACCCGAACATCCTGTCGGTACTGAA
                                                                              +264
GGAGTCCTGCCAACGTTTTGCCACCAAGCCCGCGTTCACCAACCTGGGCAAGACCCTGACCTACGGCGAGCTGTACAA
GCTGTCCGGCGACTTCGCCGCCTACCTGCAGCAGCACACCGATCTCAAGCCCGGCGACCGCATCGCCGTCCAGTTG...
```

e

Target	Position	Query	Position	Energy
fadD1	66 -- 85	prrf1	62 -- 79	-6.72706

Details of Selected Interaction — Download Interaction Details

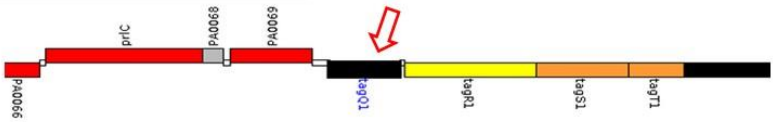
```
                    65                  86
                    |                   |
          5'-GCC...GUGAA        GU   CUU     UGGGC...CAA-3'
Target                GAUUAGCU    UG   AGGAG
                      |||||||:    ||   |||||
 Query                CUAAUCGG    AC   UCCUC
          3'-UUU...UGGCA        ACU         UAUUA...CAA-5'
                    |                       |
                    80                      61
```

Energy	**-6.72706 kcal/mol**	Position - Target RNA	66 -- 85
Hybridization Energy	-17.5	Position - Query RNA	62 -- 79
Unfolding Energy - Target	5.80366	Position Seed - Target RNA	67 -- 73
Unfolding Energy - Query	4.96928	Position Seed - Query RNA	72 -- 78

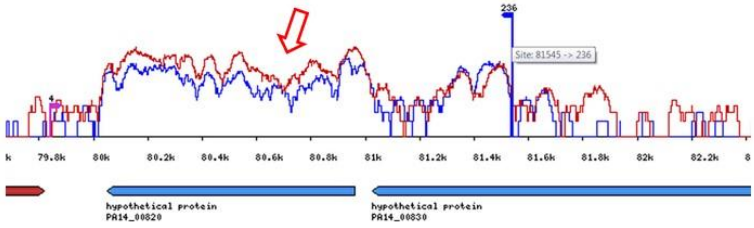
Supplementary Figure 7 - #11

#11 tagQ1, PA0070 : T6SS

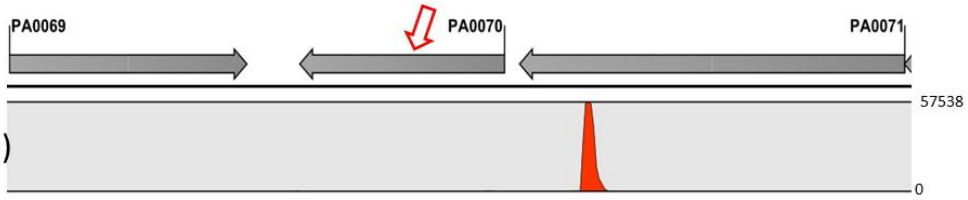
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

tagR→ +1→

TAGCGGCATTGACCGATGCCATCGATGACGCTCAAGATCCCACCA**A**GCAATTGGACGGAATCATTGCCGCCACCCGCG

ACCCGCAGATGCAGGCGCAGTTGGCCCAGATAAACGAGGAACTCAAGCGTAACGTTTCGTTGATCGCCCGGCAGCGC

GAAGAGGCCGCCGGAAACCTGATACAGTCGGCGGCACTGGTCGCCGAGACGATCAACAACTACAACATACGCCTGAC

+209 +224

CAACCTCAAGAAGGACAGGGA**GAAAGCCGTGGCAGCC**AGGGATCAGGCGACCGCCCAGCTCTATGCCGGCGCCATC

GCCAACGGGCGCAGCGCCCTCGATGGCGCATTGGCCATCTACATCGACAACCTCGCCACCGGCACCCGTTACACCGAC

GCGGTGATCCAGGCGCAGTTCCAGCGCATCCAGGAAGAACTCAATCGCAACCCGGTTCTGGGCAAGAGCCTGGTCAA

GCGCGCGACCCTGTTCGTCAAACACGTCGGAGATTATCGCCAGCAGCACCGGGCCGAGCCGGAGGCGGTATTGAAGG

S/D

AATTGCTCGCGTCCAGCGGGCGT**TGA**AGGAGTTCCGTCCGGCCCCTCTCGGCAGCCGGGCGGTTCATCAAACCAGGA

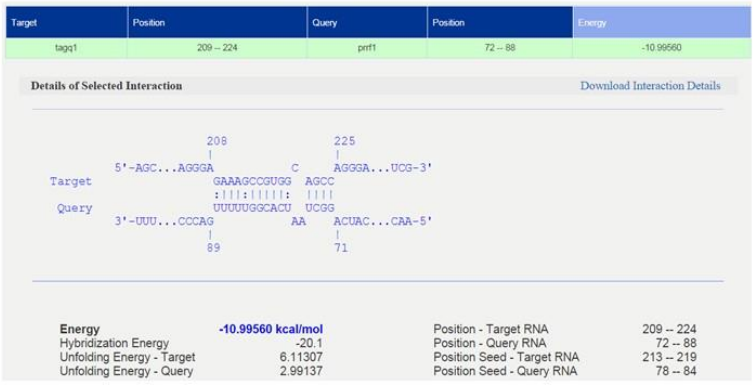
+583 tagQ1→

GTGAGATGCA**ATG**AGCCAACCCAGCGAAAACCGTTTGATCACCTCGGCGCGCTACGCGCTCTGCCTGTTGACCGCCAG

+185

CGGCGTGCTGCTCAGCGGCTGCGCCAGCAGCGGCGTCG....

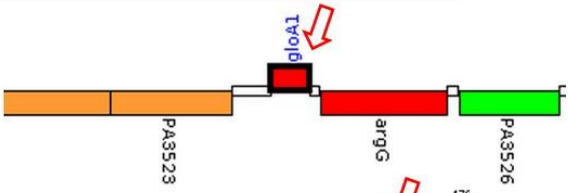
e



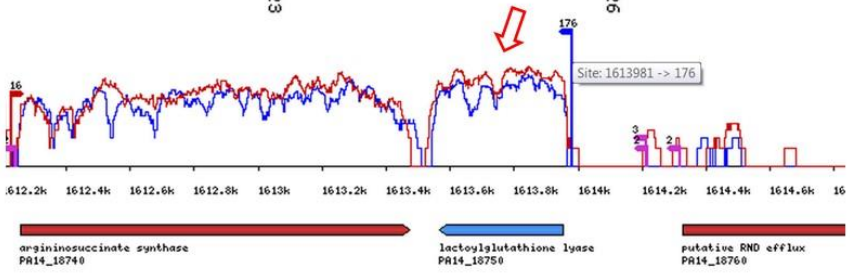
Supplementary Figure 7 - #12

#12 gloA1, PA3524 : lactoylglutathione lyase

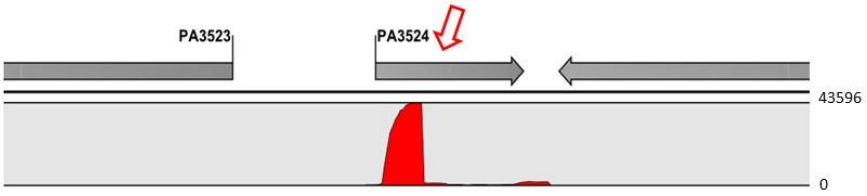
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

+1 +5 +19S/D

GGGACAATCCCGATCTCGCAGCCGGTTGCCGGCCACATGGTAAAATCCGCCTTTGACCGTGAAGCTCGAGGCAATGA

+30

AAATGCGCATTCTCCATACCATGATCCGGGTCGGAAACATCGATCGCTCGATCGATTTCTATACCCGGGTGCTAGGCATG

ACCCTGCTCAGGAAGAACGACTATCCCGACGGCCAGTTCACCCTGGCCTTCGTCGGCTATGGCAACGAGGCGGATTCC

+186

GCCGTGATCGAGCTTACCCACAACTGGGGCGTGGATGCCTACGAGATCGGCACCGGCTACGGCCATATCGCCATCGAG

....

e

Target	Position	Query	Position	Energy
gloA1	5 -- 19	prrf1	69 -- 85	-11.64340

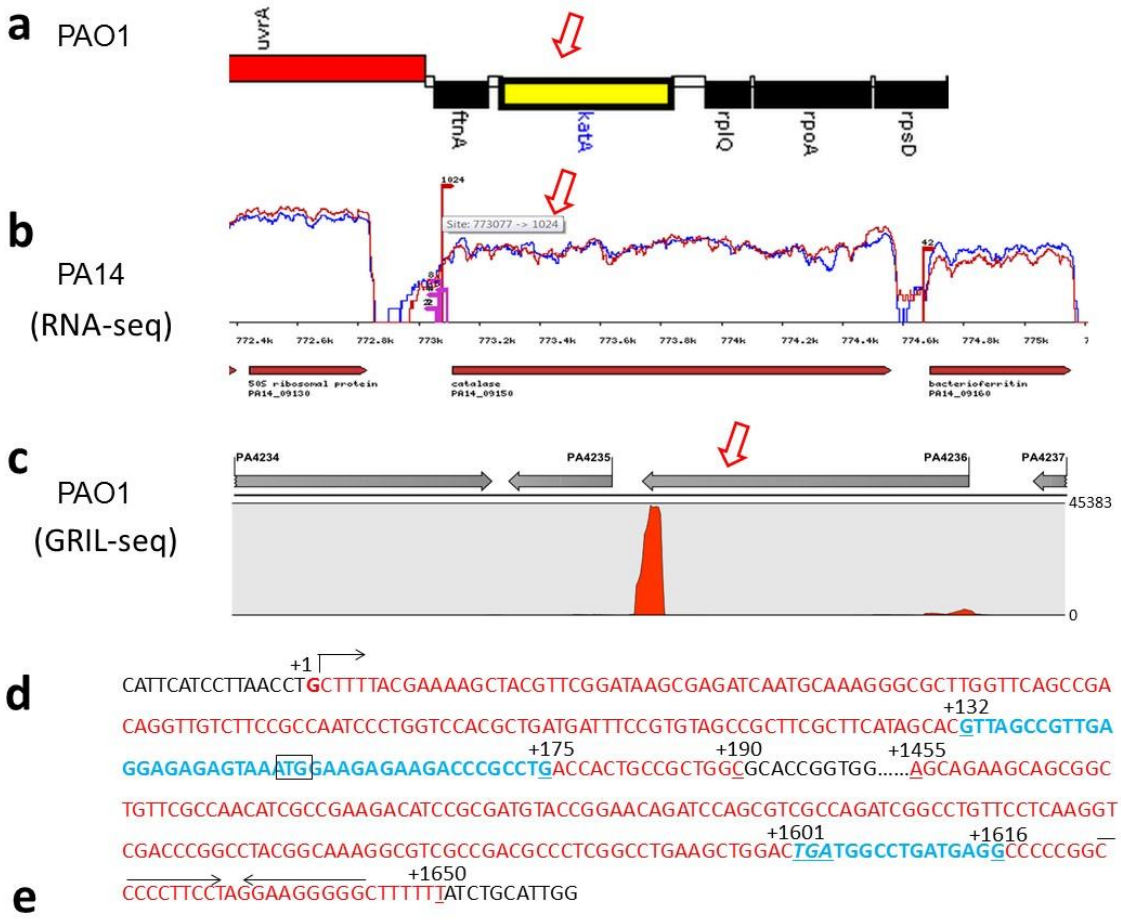
Details of Selected Interaction Download Interaction Details

```
                    4                20
                    |                |
               5'-CUUU             UC  GGCAA...CCG-3'
Target                  GACCGUGA  AGC  GA
                        :|||||||  |||  ||
Query                   UUGGCACU  UCG  CU
          3'-UUU...AGUUU        AA   GA  ACUCC...CAA-5'
                        |                |
                        86               68
```

Energy	**-11.64340 kcal/mol**	Position - Target RNA	5 -- 19
Hybridization Energy	-17.4	Position - Query RNA	69 -- 85
Unfolding Energy - Target	2.76917	Position Seed - Target RNA	6 -- 12
Unfolding Energy - Query	2.98745	Position Seed - Query RNA	78 -- 84

Supplementary Figure 7 - #13

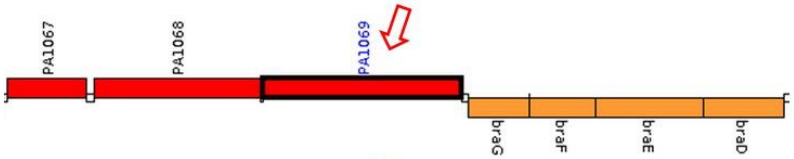
#13 katA, PA4236 : catalase



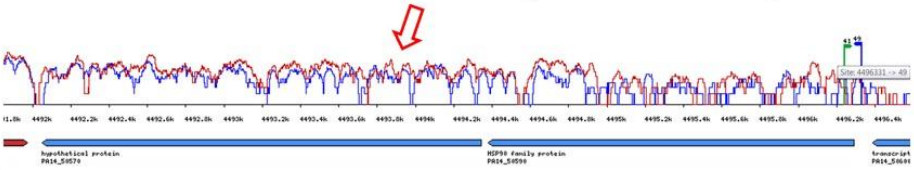
Supplementary Figure 7 - #14

#14 PA1069 : hypothetical protein

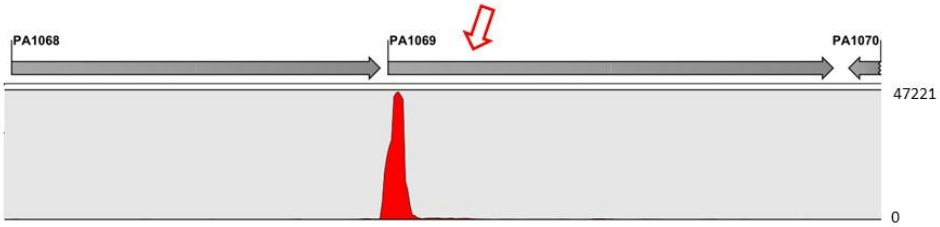
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

+1

CGCGACGCGGCTCTATTGCACTCCGGGCCGCTTCCATGCGGCCTGAAGCTCATCGCGGGCACGTCGTTGCACAAGGAA

PA1068→ +1′

CGTGACGGAACGTGGCAGAATCCCGCACC....CGACCTCGCTGAACCAGGCCCTGGCCGGACTCGCCGATTCGGTGAA

+81 ′ S/D PA1069→ +123 ′

GCGCCTGCTGGAGCAA***TAA***GAAAATTCGCAACACGCCTGATGGAGACTGAAGCATATGGATATCTGGAACTGGGTGG

AGAAGCTCCGCGCCGACCTGCGTGAGGCCGGCCAGGAGCAGAGTGCGCGGCTGATCGACGAACTCACCGCGTACGT

CTGCGACCTCGAGGTGGAGCGCGCCGAGGCGCTGTTGCCGGAGGTCAAGGCGCTGAGCCGGACGTTGAACAACCCG

+347 ′

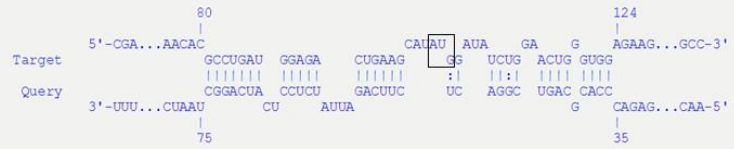
TGGCTGGAAATCTTCGTCCGCCACTGGGAAATGCGTAACCGGGTCGGCAACCAACTGGAGGGTGAGACCGCC....

′: arbitrary number

e

Target	Position	Query	Position	Energy
pa1069	81 -- 123	prrf1	36 -- 74	-14.42270

Details of Selected Interaction Download Interaction Details

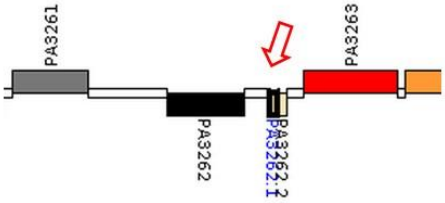


Energy	**-14.42270 kcal/mol**	Position - Target RNA	81 -- 123
Hybridization Energy	-37.2	Position - Query RNA	36 -- 74
Unfolding Energy - Target	9.57013	Position Seed - Target RNA	81 -- 87
Unfolding Energy - Query	13.20720	Position Seed - Query RNA	68 -- 74

Supplementary Figure 7 - #15

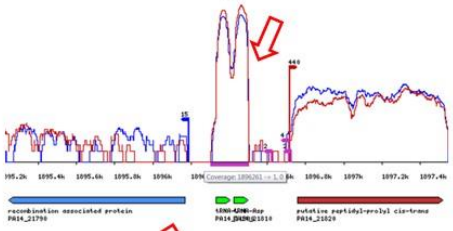
#15 PA3262.1 : Asp tRNA

a PAO1



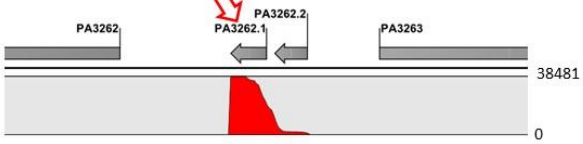
b

PA14
(RNA-seq)



c

PAO1
(GRIL-seq)

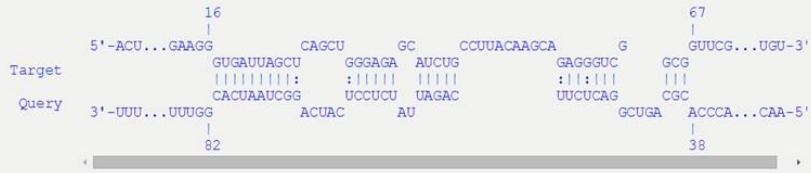


d

e

Target	Position	Query	Position	Energy
pa1750	17 -- 66	prrf1	39 -- 81	-9.37551

Details of Selected Interaction Download Interaction Details

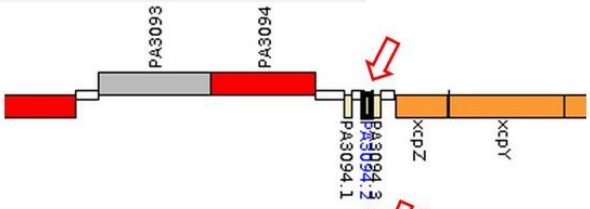


Energy	**-9.37551 kcal/mol**	Position - Target RNA	17 -- 66
Hybridization Energy	-39.6	Position - Query RNA	39 -- 81
Unfolding Energy - Target	16.74420	Position Seed - Target RNA	56 -- 62
Unfolding Energy - Query	13.48030	Position Seed - Query RNA	47 -- 53

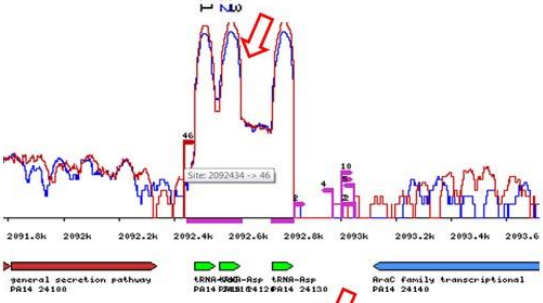
Supplementary Figure 7 - #16

#16 PA3094.2: Asp tRNA

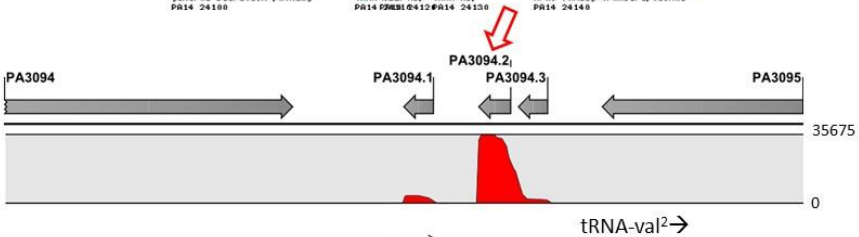
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

tRNA-val[2]→ (+16, +17)

+1

ATTGACTTAGGTTTTTCGGTCGGTAGAATGCGCCCCACTTCGAGAGTGAAGGGTGATTAGCTCAGCTGGGAGAGCAT

+66 tRNA-asp[2]→

CTGCCTTACAAGCAGAGGGTCGGCGGTTCGATCCCGTCATCACCCACCAATCTCGCAAGTTACGCGCAGCGGTAGTTC

AGTCGGTTAGAATACCGGCCTGTCACGCCGGGGGTCGCGGGTTCGAGTCCCGTCCGCTGCGCCATTCACTTCCCCTCCC

GAGTTCACCACCCCGAACTCGCCGTCTTCCACCAGAAGGCGGGCAGCCTGGCTGCAACGAGAGGAAGTCGCTTCACC

tRNA-asp[3]→

GGGCATCTTGCCCAGCCGACGCGCAGCGGTAGTTCAGTCGGTTAGAATACCGGCCTGTCACGCCGGGGGTCGCGGGT

+402

TCGAGTCCCGTCCGCTGCGCCATACATCTTCACCACGCCGAGGCGGGGTG.....

e

Target	Position	Query	Position	Energy
pa3094.2	17 -- 66	prrf1	39 -- 81	-9.37551

Details of Selected Interaction — Download Interaction Details

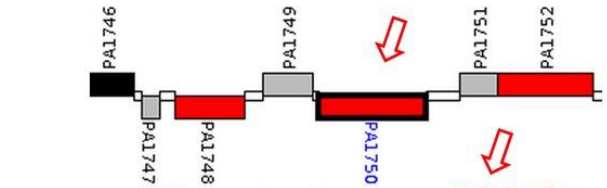
```
                16                                                  67
                |                                                   |
5'-ACU...GAAGG          CAGCU      GC     CCUUACAAGCA       G      GUUCG...GUG-3'
Target        GUGAUUAGCU     GGGAGA  AUCUG           GAGGGUC     GCG
              |||||||||:     :|||||  |||||           :||:|||     |||
Query         CACUAAUCGG     UCCUCU  UAGAC           UUCUCAG     CGC
3'-UUU...UUUGG          ACUAC      AU                     GCUGA   ACCCA...CAA-5'
                |                                                   |
                82                                                  38
```

Energy	**-9.37551 kcal/mol**	Position - Target RNA	17 -- 66
Hybridization Energy	-39.6	Position - Query RNA	39 -- 81
Unfolding Energy - Target	16.74420	Position Seed - Target RNA	56 -- 62
Unfolding Energy - Query	13.48030	Position Seed - Query RNA	47 -- 53

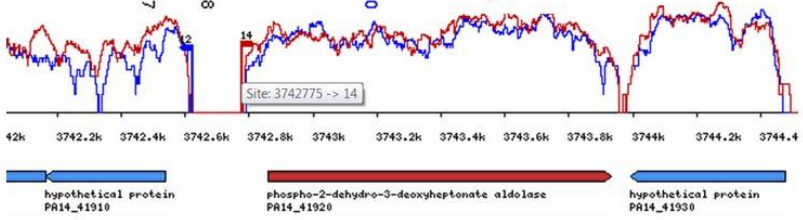
Supplementary Figure 7 - #17

#17 PA1750 : phospho-2-dehydro-3-deoxyheptonate aldolase

a PAO1



b PA14 (RNA-seq)



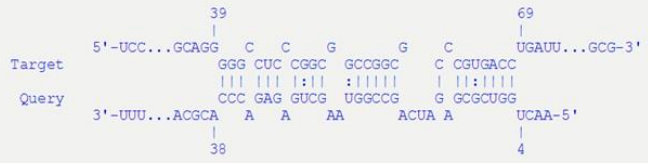
c PAO1 (GRIL-seq)

d

e

Target	Position	Query	Position	Energy
pa1750	40 -- 68	prrf1	5 -- 37	-5.71146

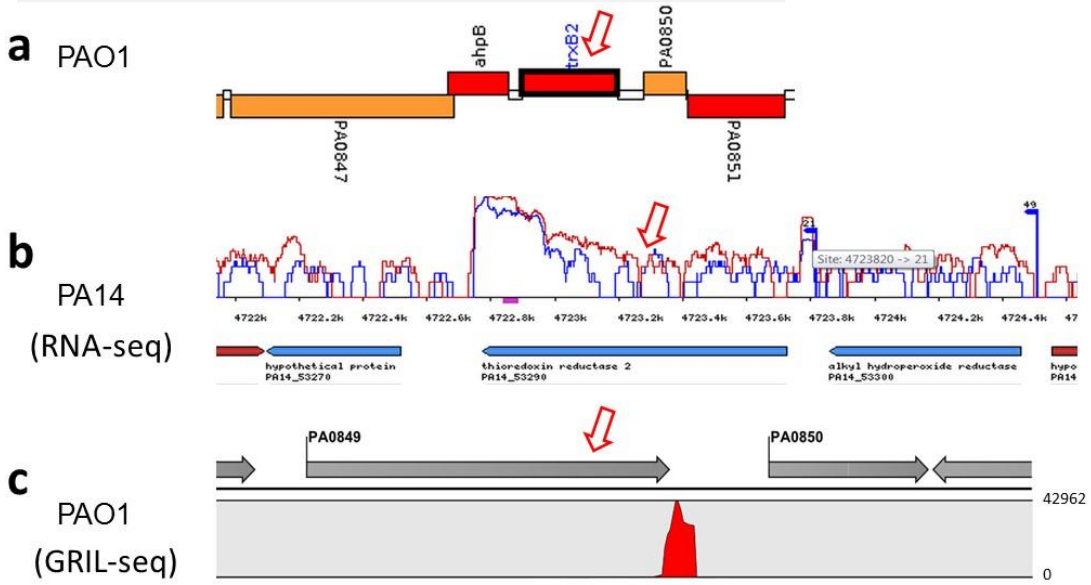
Details of Selected Interaction — Download Interaction Details



Energy	**-5.71146 kcal/mol**	Position - Target RNA	40 -- 68
Hybridization Energy	-41.3	Position - Query RNA	5 -- 37
Unfolding Energy - Target	15.89080	Position Seed - Target RNA	62 -- 68
Unfolding Energy - Query	19.69770	Position Seed - Query RNA	5 -- 11

Supplementary Figure 7 - #18

#18 trxB2, PA0849 : Thioredoxin reductase



d

+1

GCTGCCGGGCGCGAAGTGTGCCCGACCGACCTTTTTCCGGGGCGACCGCTCCTTCCAAGCCTTCCCCATGTGCTTTGGTGGCC

+83 S/D +93

CCGTTTTTATTCGCGCCGACGCTGATGTCGGCAGGAGTCCGACGAGATGCCCGATACCCTCCGCCACGCCCGAGTGATCATC

+196

CTCGGTTCCGGTCCCGCCGGTTACAGCGCGGCTGTCTACGCCGCGCGCGCCAACCTCAAGCCGCTGCTGATCACCGG...

+887

..CCCTGAAGGACGGCTACCTGGTGGTCAACGGCGGGCGCGAAGGCAACGCCACCGCGACCAACGTACCGGGTGTGTTCGCCG

CCGGCGACGTGGCCGACCACGTCTACCGCCAGGCGATCACCTCGGCCGGCGCCGGCTGCATGGCCGCGCTGGATGTGGAGCG

+1037 +1075 +1108

CTATCTCGATTCGCTCTGATGAGAAGACAGCCCTGAGTGCTGCGTGCAAGGAAGGCCCCGCTTCGGCGGGGCTTTTCTTTGTC

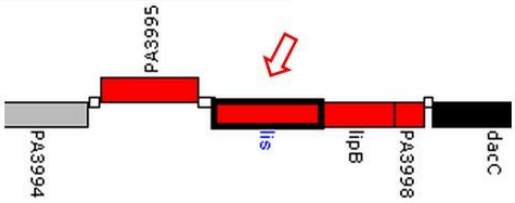
CGTTCGGAAGGCCGCGGGCAGTGGGTCGTCCGGTTA

e

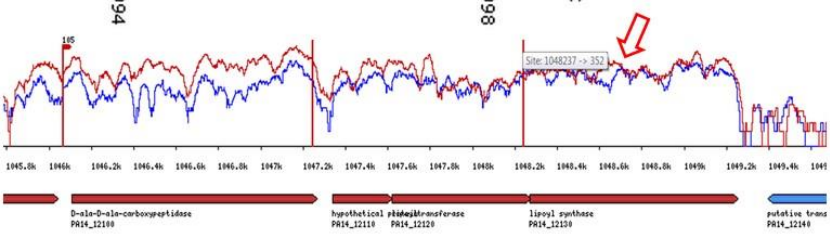
Supplementary Figure 7 - #19

#19 lis, PA3996 : lipoate synthase

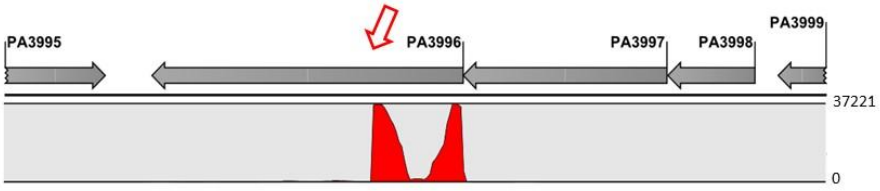
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

PA3997→ +1 +20S/D +34 PA3998→

GAGCTCGTCTCACGCCTTGGCTACGCTGAACAGAAGACCCTTACGGGCCGGATCGAACTGACATGAGCACTGTTGTG

+66 +68

GAGAAGTCCGGAGAAGCCAAGCCGGGCAAGGTGGAAGTCGGCGTGAAGCTGCGCGGCGCGGAAAAGGTCGCGCG

+159 +173

GATTCCGGTGAAGATCATTCCCACCGAGGAACTGCCGAAGAAACCCGACTGGATCCGCGTGCGCATCCCGGTTTCCCC

CGAGGTCGACCGCATCAAGCAACTGCTGCGCAAGCACAAGCTGCACAGCGTCTGCGAAGAGGCGTCCTGCCCGAACC

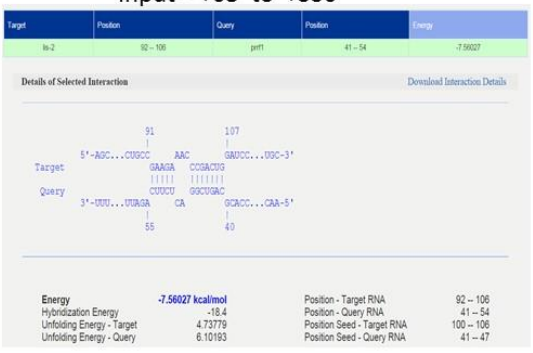
+333

TGGGCGAGTGCTTCTCCGGCGGCACCGCGACCTTCATGATCATGGGCGATATCTGC.....

e

* Input = +1 to +333

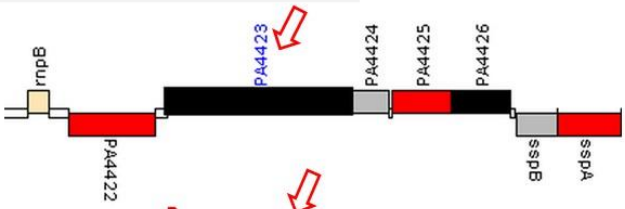
* Input = +68 to +330



Supplementary Figure 7 - #20

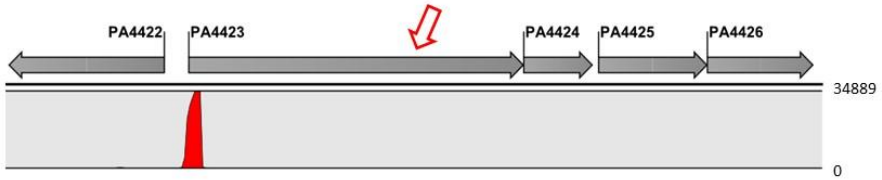
#20 PA4423 : putative lipoprotein

a PAO1



b PA14 (RNA-seq)

c PAO1 (GRIL-seq)



d

+1 S/D +31

CCATCGCACCCGAGGCGGGCCTCCGGTACAATCGGCGACTCTTCGTGAATGCGCCATGAGAAGCCCTATATGATCGCTT

+90 +96

GCCTGCGTCCGCTTTCTGCTCTGATCCTGGCCGGTCTGCTGACCGCCTGCGCGACCTCGTCCAATTCCGGACTGGGCGA

+161

ACTTCCCCGTACGCCGAATGCCAGCATCGAGCAGTTGTTGCAACAGGCCAGCCAGAGCAAGCCCGAGGAAGCGGCGC

TGCTGCGCCTCTCGGCAGCGGACCTTGCCTACCAGCAGAAGGACCTCGCCAGGTCCACCGCCATCCTGGGACAGATTC

CGCTGGAAAGCCTGAAGCCGGCCCAGCAGGTCTTCGCCAGCACCCTGAACGCCGAA

e

Target	Position	Query	Position	Energy
pa4423	90 -- 96	prrf1	5 -- 11	-5.10220

Details of Selected Interaction Download Interaction Details

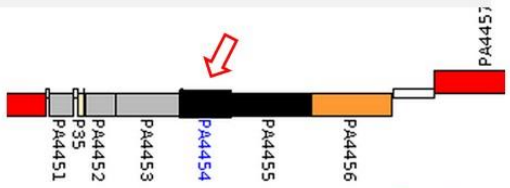
```
                    89      97
                    |       |
         5'-UCU...GCCUG       UCGUC...CGA-3'
Target                CGCGACC
                      |||||||
 Query                GCGCUGG
         3'-UUU...CUAGA       UCAA-5'
                    |       |
                    12      4
```

Energy	**-5.10220 kcal/mol**	Position - Target RNA	90 -- 96
Hybridization Energy	-14.5	Position - Query RNA	5 -- 11
Unfolding Energy - Target	3.51155	Position Seed - Target RNA	90 -- 96
Unfolding Energy - Query	5.88625	Position Seed - Query RNA	5 -- 11

Supplementary Figure 7 - #21

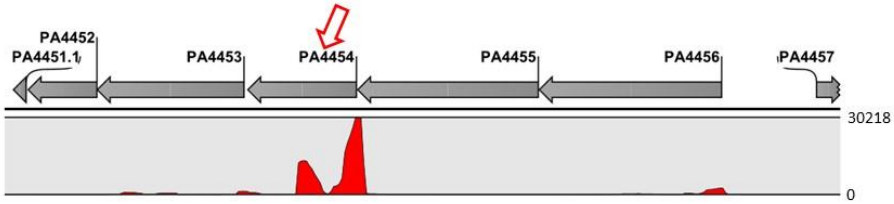
#21 PA4454 : conserved hypothetical protein

a PAO1



b PA14 (RNA-seq)

c PAO1 (GRIL-seq)



d

+1′ PA4453→ +71′

AAGGAATCAGCCGGGCGACGACCCGGACCGTGGTCTATGCCTCCCTGGCGGTGCTGGGGCTCGACTTCATTCTGACTG

+79′ S/D **PA4454**→

CTTTGATGTTTGGAGATTTC***TGA***ATGCAAACCCGCACCCTGGAAATCGGTGTCGGCCTGTTCCTCCTGGCCGGCCTGCT

GGCCCTGTTGCTGCTGGCCCTGCGGGTCAGCGGCCTGAGCGTGGGCAACGCCGGCGATACCTACAAGGTCTACGCCTA

CTTCGACAACATCGCCGGTGTTACCGTGCGCGGCAAGGTCACCCTGGCCGGCGTGACGATCGGCAAGGTGACGGCGG

+380′

TCGACCTGGATCGCGACAGCTACACTGGTCGCGTGACCATGGAGATCAACCAGAACGTGAACAACCTGCCGGTCGATT

′ : arbitrary number

e

Target	Position	Query	Position	Energy
pa4454	71 -- 79	prrf1	40 -- 48	-8.40039

Details of Selected Interaction Download Interaction Details

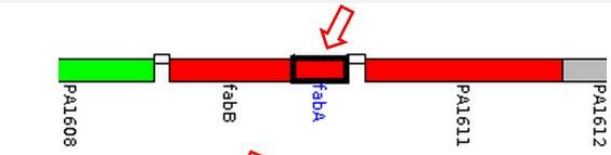
```
                    70        80
                    |         |
          5'-AAG...UUCAU        UUUGA...CUG-3'
Target                UCUGACUGC
                      ||:||||||
 Query                AGGCUGACG
          3'-UUU...UUCUC        CACCC...CAA-5'
                    |         |
                    49        39
```

Energy	**-8.40039 kcal/mol**	Position - Target RNA	71 -- 79
Hybridization Energy	-14.9	Position - Query RNA	40 -- 48
Unfolding Energy - Target	2.38923	Position Seed - Target RNA	73 -- 79
Unfolding Energy - Query	4.11038	Position Seed - Query RNA	40 -- 46

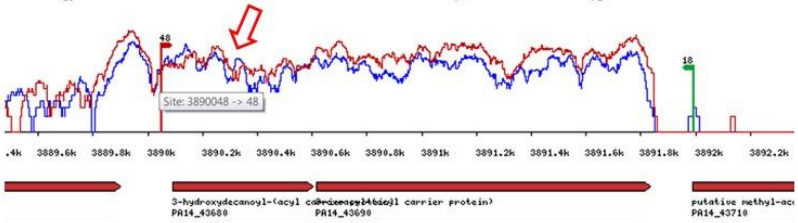
Supplementary Figure 7 - #22

#22 fabA, PA1610 : 3-hydroxydecanoyl-ACP dehydratase

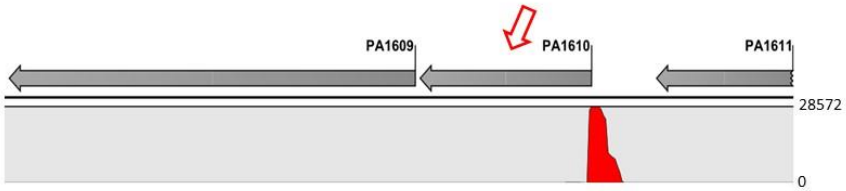
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

+1 +29 S/D +42

TACAATAACCCGGCGCGACGGCCGCTGGACGAACCGCCACAACCCTGCAGTTCAGGGATTTTTGAGGAGCTCGCATG

ACCAAACAACACGCCTTCACCCGAGAAGACCTGCTGCGCTGCAGTCGCGGCGAGCTGTTCGGCCCGGGTAACGCGCA

+138

ACTTCCCGCCCCCAACATGCTGATGATCGATCGCATCGTTCACATCAGCGATGTCGGCGGCAAGTATGGCAAG.....

e

Target	Position	Query	Position	Energy
pa1610	29 -- 42	prrf1	43 -- 57	-4.55214

Details of Selected Interaction Download Interaction Details

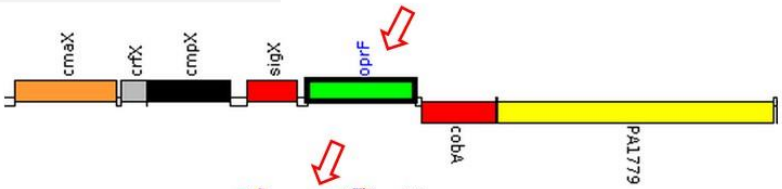
```
                    28              43
                     |               |
          5'-ACC...GGAUU         CU    AUGAC...AUG-3'
Target                 UUUGAGGAG  CG C
                       |:|||:|||  || |
 Query                 AGACUUCUC  GC G
          3'-UUU...CUAUU         AG  U ACGCA...CAA-5'
                     |               |
                    58              42
```

Energy	**-4.55214 kcal/mol**	Position - Target RNA	29 -- 42
Hybridization Energy	-15.2	Position - Query RNA	43 -- 57
Unfolding Energy - Target	4.37064	Position Seed - Target RNA	31 -- 37
Unfolding Energy - Query	6.27721	Position Seed - Query RNA	49 -- 55

Supplementary Figure 7 - #23

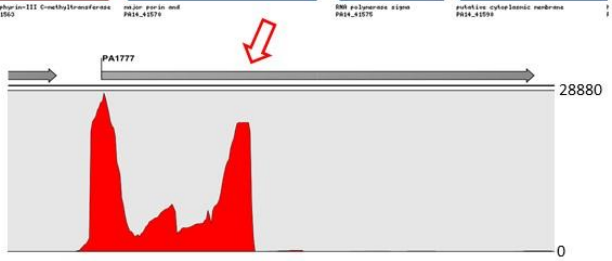
#23 oprF, PA1777 : porin

a PAO1



b PA14 (RNA-seq)

c PAO1 (GRIL-seq)



d

+1

AATATTGTCTCTCTATGCGGGAAGTTCTGATAAACTTGCCACCCAAGTTGTGCGGCTGATTGTTGGACAACTAACTGACC

+42 S/D +31 +100

ATCAAGATGGGGATTTAACGGATGAAACTGAAGAACACCTTAGGCGTTGTCATCGGCTCGCTGGTTGCCGCTTCGGC

AATGAACGCCTTTGCCCAGGGCCAGAACTCGGTAGAGATCGAAGCCTTCGGCAAGCGCTACTTCACCGACAGCGTTCG

+270

CAACATGAAGAACGCGGACCTGTACGGCGGCTCGATCGGTTACTTCCTGACCGACGACGTCGAGCTGGCGCTGTCCTA

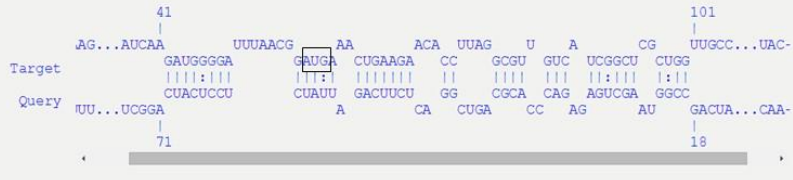
CGGTGAGTACCATGACGTTCGTGGCACCTACGAAACCGGCAACAAGAAGGTCCACGGCAACCTGACCTCCCTGGACG

CCATCTACCACTTCGGTACCCCGGGCGTAGGTCTGCGTCCGTACGTGTCGGCTGGTCTGGCTCACCAGAACATCAC.....

e

Target	Position	Query	Position	Energy
pa1777	42 -- 100	prrf1	19 -- 70	-15.25010

Details of Selected Interaction Download Interaction Details

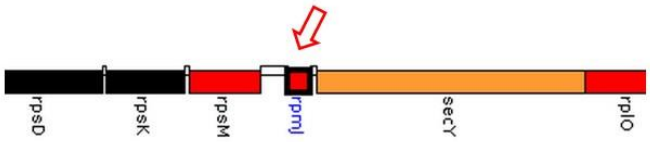


Energy	**-15.25010 kcal/mol**	Position - Target RNA	42 -- 100
Hybridization Energy	-49.4	Position - Query RNA	19 -- 70
Unfolding Energy - Target	18.12010	Position Seed - Target RNA	64 -- 70
Unfolding Energy - Query	16.03980	Position Seed - Query RNA	50 -- 56

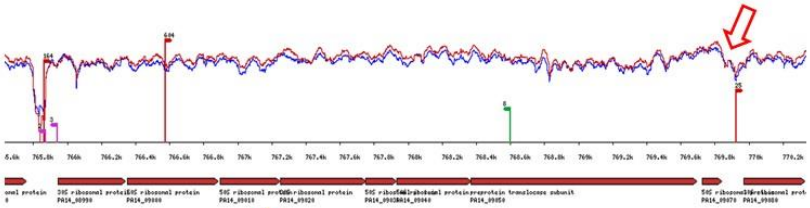
Supplementary Figure 7 - #24

#24 rpmJ, PA4242 : 50S ribosomal protein L36

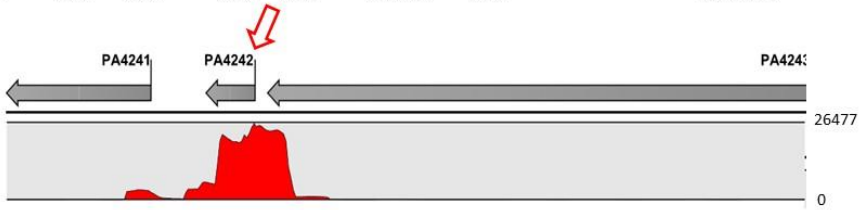
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

PA4243→

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TCTGCCTGCTGCCCCAGTTCCTGGTGGTTGCGGCGCACGTGCCGTTCTATCTTGGCGGGACCTCGCTGTTGATCGTGGT

+1 ′ +58 ′ +74 ′

CGTGGTTGTCATGGACTTTATGGCCCAGGTGCAATCTCACCTCGTTTCTCACCAGTATGAATCCCTGATGAAGAAAGCC

S/D PA4242→

AACCTGAAGGGCTACGGCAGCGGCATGCTGCGCTGATAGACCCGTAAGGTTCGAGGAGTTGGTGATGAAAGTTCGTG

CATCGGTCAAGAAGCTGTGCCGTAACTGCAAAATCATCCGTCGCGATGGCATCGTGCGGGTGATCTGCAGCGCGGAAC

+260 ′

CGCGTCACAAGCAGCGCCAAGGCTGA

′ : arbitrary number

e

Target	Position	Query	Position	Energy
pa4242	58 -- 74	prrf1	63 -- 80	-8.51197

Details of Selected Interaction Download Interaction Details

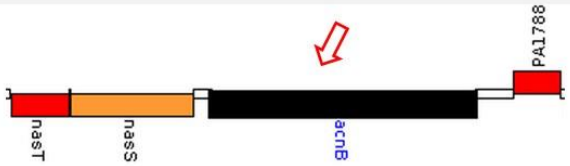
```
                    57                  75
                    |                   |
          5'-CGU...CAGUA    AUC         A  AAGCC...UGA-3'
Target                  UGA     CCUGAUGA GA
                        |||     |||||||| ||
 Query                  ACU     GGACUACU CU
          3'-UUU...UUGGC    AAUC        C  CUAUU...CAA-5'
                    |                   |
                    81                  62
```

Energy	**-8.51197 kcal/mol**	Position - Target RNA	58 -- 74
Hybridization Energy	-14.4	Position - Query RNA	63 -- 80
Unfolding Energy - Target	2.94105	Position Seed - Target RNA	65 -- 71
Unfolding Energy - Query	2.94698	Position Seed - Query RNA	66 -- 72

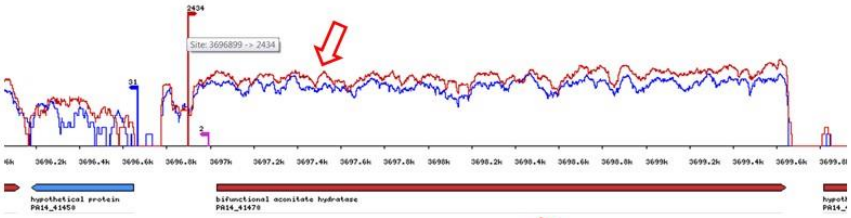
Supplementary Figure 7 - #25

#25 acnB, PA1787 : aconitate hydratase 2

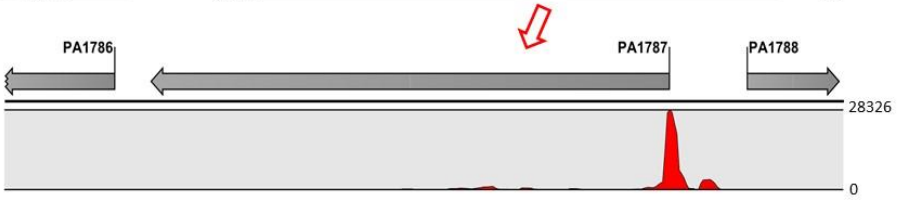
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

+1

TCTCGCCAGCCCCACCCCAGCCCGCCGGCGTAAAACTTTGAAAACAGCTTCTGTCTGGAACCTGCCGATGCCCTCGTAG

+122

GTTGGTGGATTACCGTCCACCTGGGCCTGACGAGCGGATCTGCGTCGGTTCCTGGATACGTGTCCCCCGGGACCACTG

S/D +131 +201

ATGAGGGAAATACTGTGCTTGAAGCCTACCGCAAACACGTAGAAGAGCGTGCCGCCCAGGGTGTCGTGCCCCAACCG

CTGAACGCCGAACAGACCGCAGGCCTGGTCGAGCTGCTGAAGAACCCGCCGGCCGGCGAAGAAGAATTCCT.....

e

Target	Position	Query	Position	Energy
pa1787	122 -- 131	prrf1	63 -- 72	-11.64370

Details of Selected Interaction Download Interaction Details

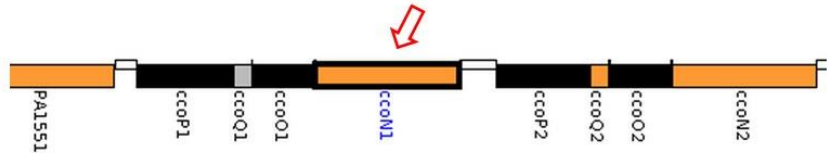
```
                    121         132
                     |           |
          5'-AAC...GACCA         AAAUA...CCG-3'
Target                CUGAUGAGGG
                      |||||||||:
 Query                GACUACUCCU
          3'-UUU...AAUCG         CUAUU...CAA-5'
                     |           |
                    73          62
```

Energy	**-11.64370 kcal/mol**	Position - Target RNA	122 -- 131
Hybridization Energy	-17.1	Position - Query RNA	63 -- 72
Unfolding Energy - Target	2.64359	Position Seed - Target RNA	125 -- 131
Unfolding Energy - Query	2.81272	Position Seed - Query RNA	63 -- 69

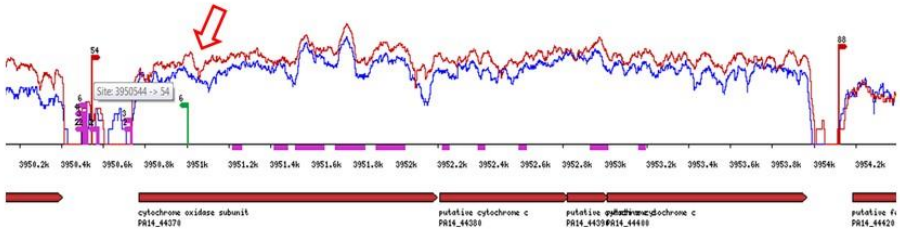
Supplementary Figure 7 - #26

#26 ccoN1, PA1554 : Cytochrome c oxidase

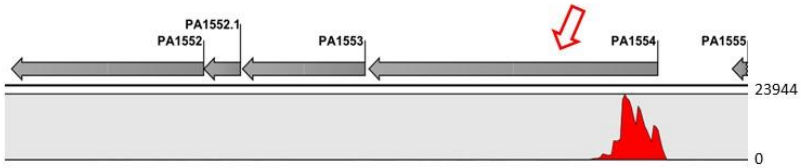
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

+1

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CCGGCCGAACCCACTCGCCCGGATAGCGCCAGAAAGCCATACGGCCGCCTTGGAAAGCGCTCTGAAGCAGGCATCAA

ACTGGCGTCGCCCCTGGCCTGGCGCGTTGCAATGCCCACCCGCTTTCTCCATACTTGCCGCCGATTTTTTGTCCCTAAAA

+208 S/D +250

AAGTCCATTAACCGTGGAACCCCTAGATGAGCACAGCAATCAGTCAGACTGCTTATAACTATAAGGTGGTCCGCCAGT

TCGCCGTTATGACGGTGGTCTGGGGGGGTCATTGGAATGGGTCTCGGTGTCCTGATCGCCGCCCAACTGGTATGGCCCG

+355

AATTGAACTTCGACCTGCCGTGGACGAGCTTCGGCCGCCTTCGCCCCCTGCACACCAACGCGGTGATCTTCGC.....

e

Target	Position	Query	Position	Energy
pa1554	208 -- 250	prrf1	40 -- 85	-11.72480

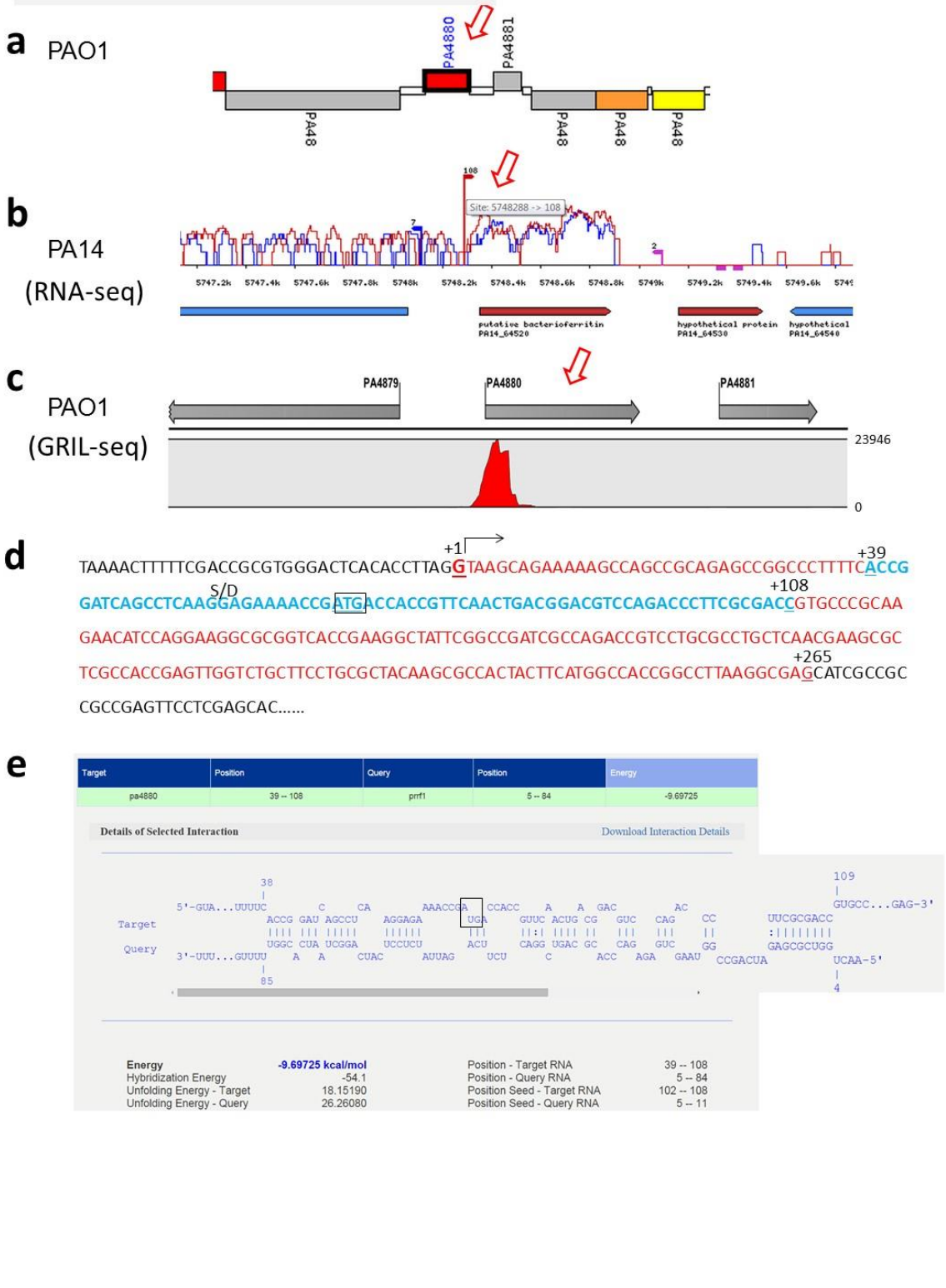
Details of Selected Interaction Download Interaction Details

```
                207                                 251
                |                                   |
      5'-AUU...CCAUU      AACC   A     CACAGC          A     UUAUA...CGA-3'
Target              AACCGUGG    CCU GAUGAG     AAUC     AGUC GACUGC
                    |||||||:    ||| ||||||     ||||     |||| ||||||
 Query              UUGGCACU    GGA CUACUC     UUAG     UCAG CUGACG
      3'-UUU...AGUUU      AAUC         CUCUA     ACUUC    G     CACCC...CAA-5'
                |                                               |
                86                                              39
```

Energy	**-11.72480 kcal/mol**	Position - Target RNA	208 -- 250
Hybridization Energy	-36.9	Position - Query RNA	40 -- 85
Unfolding Energy - Target	11.01640	Position Seed - Target RNA	209 -- 215
Unfolding Energy - Query	14.15880	Position Seed - Query RNA	78 -- 84

Supplementary Figure 7 - #27

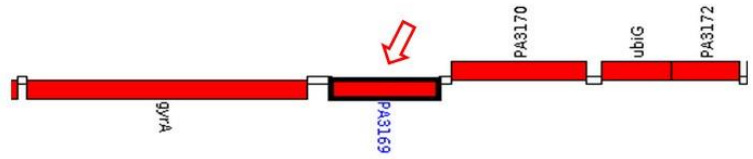
#27 PA4880 : bacterioferritin



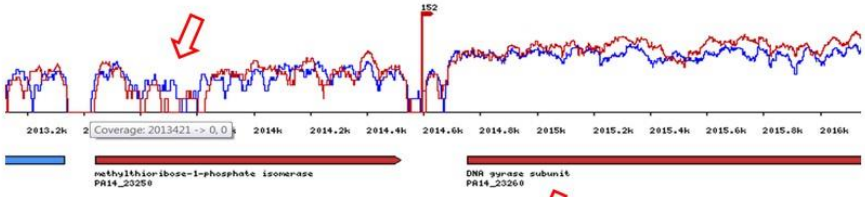
Supplementary Figure 7 - #28

#28 PA3169 : 5-methylthioribose-1-phosphate isomerase MtnA

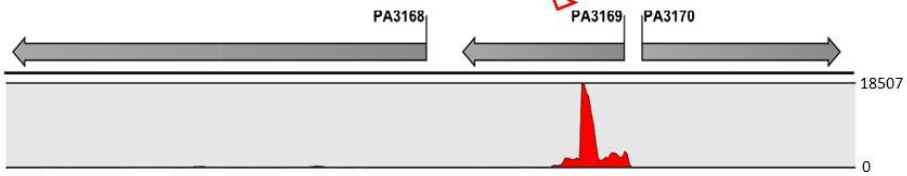
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

+1 S/D

GGGCTCGTTATAATCCGCAGCTTTTCCGTGATTCAGCGAGAGGTGTGTGATTCAGCGAGAGGTGTGTGCATGCGAGAG

CGACTGCTGGCGGCCGAGCGGGTCAAGGCGATCGAGTGGCGGGACGGTACGCTGCGGTTGCTGGATCAGCGCCTGC

+133 +140

TGCCCCAGGAGGAGGTCTGGCTCGAACACGAGTCGGCGGCCGAGGTGGCCAAGGCCATTCGCGATATGGTCGTGCG

+201

CGGCGCGCCGGCCATCGGCATCAGCGCCGCCTACGGCATCGTCCTTGGCGCCCGCGCGCGCCTGGCGCAGGGCGGCG

ACTGGCGCGCCGCGCTGGAGGAGGACTTCCGCCTGCTGGCCGACTCGCGACCCACGGCGGTCAACCTGTTCT……

e

Target	Position	Query	Position	Energy
pa3169	133 -- 140	prrf1	60 -- 67	-5.99550

Details of Selected Interaction Download Interaction Details

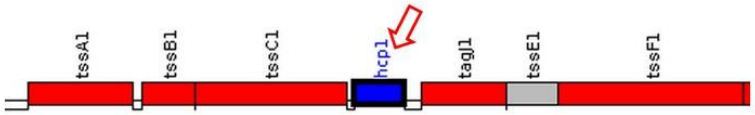
```
                    132      141
                     |        |
        5'-AUU...CCCAG        CUGGC...CGC-3'
Target                GAGGAGGU
                      ||||||:|
 Query                CUCCUCUA
        3'-UUU...GACUA        UUAGA...CAA-5'
                     |        |
                    68       59
```

Energy	**-5.99550 kcal/mol**	Position - Target RNA	133 -- 140
Hybridization Energy	-12.5	Position - Query RNA	60 -- 67
Unfolding Energy - Target	3.02997	Position Seed - Target RNA	134 -- 140
Unfolding Energy - Query	3.47453	Position Seed - Query RNA	60 -- 66

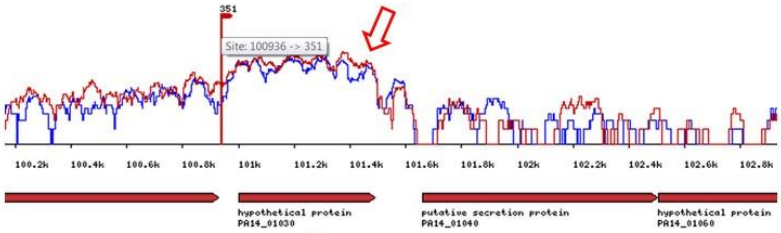
Supplementary Figure 7 - #29

#29 hcp1, PA0085 : T6SS component

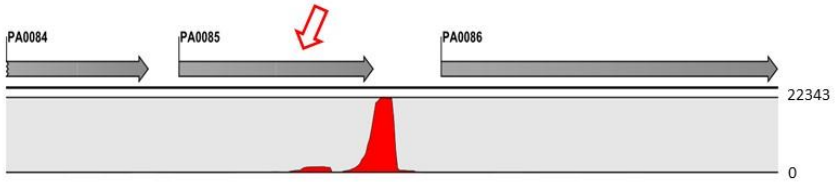
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

+1

AGCTGCCTTCGGCCAAAGAGGCCTGAGGCATTCA**A**CGACAGCGCCTGCCATGGTGGCGGGCGCTTGCATAGCGTAGT

+415

GGCGCGAGCCACACGGGAGGAAAGATGCTGTTGATATGTT.....TGGCGAGGATCGCCTGACCGAGAACGTGACCCT

GAACTTCGCCCAGGTCCAGGTCGACTACCAGCCGCAGAAGGCGGATGGCGCGAAGGACGGCGGTCCGGTCAAGTAC

+544 +560

GGCTGGAACATCCGCCAGAACGTGCAGGCCTGATGAGCCGGCTGCCGGTCATGGCGTGGCCAGGCCATGCCGTGGC

+656

CGGTGCGAGCGCCCGCCATACCGGGTGCCGAAGGCGCGCGCGGCGCGCCGCGGACCTCGTCCGCCCGACCT .....

e

Target	Position	Query	Position	Energy
pa0085	130 -- 146	prrf1	65 -- 82	-15.04160

Details of Selected Interaction Download Interaction Details

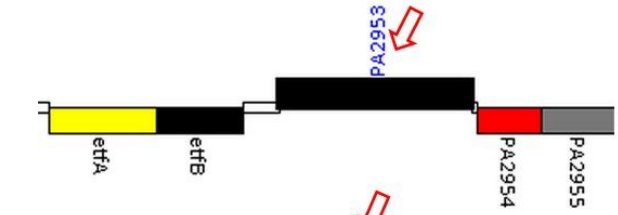
```
                    129                 147
                     |                   |
          5'-UGG...CAGAA    CA           CCGGC...CCU-3'
Target                     CGUG   GGCCUGAUGAG
                           ||||   :||||||||||
 Query                     GCAC   UCGGACUACUC
          3'-UUU...UUUUG    UAA           CUCUA...CAA-5'
                     |                   |
                    83                  64
```

Energy	**-15.04160 kcal/mol**	Position - Target RNA	130 -- 146
Hybridization Energy	-24.8	Position - Query RNA	65 -- 82
Unfolding Energy - Target	6.76085	Position Seed - Target RNA	140 -- 146
Unfolding Energy - Query	2.99753	Position Seed - Query RNA	65 -- 71

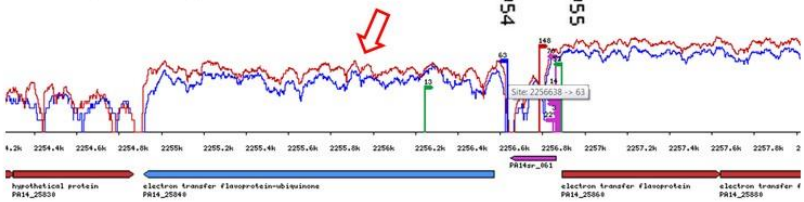
Supplementary Figure 7 - #30

#30 PA2953 : electron transfer flavoprotein-ubiquinone oxidoreductase

a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)

d

+1 +37

GGACCTGCCCATGCAGCTATATATAATGCGCCCGCTCTGATCAGGGAAAGATCTCAGCCACCAATAAACAGATACCGTG

S/D +63

AGCCTTGAGTAGGAGATCGATGTGGAACGCGAATACATGGAATTCGACGTCGTCATCGTCGGCGCCGGCCCCGCCGG

TCTGTCCGCCGCATGCCGACTGAAGCAGAAGGCCGCCGAAGCCGGTCAAGAGATCAGCGTCTGCGTGGTCGAGAAG

GGTTCCGAAGTGGGCGCCCACATCCTCTCCGGCGCGGTGTTCGAGCCGCGCGCGCTGAACGAGCTGTTCCCCGACTG

+278

GAAGGAACTCGGCG.....

e

Target	Position	Query	Position	Energy
pa2953	37 -- 63	prrf1	60 -- 88	-14.95380

Details of Selected Interaction Download Interaction Details

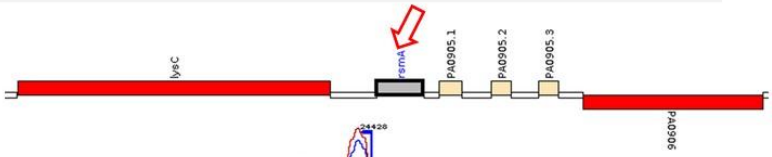
```
                    36                          64
                    |                           |
          5'-GCU...UAAAC   U             U  GU      CGAUG...GGA-3'
Target                AGA ACCGUGA   GCCU GA  AGGAGAU
                      |:| |||||||   |||| ||  |||||||
 Query                UUU UGGCACU   CGGA CU  UCCUCUA
          3'-UUU...CCCAG   U       AAU       AC      UUAGA...CAA-5'
                    |                           |
                    89                          59
```

Energy	**-14.95380 kcal/mol**	Position - Target RNA	37 -- 63
Hybridization Energy	-27.4	Position - Query RNA	60 -- 88
Unfolding Energy - Target	5.32882	Position Seed - Target RNA	57 -- 63
Unfolding Energy - Query	7.11738	Position Seed - Query RNA	60 -- 66

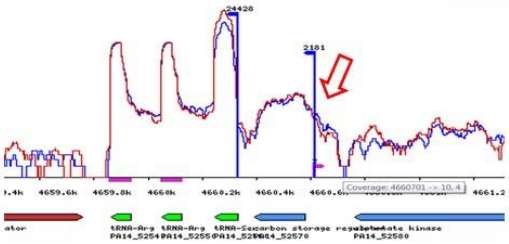
Supplementary Figure 7 - #31

#31 rsmA, PA0905 : RNA-binding global regulator

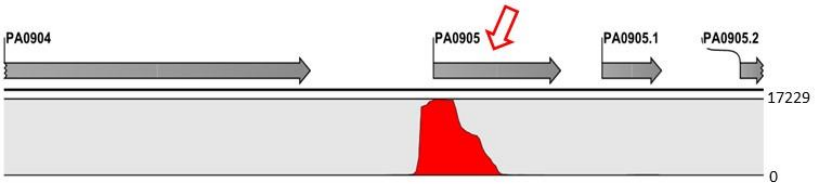
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

+1

TTCATTCCGGCGGGACTGGTCAATACTG**G**GTGAAGGATCGCGCTCTTGATTTCTGCGGATCCGCCGCCATTTCTTTTTTG

+78 S/D +106

CAGACTGTTGTCCTGAAATATTCGCGTGAGGAGAAAGGAATGCTGATTCTGACTCGTCGGGTCGGAGAGACCCTGAT

GGTAGGTGACGACGTCACCGTGACGGTACTGGGTGTCAAAGGGAACCAGGTGCGCATCGGCGTCAACGCGCCGAAG

+206

GAAGTCGCCGTACACCGGGAGGAAATTTACCAGCGCATCCAGAAAGAGAAAGATCAAGAGCCAAACCAT***TAA***TTTTTA

TCTAATTTTCCCTTTGCAAACGGGGTAAAGATGGGTATCATGCGCCCCGTGTTGCGGAGAGGTGGCCGAGTGGCCGAA

e

Target	Position	Query	Position	Energy
pa0905	78 -- 106	prrf1	42 -- 69	-7.39943

Details of Selected Interaction Download Interaction Details

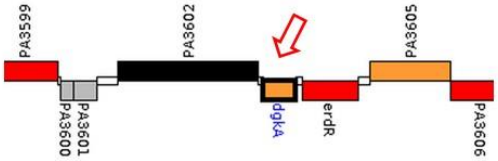
```
                  77                          107
                  |                           |
        5'-GGU...UUCGC        A  GAA  CU  U     CGUCG...AGG-3'
Target              GUGAGGAGA AG   UG  GA UCUGACU
                    :||||||||| |:   ||  || ||:||||
 Query              UACUCCUCU UU   AC  CU AGGCUGA
        3'-UUU...CGGAC        A  AG   UU  C     CGCAC...CAA-5'
                  |                           |
                  70                          41
```

Energy	**-7.39943 kcal/mol**	Position - Target RNA	78 -- 106
Hybridization Energy	-25.9	Position - Query RNA	42 -- 69
Unfolding Energy - Target	7.75759	Position Seed - Target RNA	100 -- 106
Unfolding Energy - Query	10.74300	Position Seed - Query RNA	42 -- 48

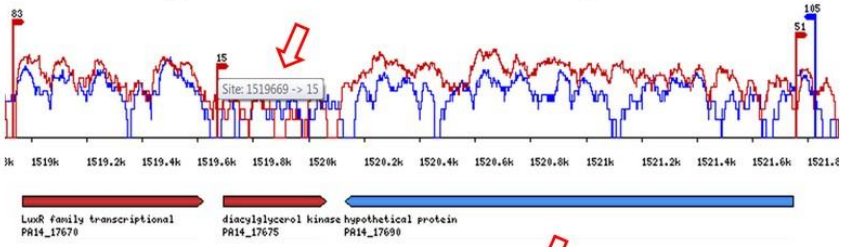
Supplementary Figure 7 - #32

#32 dgkA, PA3603 : diacylglycerol kinase

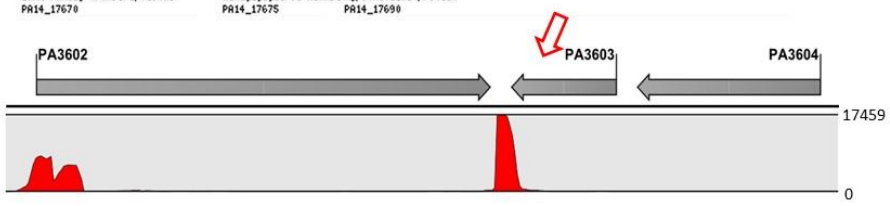
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

+1

GACCAGCCGGCGGCTAGACTGCCGGC**C**TTCCGAATCGATGAGAACCCGACG**TG**TCGCCTTCC……GCGTCTCGCTGGA

ACGCCACCCTCTGTCGAAGAATGCCAAGGACATGGGCAGCGCCGCGCAGTTCGTGGCCCTCACCGTGATCACCGTGAC

+391 +408 +446

CTGGGCGACCATCCTGCTG**G**GC*TGA*TCTACGCGGACATGAAAAAGCCGGAGGCAGCGATGCCTCCGGCTTTTTC**T**TG

CGTGCGA……

e

Target	Position	Query	Position	Energy
pa3604	98 -- 115	prrf1	4 -- 20	-7.19701

Details of Selected Interaction Download Interaction Details

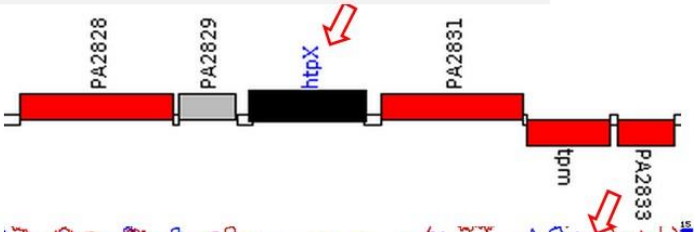
```
                  97              116
                  |               |
        5'-ACG...UGCUG         A    GA  UGAAA...CUU-3'
Target                GGCUGAUCU CGCG  CA
                      ||||||||| ||||  ||
 Query                CCGACUAGA GCGC  GU
        3'-UUU...AAUGG               UG  CAA-5'
                  |                   |
                  21                  3
```

Energy	**-7.19701 kcal/mol**	Position - Target RNA	98 -- 115
Hybridization Energy	-27.1	Position - Query RNA	4 -- 20
Unfolding Energy - Target	8.16544	Position Seed - Target RNA	100 -- 106
Unfolding Energy - Query	11.73760	Position Seed - Query RNA	12 -- 18

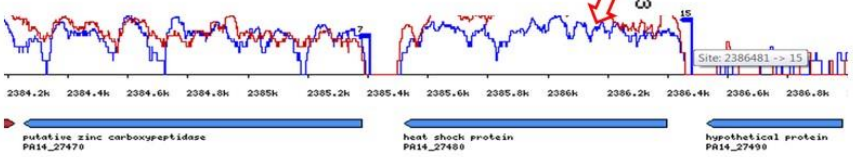
Supplementary Figure 7 - #33

#33 htpX, PA2830 : heat shock protein HtpX

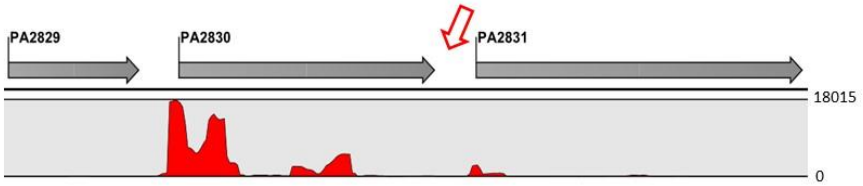
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

+1

TCGCCATGGACCGCCCCTTATATAACCCCGC**A**TGATTATCAGGAGCCCGTTCGCCCGCCCCGGCGGCCTGCGAACCGCG

+51 S/D+73 +127

CTCCGTGCCTTCAGCCGTGAGGAGAAGCTTTACACC**ATG**ATGCGCATCCTGTTGTTCCTGGCCACCAACCTGGCAGTCC

+129

TGGTGATTGCCAGCATCACCCTGAAACTGCTCGGGGTGGACCGCTTCACCGGCCAGAATTACGGCAGCCTGCTGGTCT

+234 +244

TCTGCGCCGTGTTCGGTTTCGCCGGTTCGCTGGTCTCGCTGTTCATCTCCAAGTGGATGGCGAAGATGAGCACCGGTAC

+361

CGAAGTCATCAGCCAGCCGCGCACCCGTCACGAACAGTGGCTGCTGCAAACCGTCGAAGAGCTGTCCCGCGAAGCCG

GCATCAAGATGCCGGAGGTCGGCATCTTCCCCGCCTACGAGGCCAACGCCTTCGCCACCGGCTGGAACAAGAACGAC

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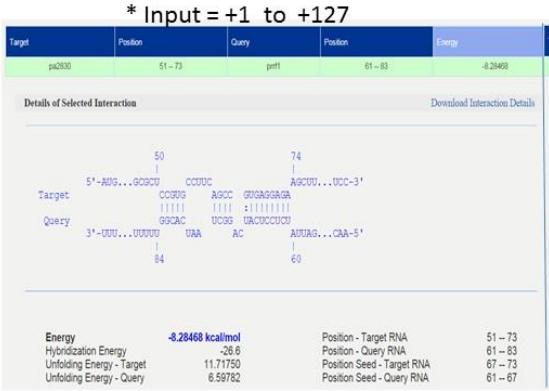
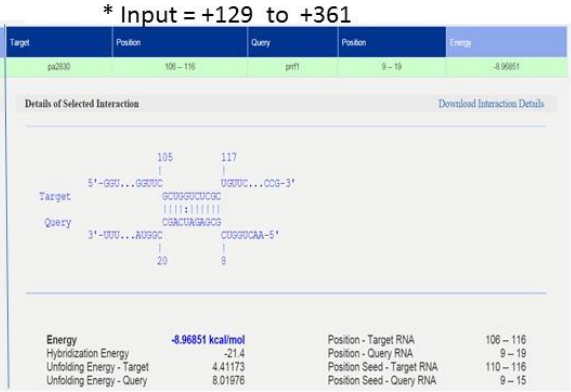
e

* Input = +1 to +127



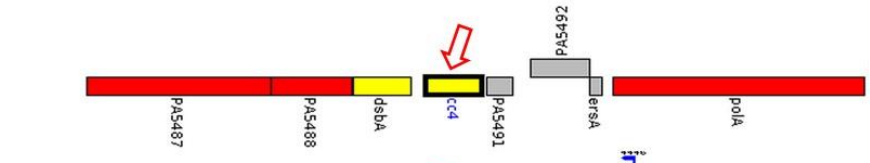
* Input = +129 to +361



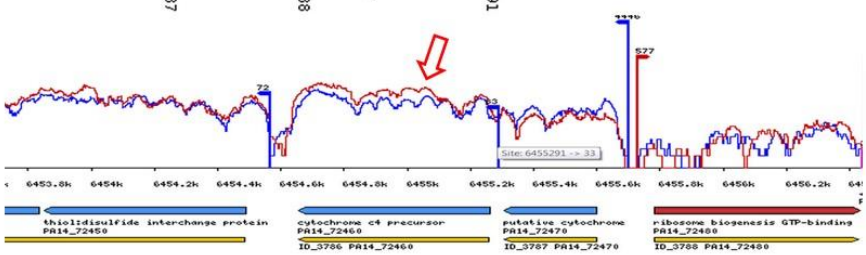
Supplementary Figure 7 - #34

#34 cc4, PA4812 : formate dehydrogenase-O, major subunit

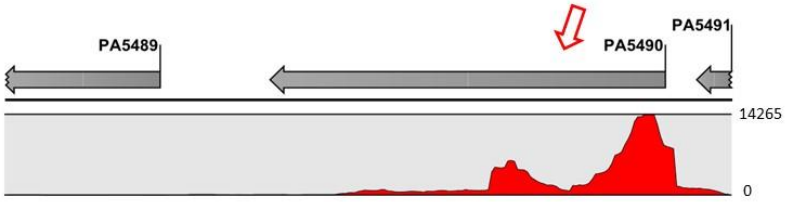
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d

+1 +11 S/D

GATGGTGAAGAAGTAGCCGAAGCCCACTATCTTTCACCCTTAGCCGTAATTGGATTAGCTGATGAACAAACTTCTCGTGAGTC

+71 +125

TGCTGTTGACCCTGGGTCTGACCGGCCTTGCCCATGCCGCTGGCGATGCCGCCGCGGGCCAGGCGAAAGCCGCGGTATGCGG

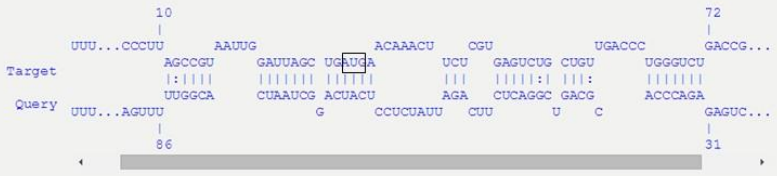
CGCCTGTCACGGTGCGGATGGCAACAGCCCGGCGCCGAACTTCCCGAAACTGGCCGGCCAGGGCGAGCGTTACCTGCTCAA

GCAGATGCATGACATCAAGGACGGCAAGCGTACCGTGCTGGAAATGACCGGCCTGCTGACCAA...

e

Target	Position	Query	Position	Energy
cc4	11 -- 71	PrrF1	32 -- 85	-17.79930

Details of Selected Interaction — Download Interaction Details

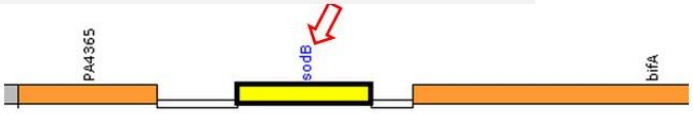


Energy	**-17.79930 kcal/mol**	Position - Target RNA	11 -- 71
Hybridization Energy	-48.6	Position - Query RNA	32 -- 85
Unfolding Energy - Target	15.79530	Position Seed - Target RNA	65 -- 71
Unfolding Energy - Query	14.96540	Position Seed - Query RNA	32 -- 38

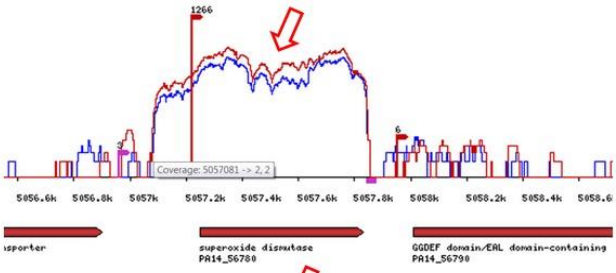
Supplementary Figure 7 - #35

#35 sodB, PA4366 : superoxide dismutase

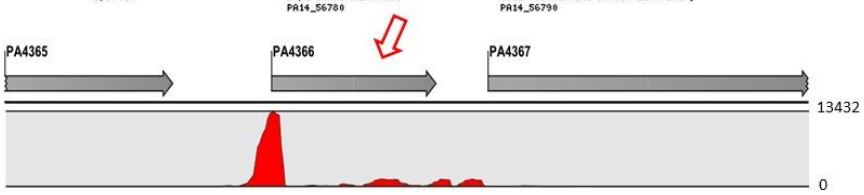
a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-Seq)



d

+1

CGTGGATATGCCCCGACACGGGTGCTATGATCCGGAGTTCGCGGCACTGGGAGCCTAGGCTCCGAGTCGCACCCGGCGCCCT

TTCCGGAGCGCTCCGCCGAACGTCCCCCCGGGGATCCAGGACGGGCTCCCGGACAGGCATCGGACCGGCCCCGTGAACCGG

+151 +157 S/D +205

TCGCGCGCTAGCCGCGCCAGTCCTGACCTGAGGAAGAATAGGAGAGACACCATGGCTTTCGAATTGCCGCCGCTGCCTTACG

+211

AAAAGAACGCCCTTGAGCCGCACATTTCCGCAGAAACCCTGGAATACCACCACGACAAGCACCACAACACCTACGTGGTGAA

CCTGAACAACCTGATCCCGGGCACCGAGTTCGAAGGCAAGAGCCTCGAAGAGATCGTCAAGAGCTCCTCCGGCGGCATCTTC

AACAACGCCGCCCAGGTGTGGAACCACACCTTCTACTGGAACTGCCTGAGCCCGAACGGCGGTGGCCAGCCCACCGG.....

e

Target	Position	Query	Position	Energy
sodB	133 -- 157	prrf1	63 -- 85	-9.22290

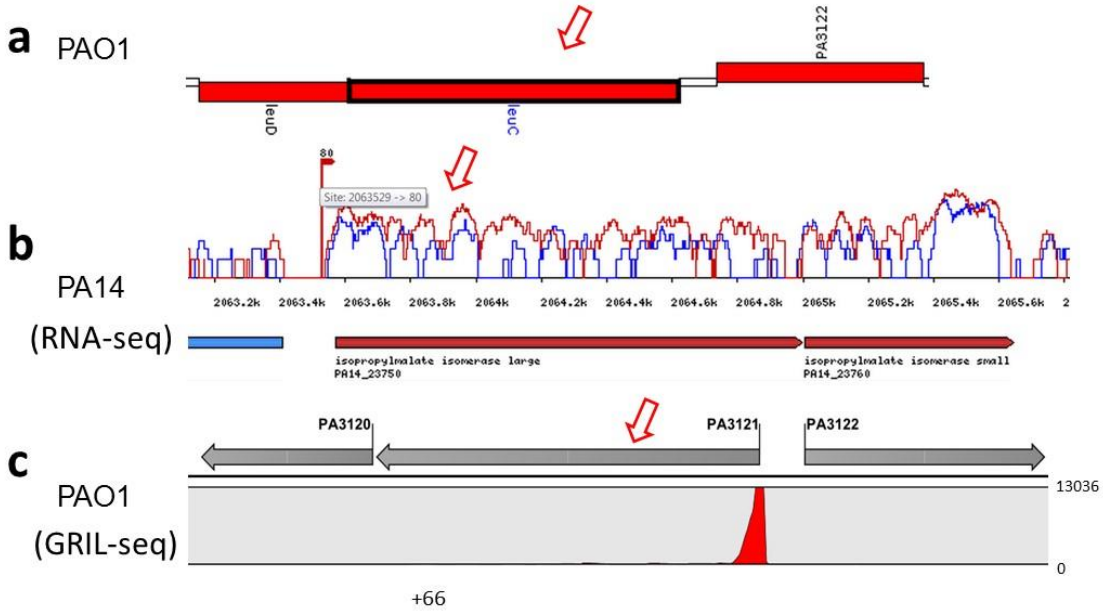
Details of Selected Interaction Download Interaction Details

```
                    132                         158
                     |                           |
          5'-GCG...GCGCU     C CC   U     CC      AGAAU...AAA-3'
Target                AGCCG G   AG CCUGA  UGAGGA
                      |:||| |   || |||||  ||||||
 Query                UUGGC C   UC GGACU  ACUCCU
          3'-UUU...AGUUU     A UAA                CUAUU...CAA-5'
                     |                           |
                    86                          62
```

Energy	**-9.22290 kcal/mol**	Position - Target RNA	133 -- 157
Hybridization Energy	-22.1	Position - Query RNA	63 -- 85
Unfolding Energy - Target	9.87189	Position Seed - Target RNA	152 -- 157
Unfolding Energy - Query	3.00521	Position Seed - Query RNA	63 -- 68

Supplementary Figure 7 - #36

#36 leuC, PA3121 : 3-isopropylmalate dehydratase large subunit



d

+1 +19 S/D+36

GAATTTGAGTTATTCGTAGACAAGCCATAGGATCATCCTCACAAGCCCTGTGGGCATAGAAATGAGCTGATGAGGAAA

TCCCGATGGCCGGCAAGACCCTCTACGACAAACTCTGGGACATGCACCTGGTCAAGCAGCGCGACGACGGTTCGGCG

CTGATCTACATCGACCGCCATATCCTGCACGAAGTGACCTCGCCGCAGGCCTTCGAGGGTCTTCGCCTGGCCGGGCGCA

+195

AGCCGTGGCGGATCGACGCCAACATCGCCACCCCGGACCACAACGTGCCGACCACCCGCACCGAGCG……

e

Target	Position	Query	Position	Energy
pa3221	19 -- 36	prrf1	63 -- 79	-11.65330

Details of Selected Interaction Download Interaction Details

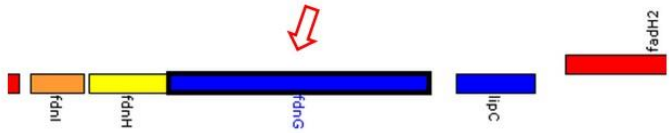
```
                    18                  37
                    |                   |
          5'-ACA...GCAUA  AAUG          AAUCC...CAA-3'
Target                GA    AGC UGAUGAGGA
                      ||    ||| |||||||||
 Query                CU    UCG ACUACUCCU
          3'-UUU...UGGCA  AA    G         CUAUU...CAA-5'
                    |                   |
                    80                  62
```

Energy	**-11.65330 kcal/mol**	Position - Target RNA	19 -- 36
Hybridization Energy	-18.9	Position - Query RNA	63 -- 79
Unfolding Energy - Target	4.32249	Position Seed - Target RNA	30 -- 36
Unfolding Energy - Query	2.92424	Position Seed - Query RNA	63 -- 69

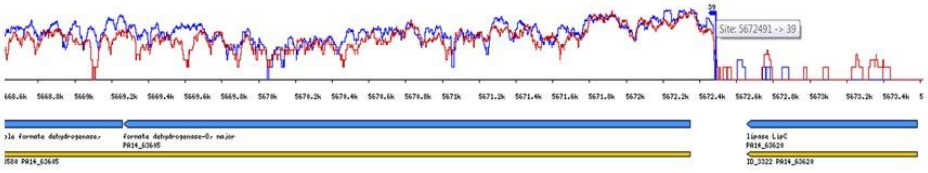
Supplementary Figure 7 - #37

#37 fdnG, PA4812 : formate dehydrogenase-O, major subunit

a PAO1



b PA14 (RNA-seq)



c PAO1 (GRIL-seq)

d

+1

TCCGCGCACCTTGCTGGACAAGGTTTGCGCCGCCGTGTAATGACTCCGGTCAAGGCAGAAAAACCCAGCGTTCGTGTAGGGTT

CGCTGCCCCGTAGCCATGCCCGGCTGGCGTACCTCGCCAGGCTGGCTATCTTTTAACCAGAGACATCCGGCCATGTCCCGGGTG

+123 S/D +137

TCCTTTGCCTGATGCAGGAGATGCACGATGGATATGAACCGTCGGCAATTCTTCAAGGTCTGCGGTATAGGCCTTGGAGGATC

+263

GAGCCTTGCGGCGCTCGGGATGGCCCCCACGGAGGCCTTCGCCGACCAGGTCCGCCACTTCAAGCTGGCGCACACGGTCGA

GAC...

e

Target	Position	Query	Position	Energy
fdnG	123 -- 137	prrF1	61 -- 74	-12.41420

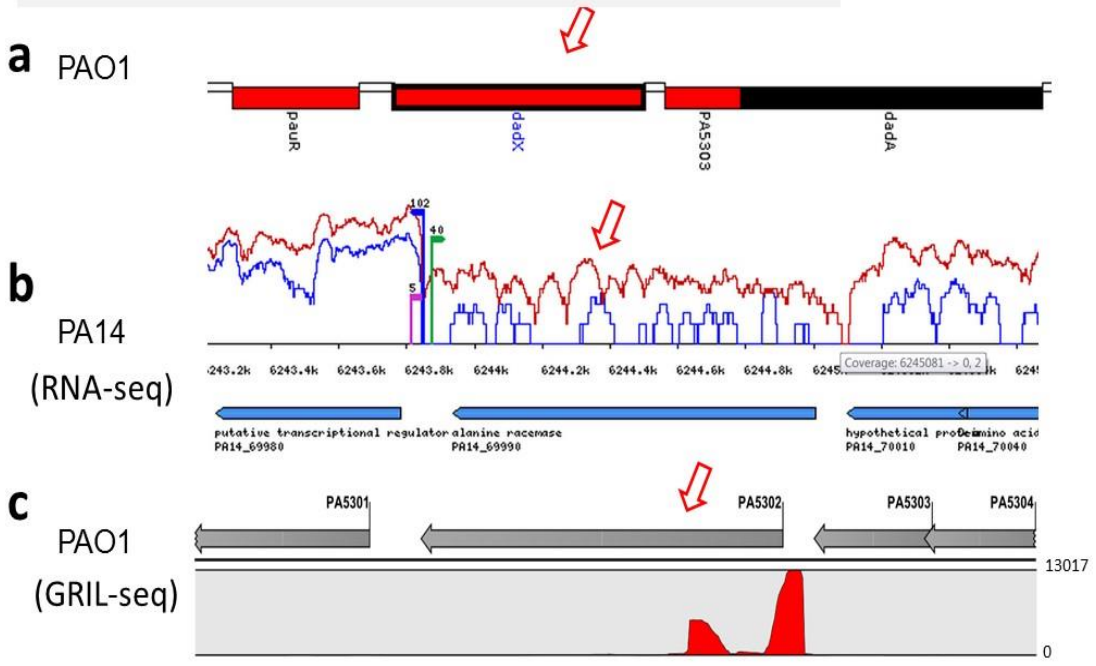
Details of Selected Interaction Download Interaction Details

```
                    122             138
                     |               |
        5'-AAG...CCUUU         C    UGCAC...AAG-3'
Target                GCCUGAUG AGGAGA
                      |||||||| ||||||
 Query                CGGACUAC UCCUCU
        3'-UUU...CUAAU               AUUAG...CAA-5'
                     |               |
                    75              60
```

Energy	**-12.41420 kcal/mol**	Position - Target RNA	123 -- 137
Hybridization Energy	-24.0	Position - Query RNA	61 -- 74
Unfolding Energy - Target	6.33575	Position Seed - Target RNA	124 -- 130
Unfolding Energy - Query	5.25009	Position Seed - Query RNA	67 -- 73

Supplementary Figure 7 - #38

#38 dadX, PA5302 : catabolic alanine racemase



d

+1 +22 +30

CGCTGCCCTGAGTTTTGTCTGGCCCGGCCGGAGAGCCGGGCTTTTTTTCGCCTGATGAAAGGTGCGCGATGCCGCAC

CTCGCTGAAGAGAGACCCGTCGCCATGCGCCCCGCCCGTGCCCTGATCGACCTGCAAGCCCTTCGTCACAACTATCGGC

+204

TGGCCCGCGAGGCCACCGGCGCCCGTGCGCTCGCGGTGATTAAAGCGGACGCATACGGCCACGGCGCGGTGCGCTGT

+239 +261

GCCGAAGCGCTGGCGGCCGAGGCCGATGGCTTCGCCGTGGCCTGCATCGAGGAGGGCCTGGAGCTGCGCGAGGCC

GGTATCCGCCAGCCGATCCTGCTGCTGGAGGGCTTCTTCGAGGCGTCCGAGCTGGAGCTGATCGTCGCCCACGACTTC

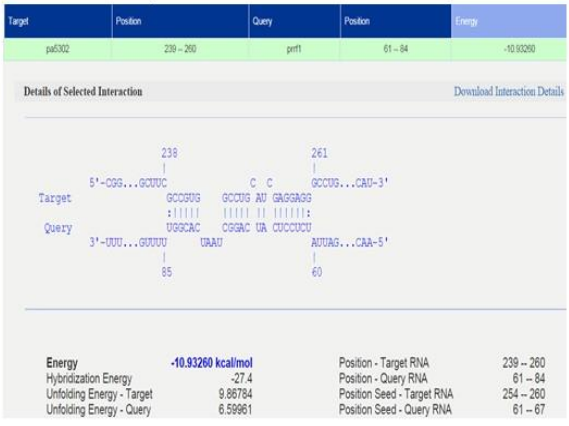
+373

TGGTGCGTGGTGCATTGCGCCTGGCAATTG.....

e

* Input = +1 to +204

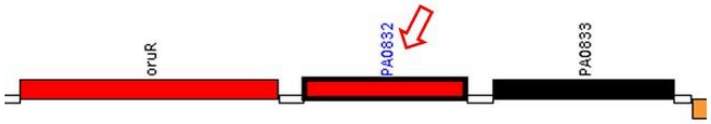
* Input = +1 to +373



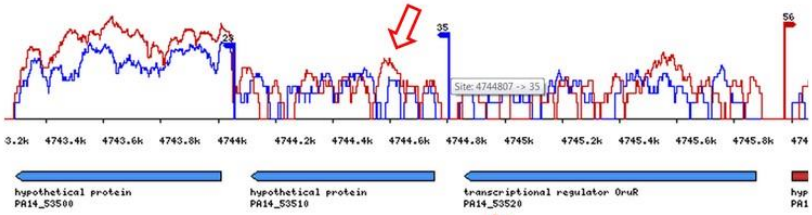
Supplementary Figure 7 - #39

#39 PA0832 : conserved hypothetical protein

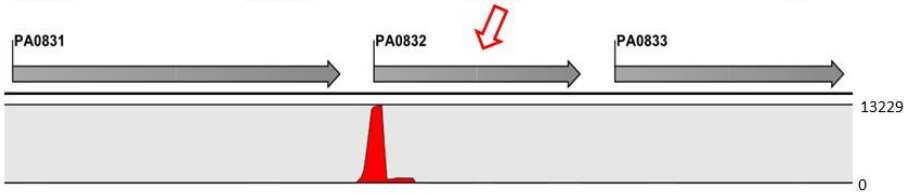
a PAO1



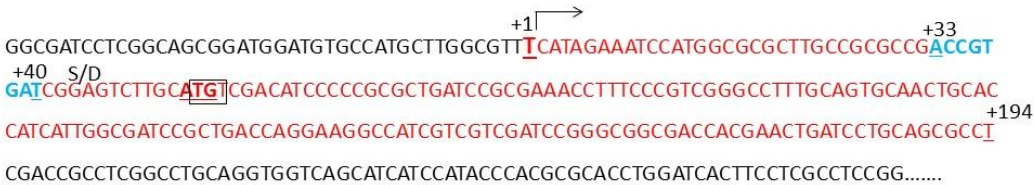
b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



d



e

Target	Position	Query	Position	Energy
pa0832	33 -- 40	prrf1	77 -- 84	-6.89877

Details of Selected Interaction Download Interaction Details

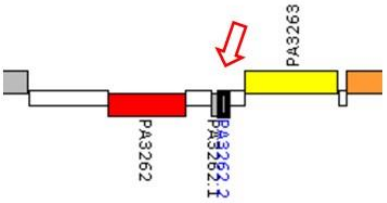
```
                    32       41
                    |        |
          5'-UCA...CGCCG       CGGAG...CCU-3'
Target                ACCGUGAU
                      ||||||||
 Query                UGGCACUA
          3'-UUU...GUUUU       AUCGG...CAA-5'
                    |        |
                    85       76
```

Energy	**-6.89877 kcal/mol**	Position - Target RNA	33 -- 40
Hybridization Energy	-11.0	Position - Query RNA	77 -- 84
Unfolding Energy - Target	2.16304	Position Seed - Target RNA	34 -- 40
Unfolding Energy - Query	1.93818	Position Seed - Query RNA	77 -- 83

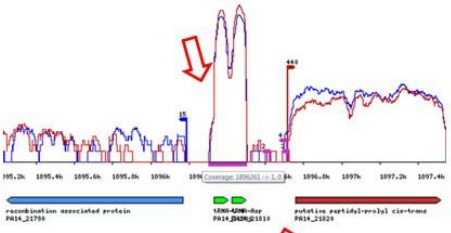
Supplementary Figure 7 - #40

#40 PA3262.2: tRNA-Val[3]

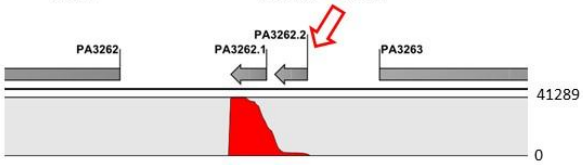
a PAO1



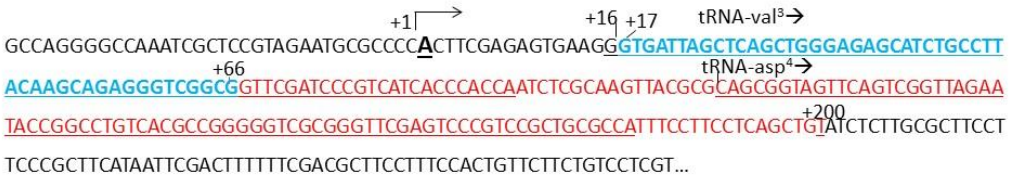
b PA14 (RNA-seq)



c PAO1 (GRIL-seq)



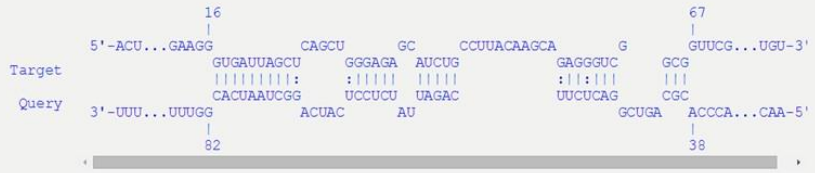
d



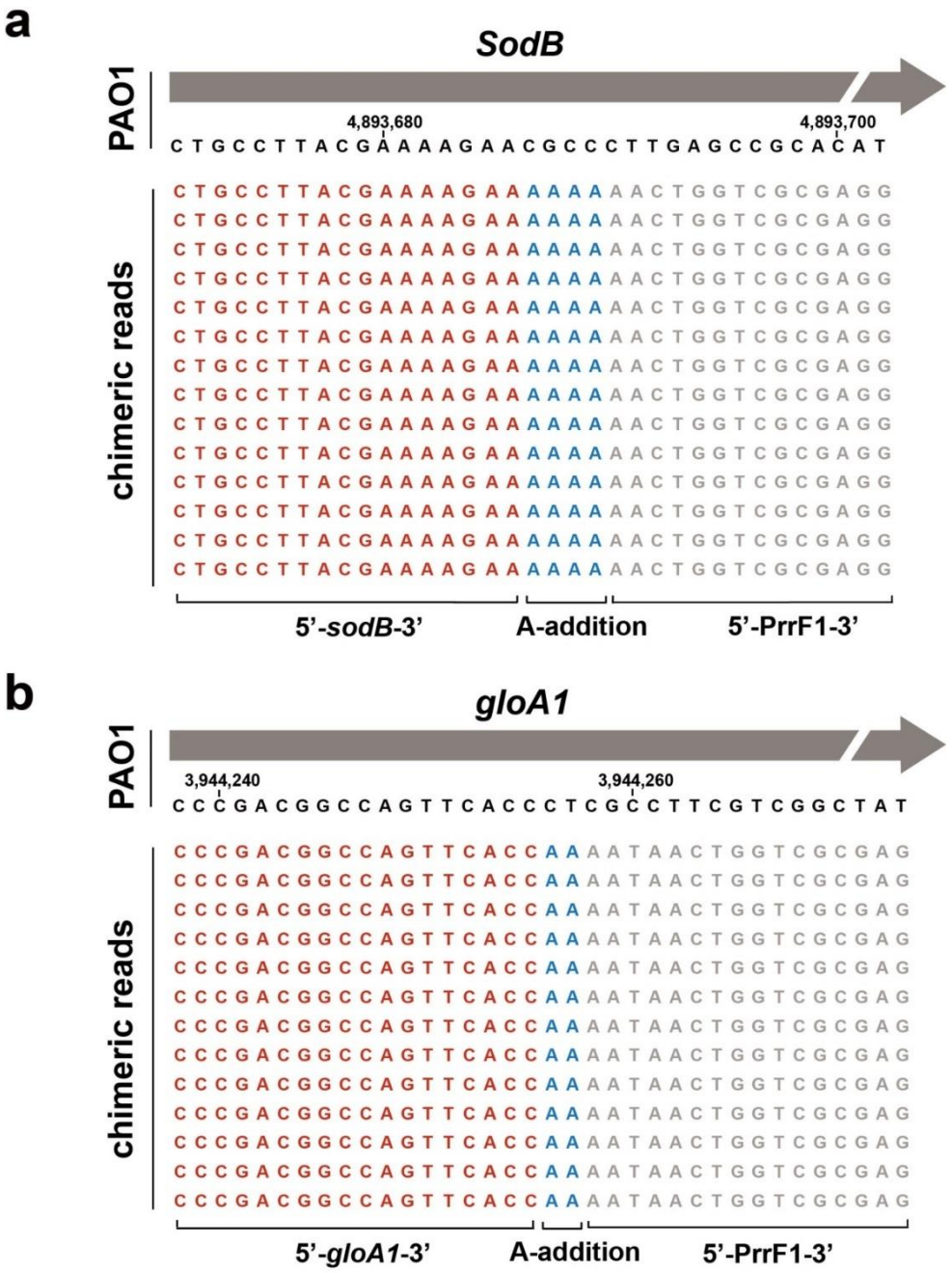
e

Target	Position	Query	Position	Energy
pa1750	17 -- 66	prrf1	39 -- 81	-9.37551

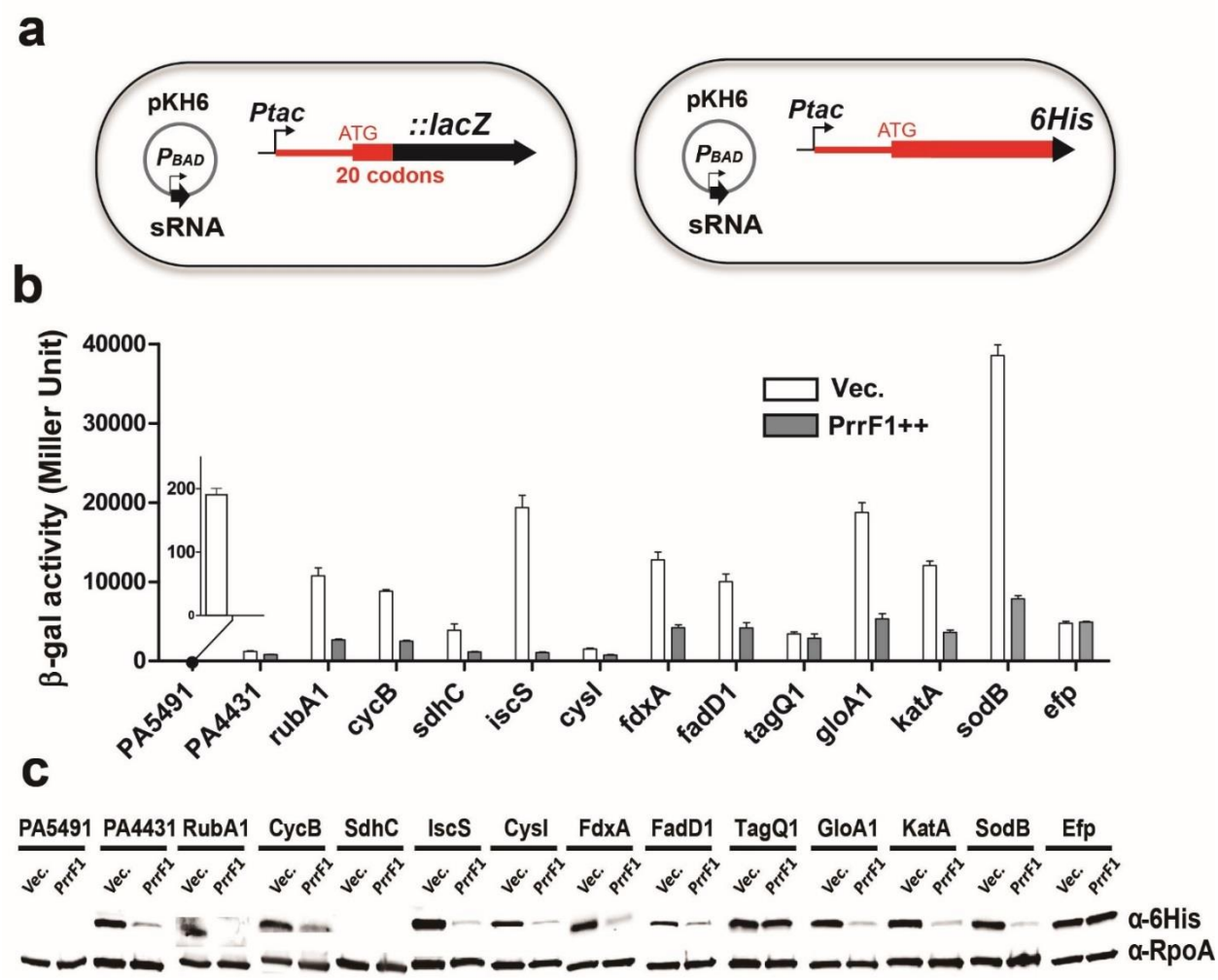
Details of Selected Interaction Download Interaction Details



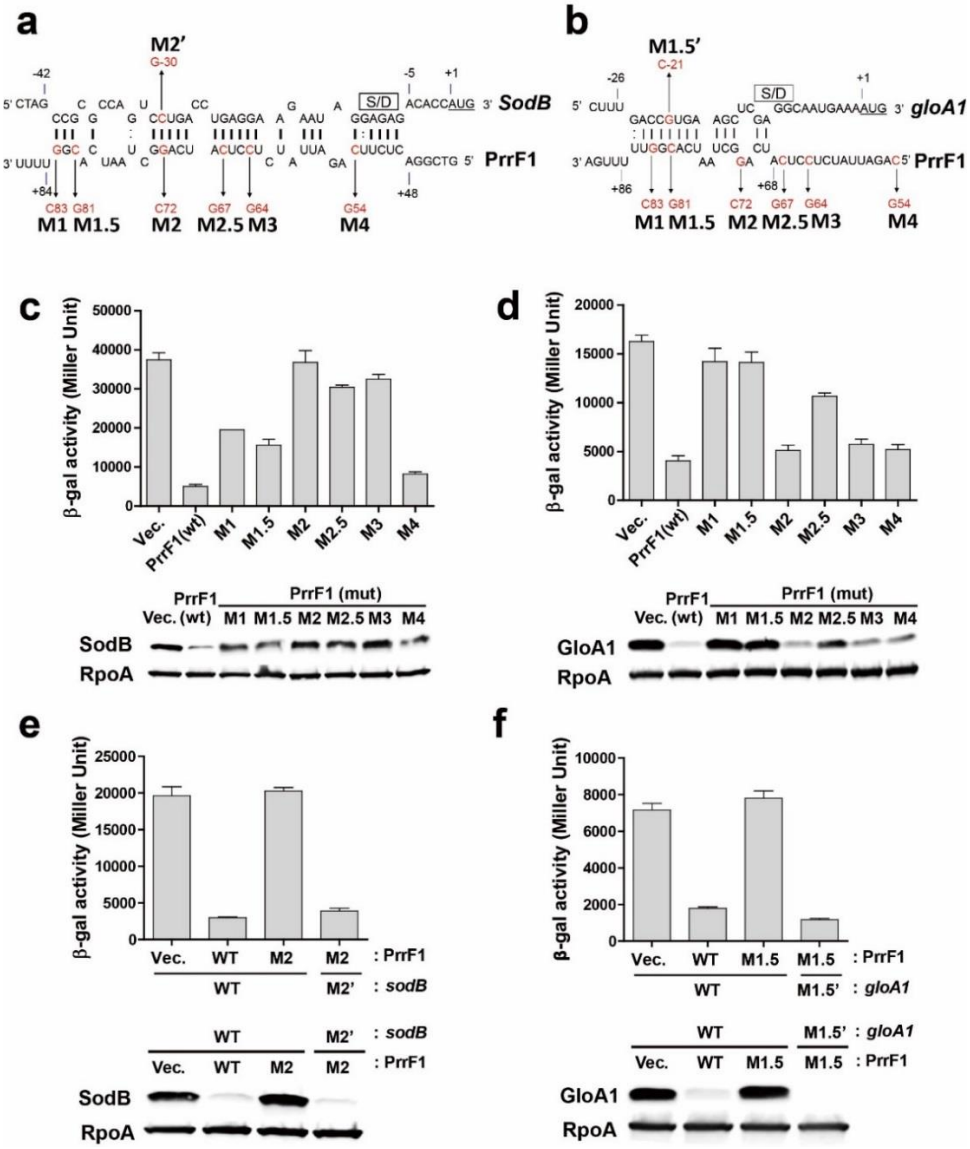
Energy	**-9.37551 kcal/mol**	Position - Target RNA	17 -- 66
Hybridization Energy	-39.6	Position - Query RNA	39 -- 81
Unfolding Energy - Target	16.74420	Position Seed - Target RNA	56 -- 62
Unfolding Energy - Query	13.48030	Position Seed - Query RNA	47 -- 53



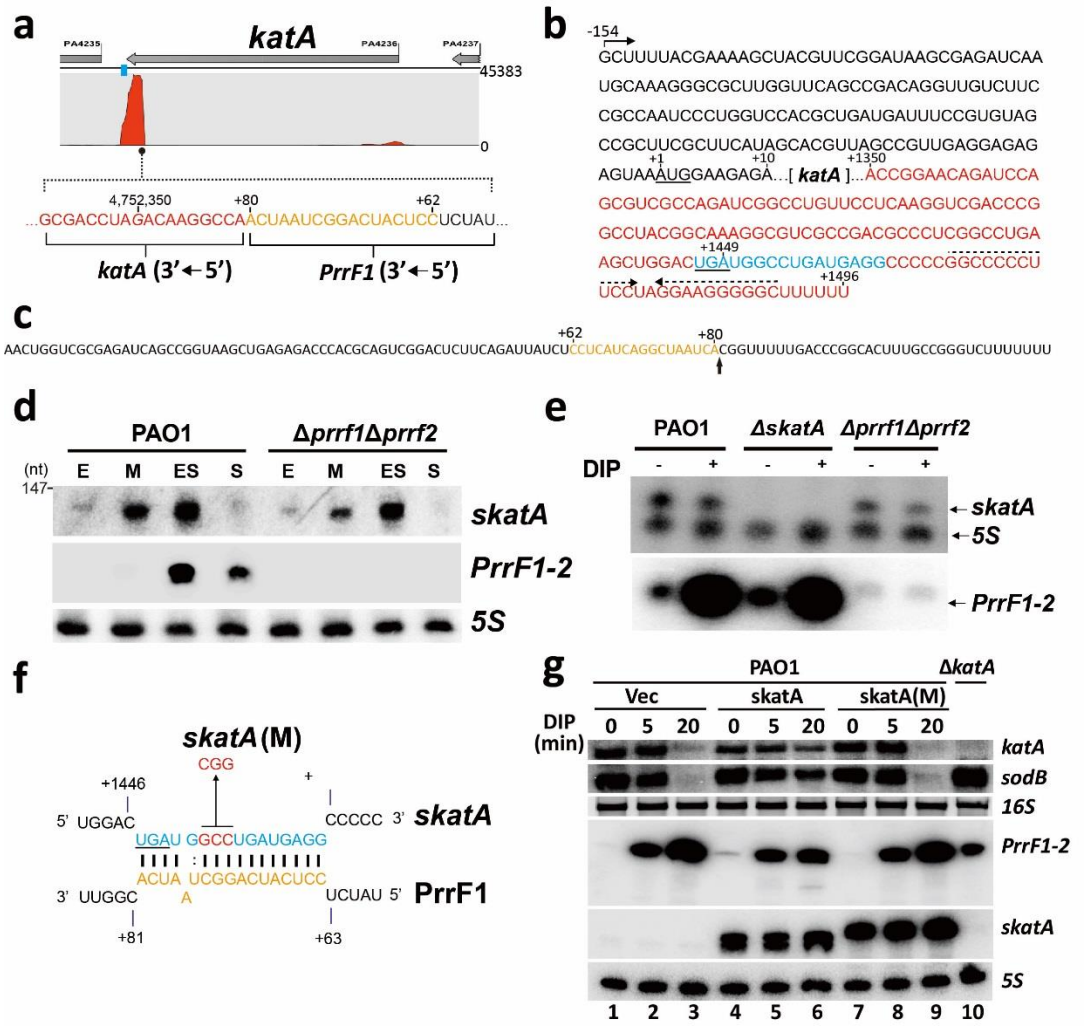
Supplementary Figure 8. Examples of A-addition (blue) at the T4 RNA ligase created junctions between PrrF1 and target transcripts. (a) A fraction of *sodB*-PrrF1 chimeric reads reveals addition of four As not found in either sequence. (b) Addition of two As detected in the *gloA1*-PrrF1 chimeras.



Supplementary Figure 9. Validation of PrrF1 targets (for top12 mRNA) obtained GRIL-Seq. (a) Schematic representation of the two types of target mRNAs translational fusions tested in *P. aeruginosa* PAO1 Δ*prrf1*Δ*prrf2*. The 5' UTRs, including codons for the first 20 amino acids of each target gene, were fused in-frame to *lacZ* (left) or the coding sequence for the entire protein and lacking the stop codons, was fused to the codons for 6His (right). The PrrF1 and mRNA expression were induced with 0.2% arabinose and 100 μM IPTG, respectively, at the mid logarithmic growth phase (OD_{600} ~1.0). After 3.5 hours, the level of the β-galactosidase activity and the Western blot signals were determined, comparing in each case the sRNA effect to that seen with the empty vector (Vec.). *SodB* and *efp* were used as positive and negative controls, respectively. (b) β-galactosidase activity of *lacZ* fusions to the top 12-PrrF1 targets. Results are the average of three independent experiments with the error bars representing the standard deviation of the average. (c) Protein levels of the top 12-PrrF1 targets determined by Western blot detection. RNA polymerase α-subunit (RpoA) was used as a loading control.

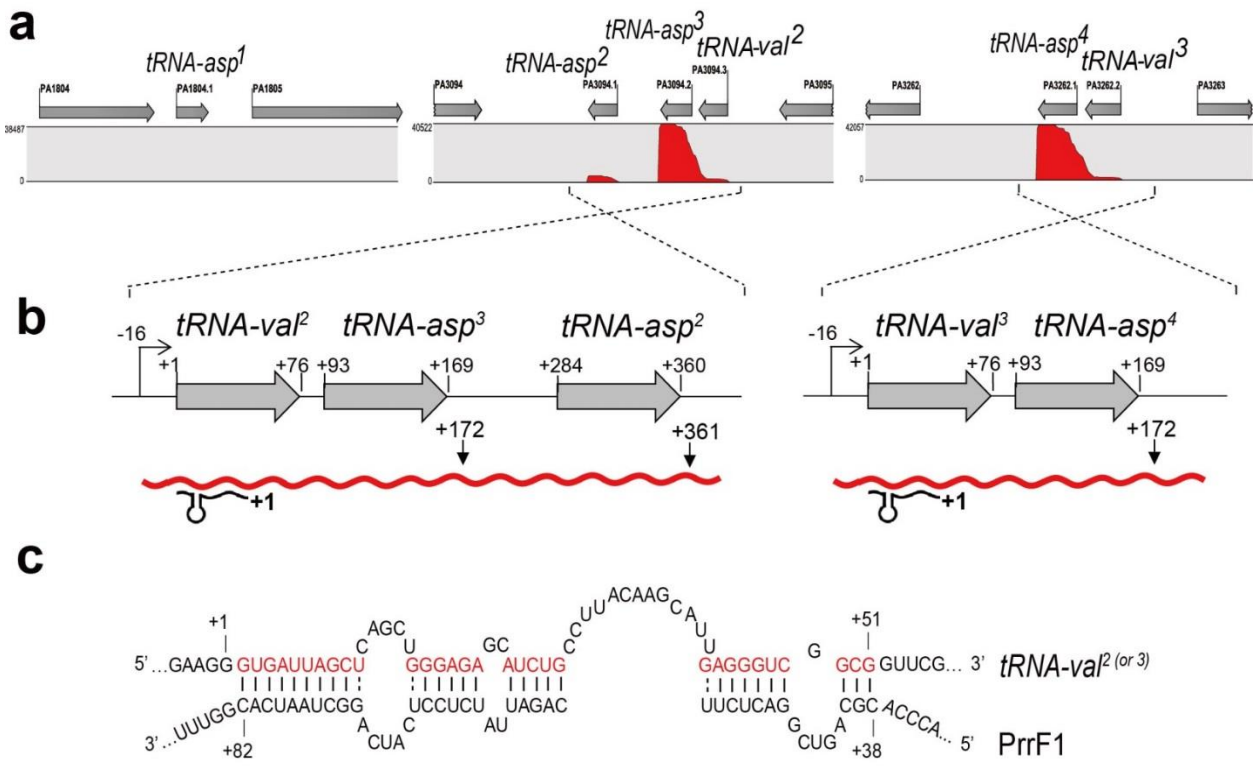


Supplementary Figure 10. Validation of predicted RNA duplexes formed by PrrF1 with *sodB* or *gloA1* mRNA. (a) Predicted RNA base pairing of PrrF1 with *sodB* or (b) *gloA1* mRNA. IntaRNA was used to predicted potential interactions. Arrows indicate the location of the substitutional mutations (in red) in the RNA. Total of six PrrF1 mutations (M1 to M4) and a single compensatory mutation (M2' or M1.5') on each mRNA was generated and analyzed using two independent assays: β-galactosidase assay using translational *lacZ* fusions and Western blot of 6His-tagged proteins. Each reporter strain PAO1 *Δprrf1Δprrf2 sodB::lacZ* or SodB::6His was transformed with the control vector (Vec.), the plasmid overexpressing PrrF1 wild type (PrrF1 (wt)), or plasmids containing the six PrrF1 mutants. (c) The level of β-galactosidase activity (upper panel) and Western blot analysis (lower panel) of *sodB::lacZ* and SodB::6His, respectively are shown. (d) Same as (c), but the level of β-galactosidase activity and the signal of Western blot of GloA1 are shown. RpoA was used as loading control. (e) Compensatory effect of *sodB (M2')::lacZ* or SodB::6His reporters with respect to PrrF1 mutant (M2) (f) Compensatory effect of *gloA1 (M1.5')::lacZ* or GloA1::6His reporter on the PrrF1 mutant (M1.5). Error bars represent the standard deviation of the average of three biological replicates

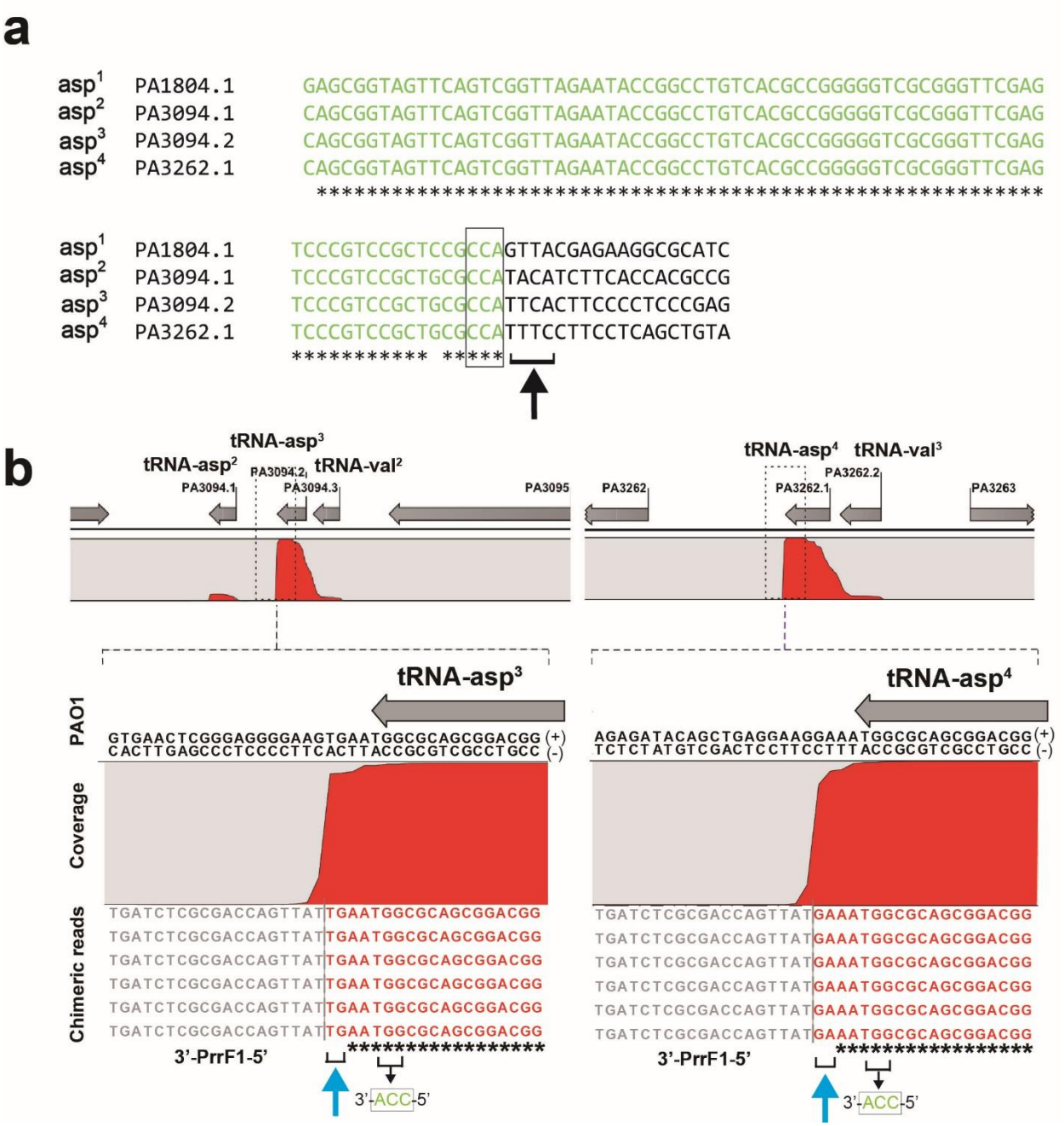


Supplementary Figure 11. Interaction of PrrF1 with the 3' end of *katA*. (a) Coverage of *katA*-PrrF1 chimeric reads in GRIL-Seq. The sequence below shows the junction between PrrF1 and katA. This information was used to construct the plasmid expressing *skatA*, ~140 nt 3' fragment of *katA*. Orange color indicates the predicted base-paring region of PrrF1 relative to 3' end of *katA*. (b) Sequence of the portion of *katA* mRNA, showing the location of *skatA* (in red). Numbers indicated are relative to the A of start codon AUG of *katA* (+1); the first nucleotide of *skatA* is at (+1350). Also shown are the *katA* stop codon (+1449) and the inverted repeat of the predicted ρ-independent terminator sequence. The predicted base-pairing sequence of *skatA* relative to PrrF1 is shown in blue. (c) The locations of the predicted base-pairing region of PrrF1 and the major ligated site with *skatA* are shown in orange and the arrow, respectively. (d) Accumulation of *skatA* at stationary phase analyzed by Northern blotting. RNA was isolated from wild type PAO1 and PAO1 lacking PrrF1 and PrrF2 (*Δprrf1Δprrf2*) at four stages of growth in LB: early, mid exponential (E, M) and early, mid stationary (ES, S) based on a growth curve generated by monitoring bacterial growth (optical density at 600 nm). 5S rRNA serves as a loading control. (e) Effect of PrrF1-2 on *skatA* levels. PrrF1 and PrrF2 were induced by addition of 2,2'-dipyridil (DIP) and the Northern blot was first probed with the 5S RNA probe followed by a *katA* probe. RNA was isolated from PAO1, *skatA* mutant and a PrrF1-2 mutant. (f) Predicted base paring of PrrF1 at 3' end of *katA* mRNA. The stop codon of *katA* mRNA is underlined. Three nucleotide mutations (GCC → CGG) to

generate *skatA* (M) are shown in red and with an arrow. (g). Effect of *skatA* overexpression on PrrF1 regulated transcripts. Strains of PAO1 harboring the empty vector (Vec.), plasmid overexpressing *skatA*, or mutant *skatA* (M) were grown to mid logarithmic phase in LB. After 15 min induction of *skatA* or mutant *skatA* (M), PrrF1 and PrrF2 were induced by addition of DIP. Cells were harvested and total RNA isolated at the time indicated, followed by Northern blot analysis, using various probes indicated on the right column. RNA from a *katA* mutant, grown overnight, was also included in the analysis (lane 10). 5S RNA and 16S RNA serve as a loading control. Results are representative of duplicate experiments.



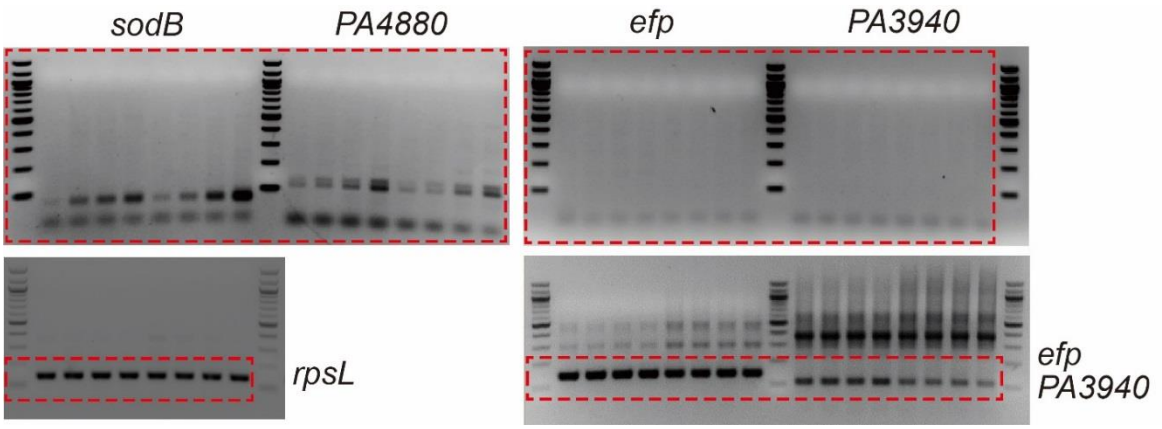
Supplementary Figure 12. PrrF1 in chimeras with tRNAs. GRIL-Seq identified three aspartic acid tRNAs (tRNA-asp^2, -asp^3 and -asp^4) ligated to PrrF1 by base-pairing with two valyl tRNAs in the un-processed precursor RNA. (a) Coverage (in red) of PrrF1 chimeric reads at locus of four tRNA-asps and two tRNA-vals in PAO1. (b) Location of the three ligation sites (arrow: +172, +361, and +172) between PrrF1 and the 3' end of tRNA-asp^2, -asp^3 and -asp^4. (c) The predicted hybridization between tRNA-val^2 (or val^3) and PrrF1 using IntaRNA. (d) DNA sequence comparison between three tRNA-vals. Red denotes the corresponding nucleotides in the transcripts involved in base-pairing with PrrF1.



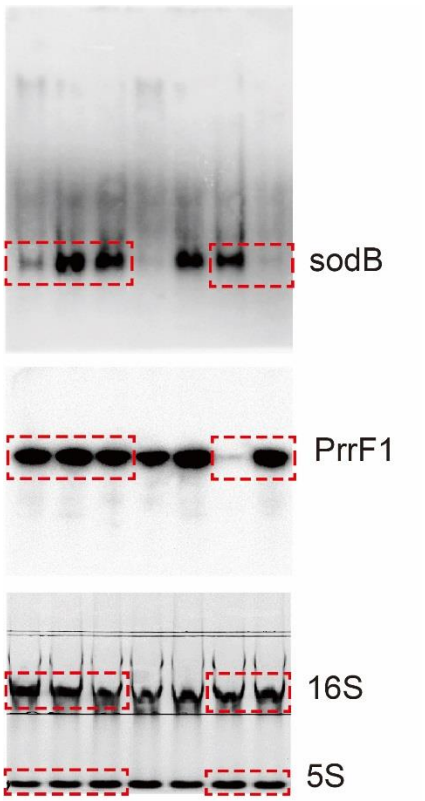
Supplementary Figure 13. Ligation of PrrF1 to precursor tRNA-asp^2, -asp^3, and -asp^4. (a) DNA sequence alignment of tRNA-asps and partial precursor sequences at their 3' ends. The mature tRNA-asps are shown in green and the CCA sequences are boxed. The arrow in black indicates the unique sequences in the precursor tRNA molecules that allowed differentiating among the four tRNAs. (b) A detailed examples showing how the PrrF1 chimeric reads with two precursor tRNAs (asp^3 and asp^4) mapped on the PAO1 genome. The arrows in blue indicate the specific sequences found in the precursor transcripts containing the individual tRNA-asp while the identical sequences of these tRNAs are marked with stars. The location corresponding to the ends of mature 3' tRNAs (CCA) in the chimeras is also shown in boxes.

Supplementary Figure 14. Uncropped images of Figs.1c, 2c, 2d, and 2e and Supplementary Figs. 4a, 4b, 9c, 10c, 10d, 10e, 10f, 11d, 11e, and 11g.

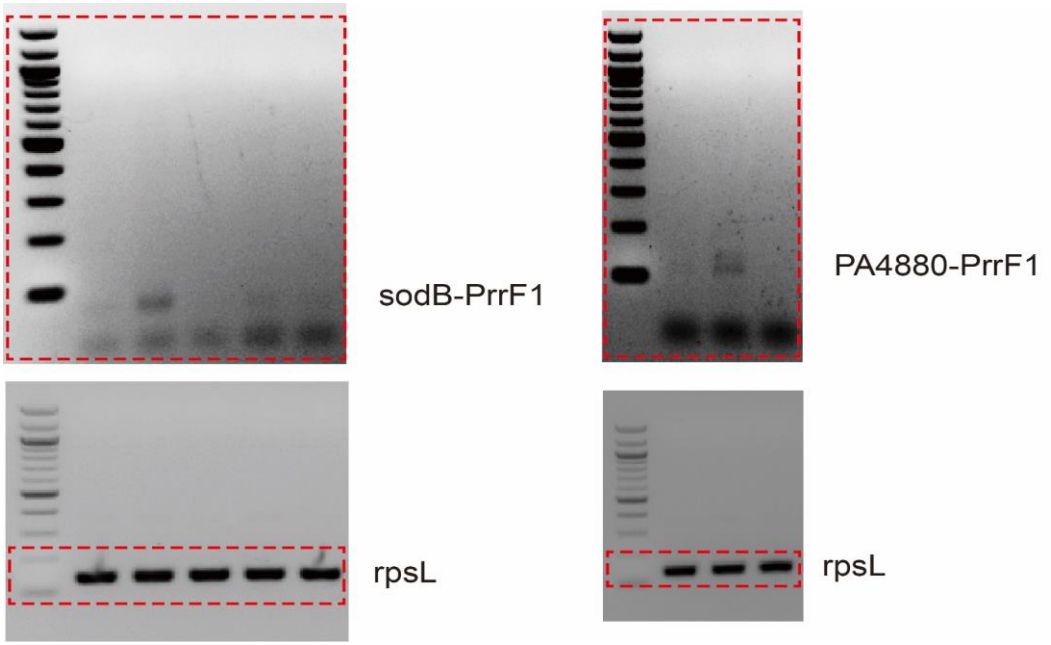
Uncropped Figure 1c



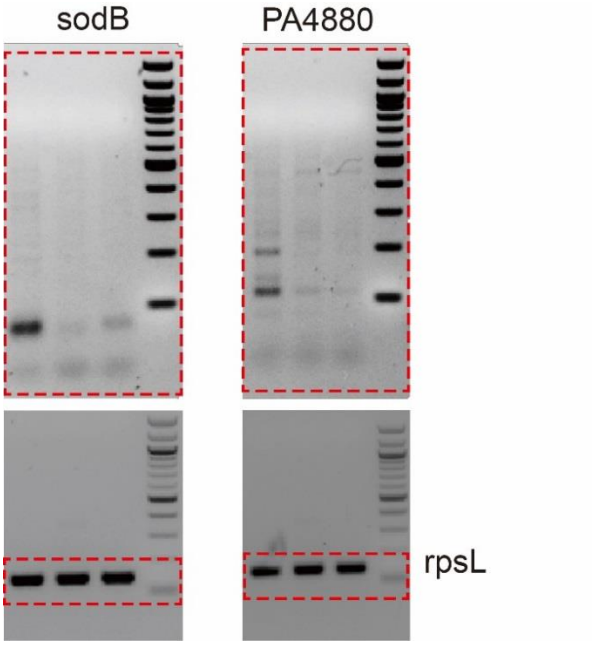
Uncropped Figure 2c



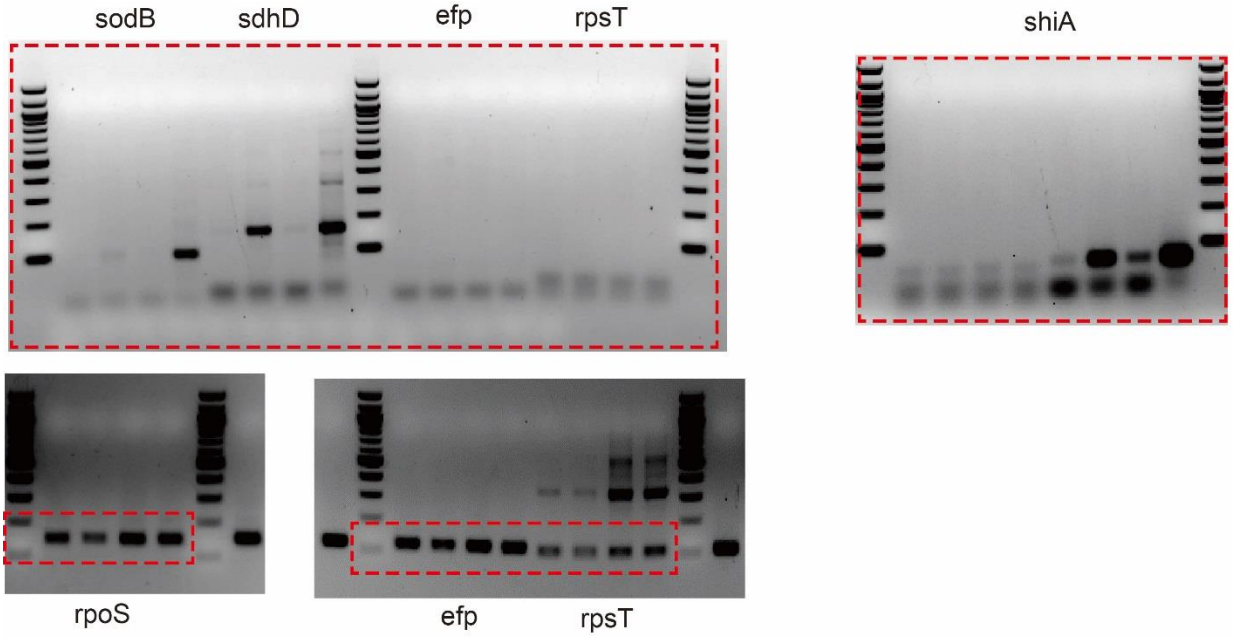
Uncropped Figure 2d-e



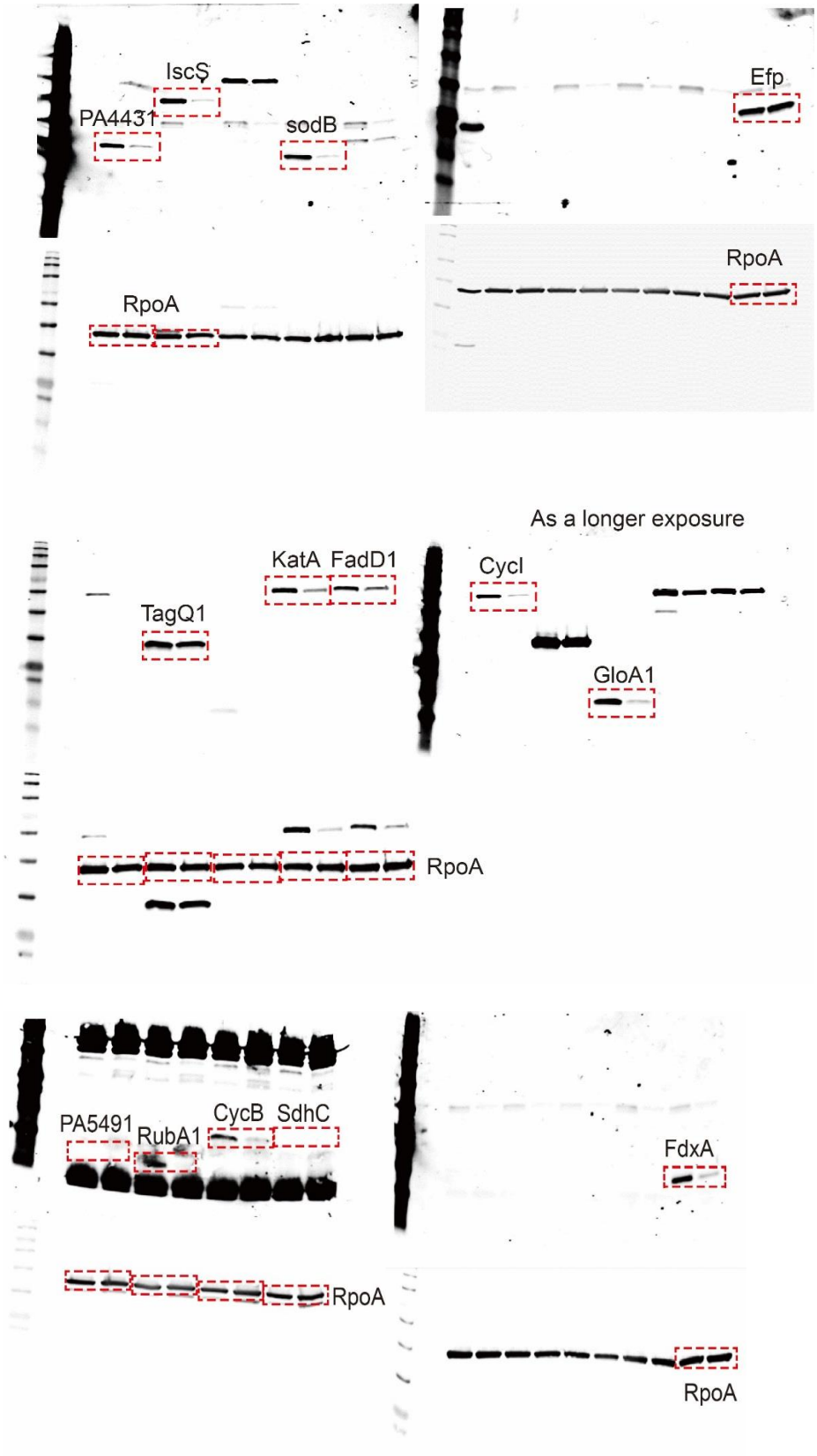
Uncropped Figure 2g



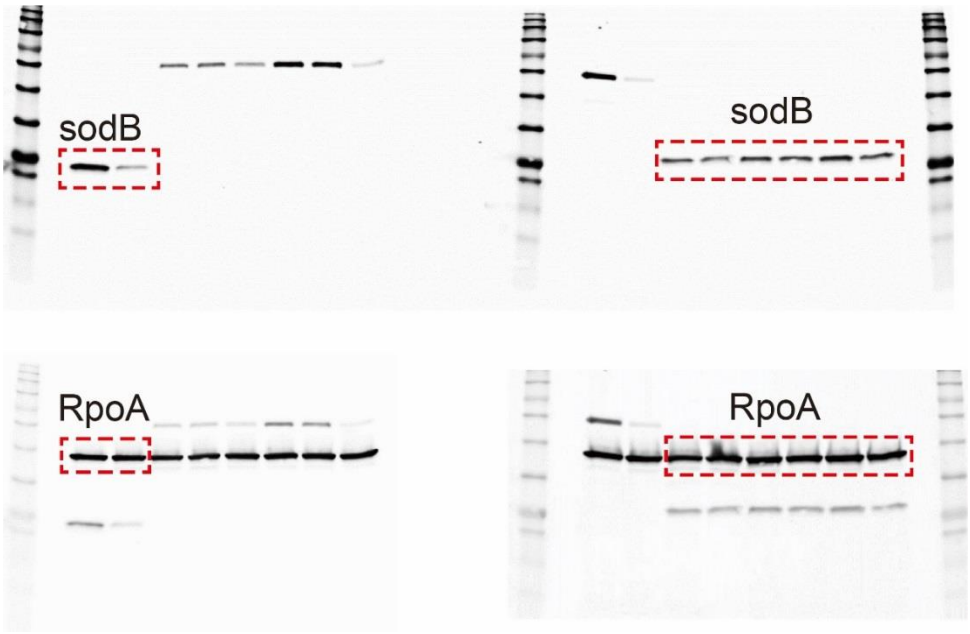
Uncropped Supplementary Figure 4a-b



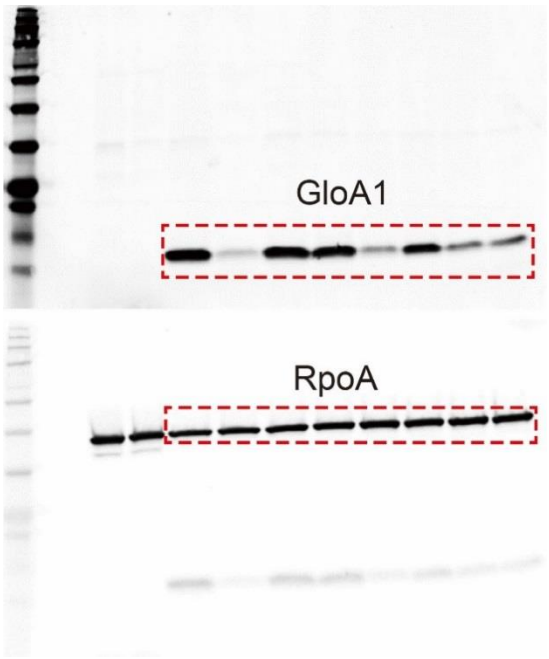
Uncropped Supplementary Figure 9c



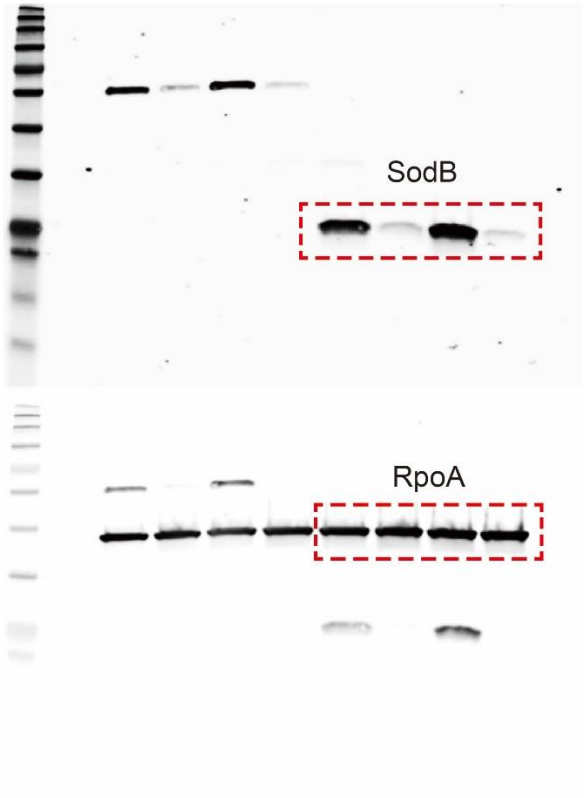
Uncropped Supplementary Figure 10c



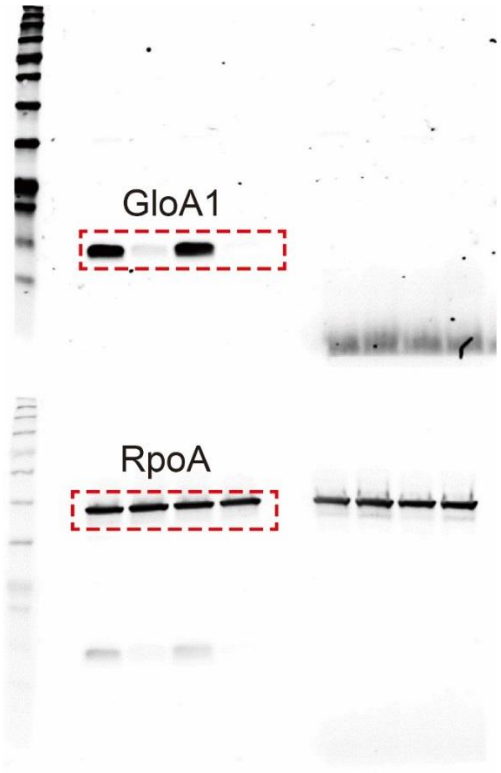
Uncropped Supplementary Figure 10d



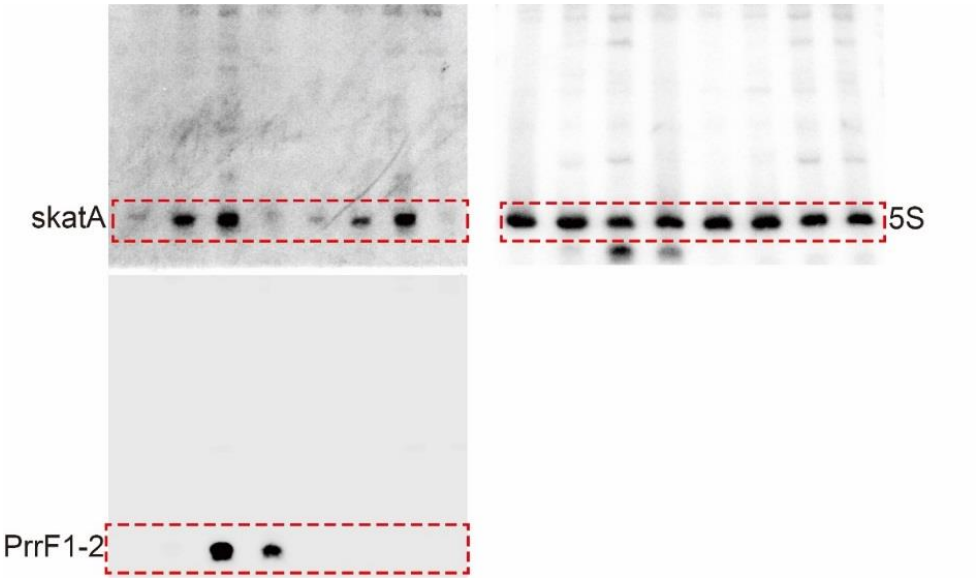
Uncropped Supplementary Figure 10e



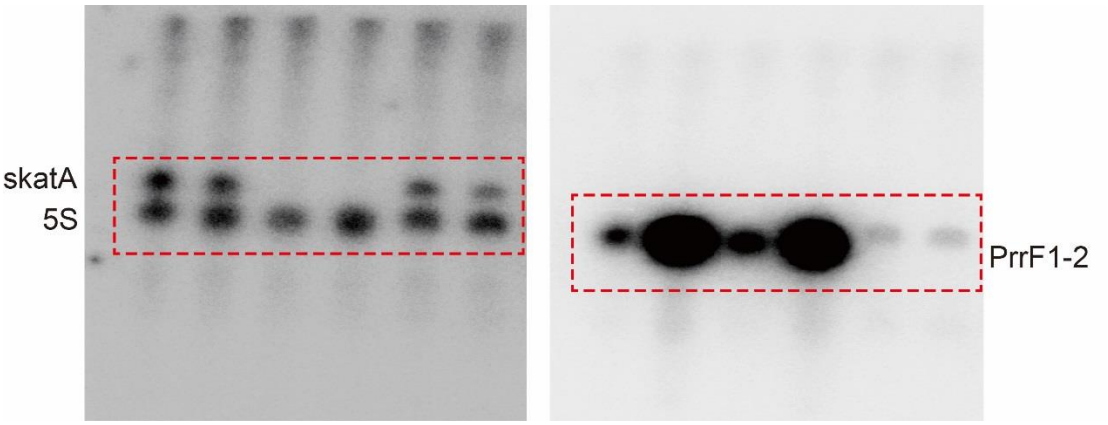
Uncropped Supplementary Figure 10f



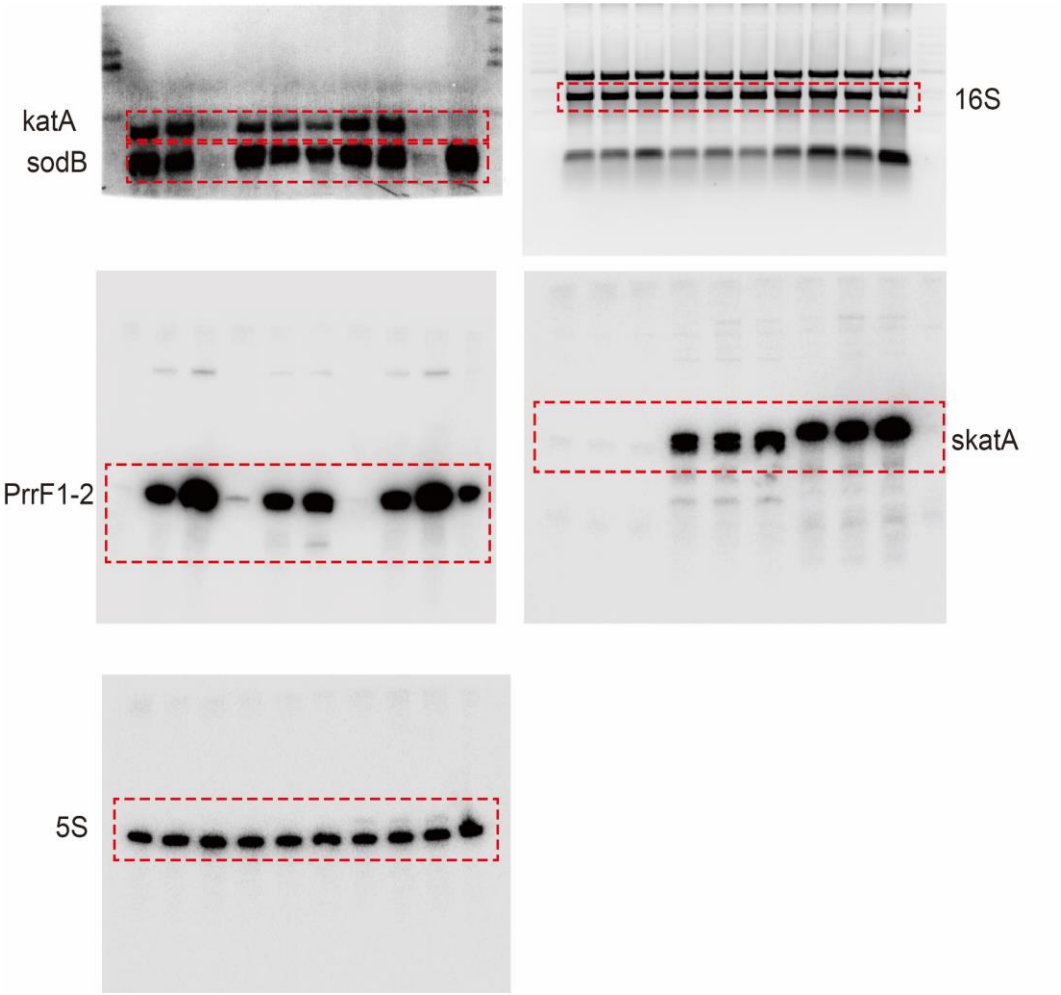
Uncropped Supplementary Figure 11d



Uncropped Supplementary Figure 11e



Uncropped Supplementary Figure 11g



Supplementary Figure 15. Sequences used in this study.

PrrF1 sRNA

AACTGGTCGCGAGATCAGCCGGTAAGCTGAGAGACCCACGCAGTCGGACTCTTCAGATTATCTCCT
CATCAGGCTAATCACGGTTTTTGACCCGGCACTTTGCCGGGTCTTTTTTT

RyhB sRNA

GCGATCAGGAAGACCCTCGCGGAGAACCTGAAAGCACGACATTGCTCACATTGCTTCCAGTATTACT
TAGCCAGCCGGGTGCTGGCTTTT

skatA sRNA

ACCGGAACAGATCCAGCGTCGCCAGATCGGCCTGTTCCTCAAGGTCGACCCGGCCTACGGCAAAG
GCGTCGCCGACGCCCTCGGCCTGAAGCTGGACTGATGGCCTGATGAGGCCCCCGGCCCCCTTCCT
AGGAAGGGGGCTTTTTT

T4 RNA ligase (*t4rnl1*)

ATGCAAGAACTTTTTAACAATTTAATGGAACTATGTAAGGATTCGCAGCGTAAGTTTTTTTACTCAGAT
GATGTAAGTGCATCTGGAAGAACTTACAGAATTTTCTCATATAATTATGCATCTTATTCTGATTGGTTA
CTTCCAGATGCACTAGAATGTCGTGGAATTATGTTTGAAATGGATGGAGAAAAACCAGTAAGAATTGC
TTCTCGTCCTATGGAAAAGTTTTTTAACTTGAATGAAAATCCGTTCACGATGAATATCGATTTAAACGA
TGTTGATTATATTCTAACAAAAGAAGACGGGTCTTTGGTATCAACTTATTTAGACGGTGATGAAATTCT
GTTCAAATCAAAGGGTTCAATCAAATCTGAGCAGGCTTTAATGGCTAATGGAATTTTGATGAATATTAA
TCACCATCGGTTGCGTGATAGACTTAAAGAATTAGCTGAAGATGGATTTACTGCTAACTTCGAATTCG
TTGCCCCGACGAATAGAATCGTTCTTGCTTATCAAGAGATGAAAATTATTTTACTGAATGTTCGTGAAA
ACGAAACGGGTGAATACATTTCATACGATGATATTTATAAAGATGCTACTCTTCGTCCGTATCTAGTT
GAACGATACGAAATCGATAGCCCTAAATGGATAGAAGAAGCTAAAAATGCAGAAAACATCGAAGGCT
ATGTTGCTGTGATGAAAGATGGTTCTCATTTTAAAATTAAGTCTGACTGGTACGTGTCTCTTCATAGTA
CAAAAAGTTCATTAGATAATCCAGAAAAATTGTTTAAGACTATTATTGATGGTGCATCAGATGATCTTA
AAGCAATGTATGCTGACGATGAATATTCATACAGAAAAATTGAAGCATTTGAAACGACTTATCTGAAG
TACTTAGACCGAGCTCTGTTTTTAGTTCTTGACTGTCATAATAAGCATTGCGGTAAGGATAGAAAGAC
TTATGCAATGGAAGCACAAGGTGTTGCTAAAGGTGCTGGAATGGATCACCTGTTCGGTATCATCATG
AGCTTATACCAGGGGTACGATAGTCAAGAAAAGGTCATGTGTGAAATCGAACAGAATTTTTTGAAAAA
TTATAAAAAATTTATCCCAGAAGGATACTAA

Supplementary Table 1 [Separate File]: RNA-seq analysis

Supplementary Table 2 [Separate File]: GRIL-seq analysis

Supplementary Table 3: Top 40 targets ranked according to the maximal coverage in GRIL-Seq.

Rank	Locus tag	Name	Max coverage	Product Name	a	b	c	d
1	PA4726.11	crcZ	338186	ncRNA				
2	PA5491		272196	cytochrome				
3	PA4431		204548	iron-sulfur protein				71
4	PA5351	rubA1	158050	rubredoxin				9
5	PA5300	cycB	154313	cytochrome C5				13
6	PA1581	sdhC	150967	succinate dehydrogenase subunit C	(1,2)			2
7	PA3814	iscS	141040	cysteine desulfurase				3
8	PA1838	cysI	80581	sulfite reductase				
9	PA3621	fdxA	56776	ferredoxin I				4
10	PA3299	fadD1	53252	long-chain-fatty-acid--CoA ligase				
11	PA0070	tagQ1	52081	T6SS				
12	PA3524	gloA1	46443	lactoylglutathione lyase				
13	PA4236	katA	44545	catalase	(1)			
14	PA1069		43627	hypothetical protein				
15	PA3262.1		41289	Asp tRNA				
16	PA3094.2		40509	Asp tRNA				
17	PA1750		38745	phospho-2-dehydro-3-deoxyheptonate aldolase				
18	PA0846	trxB2	37196	thioredoxin reductase				
19	PA3996	lis	35545	lipoate synthase				
20	PA4423		34345	putative lipoprotein				
21	PA4454		30002	conserved hypothetical protein				57
22	PA1610	fabA	29790	3-hydroxydecanoyl-ACP dehydratase				
23	PA1777	oprF	28783	porin				
24	PA4242	rpmJ	26892	50S ribosomal protein L36				
25	PA1787	acnB	25808	aconitate hydratase 2	(1)			16
26	PA1554	ccoN1	24260	cytochrome c oxidase, cbb3-type, CcoN subunit				
27	PA4880		22422	bacterioferritin	(1,2)			65
28	PA3169		21769	5-methylthioribose-1-phosphate isomerase MtnA				
29	PA0085	hcp1	20399	T6SS				
30	PA2953		19407	electron transfer flavoprotein-ubiquinone oxidoreductase				41
31	PA0905	rsmA	17010	RsmA				
32	PA3603	dgkA	16834	diacylglycerol kinase				
33	PA2830	htpX	15593	heat shock protein HtpX				
34	PA5490	cc4	14265	cytochrome c4 precursor				1
35	PA4366	sodB	14027	superoxide dismutase	(1,2)			
36	PA3121	leuC	13680	3-isopropylmalate dehydratase large subunit				31
37	PA4812	fdnG	13169	formate dehydrogenase-O, major subunit				
38	PA5302	dadX	12748	catabolic alanine racemase				
39	PA0832		11985	conserved hypothetical protein				
40	PA3262.2		11590	tRNA-Val				

a : Known in previous studies. The number denotes the references (1: Oglesby A. G. *et al.*[5], 2: Wilderman P. J. *et al.*[7]). **b** : Iron containing protein. **c** : Heme containing (4Fe-4S or 2Fe-2S). **d** : Ranked within top 100 analyzed by CopraRNA prediction. The number represents the rank of the corresponding gene.

Supplementary Table 4: Strains and Plasmids used in this study.

Strain	Genotype/relevant features	Source/reference
P. aeruginosa		
PAO1	wild-type	Michael Vasil's lab
PAO1Δ*hfq*	Isogenic deletion strain constructed with pEXG2-Δ*hfq*	Lab collection
PAO1Δ*rppH*	Isogenic deletion strain constructed with pEXG2-Δ*rppH*	This work
PAO1Δ*prrf1prrf2*	Isogenic deletion strain constructed with pEXG2-Δ*prrf1prrf2*	This work
PAO1Δ*katA*	Isogenic deletion strain constructed with pEXG2-Δ*katA*	This work
PAO1Δs*katA*	Isogenic deletion strain constructed with pEXG2-Δs*katA*	This work
PT4P1	PAO1 strain containing both plasmid pKH13-*t4rnl1* and pKH6-PrrF1	This work
E. coli		
SM10 λpir	*thi-1 leuB6 supE44 tonA21 lacY1 recA::RP4-2-Tc::Mu Km^R*	Simon *et al.*[17]
Stellar Competent Cell	*F–, endA1, supE44, thi-1, recA1, relA1, gyrA96, phoA, Φ80d lacZΔ M15, Δ (lacZYA - argF) U169, Δ (mrr - hsdRMS - mcrBC), ΔmcrA, λ–*	Clontech
ET4R1	*E. coli* MG1655 containing both plasmid pKH13-*t4rnl1* and pKH6-RyhB	This work
Plasmids	**Relevant features**	**Source/reference**
pEXG2	ColE1 suicide vector; mob sacB Gen^R	Rietsch *et al*.[18]
pEXG2-Δ*rppH*	pEXG2 with flanking regions to introduce an unmarked *rppH* (PA0336)	This work
pEXG2-Δ*prrf1prrf2*	pEXG2 with flanking regions to introduce an unmarked *prrf1* and *prrf2*	This work
pEXG2-Δ*katA*	pEXG2 with flanking regions to introduce an unmarked *katA* (PA4236) including 5' and 3' UTR of *katA*	This work
pEXG2-Δs*katA*	pEXG2 with flanking regions to introduce an unmarked *skatA*	This work
pEXG2-tagQ1-6H	pEXG2 with flanking regions to introduce six His tagged tagQ1	This work
pRK2013	ColE1 Tra1 Mob1 (pRK2) Km^R	Figurski and Hellinski[19]
pMMB67EH	Broad-host-range expression vector from Ptac; lacIq, Amp^R	Furste *et al.*[20]
pPSV40	pBR322 origin of replication, Gen^R	Rietsch *et al*.[21]
pPSV40-1	modification of HindIII site of pPSV40	This work
pKH4	pMMB67EH derivate vector: tac promoter with two lac operator sites at upstream of the -35 element and between -35 and -10 elements	This work
pKH7	pPSV40-1-derivated vector: lacIq, Ptac and rrnB terminator of pMMB67EH was cloned to pPSV40-1	This work
pKH11	pKH7-derivated vector: Gen^R was changed to Carb^R	This work
pBTK27	Broad host range expression vector derivated from pMMB67EH Gateway with an additional lac repressor $lacI_q$	Kulasakara *et al*.[22]
pET16b-*t4rnl1*	template plasmid for T4 RNA ligase 1	Stewart Shuman's lab
pKH13	pKH11-devated vector: Ptac promoter from pBTK27, Carb^R, IPTG inducible	This work
pKH13-*t4rnl1*	T4RNA ligase expression	This work
pKH13-*t4K99N*	T4RNA ligase mutant expression	This work
pJN105	Expression vector the arabinose-inducible PBAD promoter, Gen^R	Newman *et al.*[23]
pKH6	pJN105-derivated vector: expression of small RNA with TTS +1	This work
pKH6-PrrF1	P. aeruginosa PAO1 PrrF1 expression. Cloned from +1 to +135 of *prrf1*	This work
pKH6-PrrF1M1	PrrF1 mutant 1 expression (G83C)	This work
pKH6-PrrF1M2	PrrF1 mutant 2 expression (G72C)	This work

pKH6-PrrF1M3	PrrF1 mutant 3 expression (C64G)	This work
pKH6-PrrF1M4	PrrF1 mutant 4 expression (C54G)	This work
pKH6-PrrF1M1.5	PrrF1 mutant 1.5 expression (C81G)	This work
pKH6-PrrF1M2.5	PrrF1 mutant 2.5 expression (C67G)	This work
pKH6-skatA	skatA expression	This work
pKH6-skatA (M)	skatA mutant expression (GCC→CGG at position +1452 to +1454 of *katA* mRNA)	This work
pKH6-RyhB	*E. coli* MG1655 RyhB expression. Cloned from +1 to +113 of *prrf1*	This work
mini-CTX-lacZ	lacZ transcriptional fusion attB integration construction plasmid, Tet^R	Becher and chweizer[24]
pPtac-miniCTX::lacZTL	mini-CTX-lacZ-derivated vector: modified Ptac with two lac operator sites at upstream of the -35 element and between -35 and -10 elements, IPTG inducible	This work
pPtac-miniCTX-PA5491::lacZTL	Translational mini-ctx::lacZ fusion containing 5' UTR (103 nt) and 20 amino acid of PA5491	This work
pminiCTX-PA4431::lacZTL	mini-ctx::lacZ translational fusion containing 5' UTR (63 nt) and 20 amino acid of PA4431	This work
pPtac-miniCTX-rubA1::lacZTL	mini-ctx::lacZ translational fusion containing 5' UTR (33 nt) and 20 amino acid of rubA1	This work
pPtac-miniCTX-cycB::lacZTL	mini-ctx::lacZ translational fusion containing 5' UTR (121 nt) and 20 amino acid of cycB	This work
pPtac-miniCTX-sdhC::lacZTL	mini-ctx::lacZ translational fusion containing 5' UTR (113 nt) and 20 amino acid of sdhC	This work
pPtac-miniCTX-iscS::lacZTL	mini-ctx::lacZ translational fusion containing 5' UTR (54 nt) and 20 amino acid of iscS	This work
pPtac-miniCTX-cysI::lacZTL	mini-ctx::lacZ translational fusion containing 5' UTR (73 nt) and 20 amino acid of cysI	This work
pPtac-miniCTX-fdxA::lacZTL	mini-ctx::lacZ translational fusion containing 5' UTR (95 nt) and 20 amino acid of fdxA	This work
pPtac-miniCTX-fadD1::lacZTL	mini-ctx::lacZ translational fusion containing 5' UTR (95 nt) and 20 amino acid of fadD1	This work
pPtac-miniCTX-tagQ1::lacZTL	mini-ctx::lacZ translational fusion containing 5' UTR (583 nt) and 20 amino acid of tagQ1	This work
pPtac-miniCTX-gloA1::lacZTL	mini-ctx::lacZ translational fusion containing 5' UTR (30 nt) and 20 amino acid of gloA1	This work
pPtac-miniCTX-katA::lacZTL	mini-ctx::lacZ translational fusion containing 5' UTR (153 nt) and 20 amino acid of katA	This work
pPtac-miniCTX-sodB::lacZTL	mini-ctx::lacZ translational fusion containing 5' UTR (175 nt) and 20 amino acid of sodB	This work
pPtac-miniCTX-PA4880::lacZTL	mini-ctx::lacZ translational fusion containing 5' UTR (66 nt) and 20 amino acid of PA4880	This work
pPtac-miniCTX-efp::lacZTL	mini-ctx::lacZ translational fusion containing 5' UTR (35 nt) and 20 amino acid of efp	This work
pPtac-miniCTX-PA3940::lacZTL	mini-ctx::lacZ translational fusion containing 5' UTR (192 nt) and 20 amino acid of PA3940	This work
pPtac-miniCTX-PA5491::6His	mini-ctx::6His translational fusion containing 5' UTR (103 nt) and coding sequences lacking the stop codon of PA5491	This work
miniCTX-PA4431::6His	mini-ctx::6His translational fusion containing 5' UTR (63 nt) and coding sequences lacking the stop codon of PA4431	This work
pPtac-miniCTX-rubA1::6His	mini-ctx::6His translational fusion containing 5' UTR (33 nt) and coding sequences lacking the stop codon of rubA1	This work
pPtac-miniCTX-cycB::6His	mini-ctx::6His translational fusion containing 5' UTR (121 nt) and coding sequences lacking the stop codon of cycB	This work
pPtac-miniCTX-sdhC::6His	mini-ctx::6His translational fusion containing 5' UTR (113 nt) and coding sequences lacking the stop codon of sdhC	This work
pPtac-miniCTX-iscS::6His	mini-ctx::6His translational fusion containing 5' UTR (54 nt) and coding sequences lacking the stop codon of iscS	This work

pPtac-miniCTX-cysI::6His	mini-ctx::6His translational fusion containing 5' UTR (73 nt) and coding sequences lacking the stop codon of cysI	This work
pPtac-miniCTX-fdxA::6His	mini-ctx::6His translational fusion containing 5' UTR (95 nt) and coding sequences lacking the stop codon of fdxA	This work
pPtac-miniCTX-fadD1::6His	mini-ctx::6His translational fusion containing 5' UTR (95 nt) and coding sequences lacking the stop codon of fadD1	This work
pPtac-miniCTX-gloA1::6His	mini-ctx::6His translational fusion containing 5' UTR (30 nt) and coding sequences lacking the stop codon of gloA1	This work
pPtac-miniCTX-katA::6His	mini-ctx::6His translational fusion containing 5' UTR (153 nt) and coding sequences lacking the stop codon of katA	This work
pPtac-miniCTX-sodB::6His	mini-ctx::6His translational fusion containing 5' UTR (175 nt) and coding sequences lacking the stop codon of sodB	This work
pPtac-miniCTX-efp::6His	mini-ctx::6His translational fusion containing 5' UTR (35 nt) and coding sequences lacking the stop codon of efp	This work
pPtac-miniCTX-sodB(M2')::lacZTL	sodB mutant (C-30G) of pPtac-miniCTX-sodB::lacZTL	This work
pPtac-miniCTX-gloA1(M1.5')::lacZTL	gloA1 mutant (G-21C) of pPtac-miniCTX-gloA1::lacZTL	This work
pPtac-miniCTX-sodB(M2')::6His	sodB mutant (C-30G) of pPtac-miniCTX-sodB::6His	This work
pPtac-miniCTX-gloA1(M1.5')::6His	gloA1 mutant (G-21C) of pPtac-miniCTX-gloA1::6His	This work

Supplementary Table 5: Primers used in this study.

Name	Sequence (5' to 3')	Used for
F_ XhoI_pKH5	GTCC CTCGAG TATGCTCTTCTGCTCC	cloning for pKH7
R_ERI_rrnBT	GTCC GAATTC CAAAAGAGTT TGTAGAAACG C	cloning for pKH7
F_pKH11_bla vec_gs	GAATACTCATCGTTGCTGCTCCATAACATC	cloning for pKH11
R_pKH11_bla vec_gs	GATTAAGCATTGGTAACAATTCGTTCAAGCCGAG	cloning for pKH11
F_Bla_gsn	GAATTGTTACCAATGCTTAATCAGTGAGG	cloning for pKH11
R_Bla_gsn	GAGCAGCAACGATGAGTATTCAACATTTCC	cloning for pKH11
F_pspOMI_lacIq	CGAGACAGAACTTAATGGGCCCGCTAACAGCGCG	cloning for pKH13
R_pKH13_pBTK27	CCGCCAAAACAGCCAAGCTTCGTCGACTCTAGA GTACCGAGCTCGAATTC	cloning for pKH13
F_xbI_SD_T4RL_pKH13	ATTCGAGCTCGGTAC TCTAGA CTAAGAAGGAGATATACAT ATG CAAGAACTTTTTAAC	cloning for pKH13-*t4rnl1*
R_Hnd3_T4RL_pKH11	CCGCCAAAACAGCC AAGCTT TTAGTATCCTTCTGGG	cloning for pKH13-*t4rnl1*
F_MluI_lacIq	CTGACGCGTTGCGCGAGAAGATTGTGC	cloning for pKH4
R_Dn_lacIq	CCGCTGACATTTATGCCAGAACCGTTATG	cloning for pKH4
F_Up_Ptac	GGCATAAATGTCAGCGGATAACATTGACATTGTGAGCGGATAAC	cloning for pKH4
R_Dn_Ptac	CCCAAGCTTGTCGACTCTAGAATTATATTGTTATCCGCTCACAATG	cloning for pKH4
F_MluI_pJN150	GTCC ACGCGT AACA AAAGTGTCTA TAATCAC	cloning for pKH6
R_ERI_XbI_pJN150	GTCC GAATTC GACGTC TCTAGA AACAGTAGAG AGTTGCG	cloning for pKH6
F_PstI_rrnBT	GTCC CTGCAGAAGCTT ACTAGT GGCTGTTTTGGCGGATG	cloning for pKH6
R_sacI_rrnBT	GTCC GAGCTC CAAAAGAGTT TGTAGAAACG C	cloning for pKH6
R_phos_notI_pKH6	p-CCATGGACGCACACCGTGG	cloning for pKH6
F_phos_aac65_pKH6	p-CTAAAGGGAACAAAAGCTGGGTACC	cloning for pKH6
F_t4rIK99N	GATTATATTCTAACAAATGAAGACGGGTCTTTG	cloning for pKH13-*t4rnl1K99N*
R_t4rIK99N	CAAAGACCCGTCTTCATTTGTTAGAATATAATC	cloning for pKH13-*t4rnl1K99N*
F_XbaI_prrf1+1	GTCC TCTAGA T AACTGGTCGC GAGATCAGC	cloning for pKH6-PrrF1 and PrrF1 mutants
R_Hd3_prrf1+135	GTCCAAGCTTCTCCGGCCGAATCGCAG	cloning for pKH6-PrrF1 and PrrF1 mutants
F_prrf1_M1	CTAATCA CCGTTTTTGA CCCGGC	cloning for pKH6-PrrF1(M1)
R_prrf1_M1	GGTCAAAAACGGTGATTAGCCTGATG	cloning for pKH6-PrrF1(M1)
F_prrf1_M2re	CCTCATC ACGCTAATCA CGGTTTTTG	cloning for pKH6-PrrF1(M2)
R_prrf1_M2	GATTAGCGTGATGAGGAGAT	cloning for pKH6-PrrF1(M2)
F_prrf1_M3	GATTA TCTCGTCATC AGGCTAATC	cloning for pKH6-PrrF1(M3)
R_prrf1_M3	CCTGATGACGAGATAATCTGAAG	cloning for pKH6-PrrF1(M3)
F_prrf1_M4	GTCGGACT CTTGAGATTA TCTCCTC	cloning for pKH6-PrrF1(M4)
R_prrf1_M4	GATAATCTCAAGAGTCCGACTG	cloning for pKH6-PrrF1(M4)
F_PrrF1_M1.5	CTCATCAGGCTAATCAGGGTTTTTGACCCGGCAC	cloning for pKH6-PrrF1(M1.5)
R_PrrF1_M1.5	GTGCCGGGTCAAAAACCCTGATTAGCCTGATGAG	cloning for pKH6-PrrF1(M1.5)
F_PrrF1_M2.5	GACTCTTCAGATTATCTCCTGATCAGGCTAATCACGG	cloning for pKH6-PrrF1(M2.5)
R_PrrF1_M2.5	CCGTGATTAGCCTGATCAGGAGATAATCTGAAGAGTC	cloning for pKH6-PrrF1(M2.5)
F_xbaI_pkh6_sKatAinf	CAACTCTCTACTGTT TCTAGA T ACCGGAACAGATCCAGCG	cloning for pKH6-skatA and skatA(M)
R_Hnd3_pkh6_sKatAinf	CCGCCAAAACAGCC AAGCTT CTGATCCGGGTCAAGCC	cloning for pKH6-skatA and skatA(M)
F_skatAmut-fus	GACTGATGCGGTGATGAGGCCC	cloning for pKH6-skatA(M)
R_skatAmut-fus	GGGCCTCATCACCGCATCAGTCCAGCTTCAG	cloning for pKH6-skatA(M)
F_Hnd3_rppHdel	GCATAAATGTAAAGC AAGCTTC GTGACGAAGCGGTTGG	cloning for pEXG2-ΔrppH
F_rppH_fus	GAGGATGTCCCAGCGACACGGAGACAAGGC	cloning for pEXG2-ΔrppH
R_rppH_fus	GCCTTGTCTCCGTGTCGCTGGGACATCCTC	cloning for pEXG2-ΔrppH
R_BHI_rppHdel	GAGCTCGAGCCCGG GGATCC CTCGGCATGCGCGATG	cloning for pEXG2-ΔrppH
F_BHI_Del_katA5n3	CAGGTCGACTCTAGA GGATCC CCATCGTAAAAGTGGTC	cloning for pEXG2-ΔkatA
R_Del_katA5n3_fusre	CTAGCCGCATATGCTCCAGCCCTCCTACAATCC	cloning for pEXG2-ΔkatA
F_Del_katA5n3_fusre	GGATTGTAGGAGGGCTG GAGCATATGCGGCTAG	cloning for pEXG2-ΔkatA
F_BHI_del_sKatA	CAGGTCGACTCTAGA GGATCC GTACGAGAGCATCGAG	cloning for pEXG2-ΔskatA
F_sKatAdel_fus	GACATCCGCGATGTATCTGCATTGGCTTGACC	cloning for pEXG2-ΔskatA
R_sKatAdel_fus	CAAGCCAATGCAGATACATCGCGGATGTCTTC	cloning for pEXG2-ΔskatA
R_ERI_del_sKatA	GAAATTAATTAAGGTACC GAATTC GCCAACTGGGCCTTGAG	cloning for pEXG2-ΔskatA and pEXG2-ΔkatA
R_prrf1+26-1	CTTACCGGCTGATCTCGCGAC	RT-PCR for the detection of chimera products of 5' end of PrrF1
F_sodB-6	GAGACACCATGGCTTTCGAATTGCC	RT-PCR for the detection of chimera products of SodB-PrrF1
F_pa4880+1	ATGACCACCGTTCAACTGACGGAC	RT-PCR for the detection of chimera products of PA4880-PrrF1
F_efp-3	CGTATGAAAACCGCTCAAGAGTTCC	RT-PCR for the detection of chimera products of efp-PrrF1
F_pa3940-4	CACCATGGCACTGACCAAAGACC	RT-PCR for the detection of chimera products of PA3940-PrrF1
F_XbaI_ryhB+1A	GTCC TCTAGA TA GCGATCAGGA AGACCCTC	cloning for pKH6-RyhB
R_Hnd3_ryhB+113	GTCC AAGCTT GCACTCCCGT GGATAAATTG AG	cloning for pKH6-RyhB

R_ryhB+57	GAAGCAATGTGAGCAATGTCGTG	RT for the detection of chimera products of 5' end of RyhB
R_ryhB+45	GCAATGTCGTGCTTTCAGGTTC	RT-PCR for the detection of chimera products of 5' end of RyhB
F_ec_sodB-32	GCAAATTAATAATAAAGGAGAGTAGCAATGTC	RT-PCR for the detection of chimera products of sodB-RyhB
F_ec_sdhD-11	GGAGTCCTCGTATGGTAAGCAACG	RT-PCR for the detection of chimera products of sdhD-RyhB
F_ec_efp-11	CAGAGGGCCTTATGGCAACGTAC	RT-PCR for the detection of efp and chimera products of efp-RyhB
F_q_ec_rpsT-11	GGAGTTGGACCTTGGCTAATATCAAATC	RT-PCR for the detection of rpsT and chimera products of rpsT-RyhB
F_ec_rpos-125_q	CATTTTGAAATTCGTTACAAGGGGAAATCC	RT-PCR for the detection of rpoS
R_ec_rpos+25_q	GAACTTTCAGCGTATTCTGACTCATAAG	RT-PCR for the detection of rpoS
R_q_ec_efq+105	GCCTTTACCCGGTTTTACGAATTCAC	RT-PCR for the detection of efp
R_q_ec_rpsT+96	GATGAAAGTACGCATCATAGAGCGAC	RT-PCR for the detection of rpsT
F_shiA-62	GACGGCAATGTGAGTTACCTTTTCC	RT-PCR the detection of chimera products of shiA-RyhB
R_shiA-38	GGAAAAGGTAACTCACATTGCCGTC	RT-PCR the detection of chimera products of RyhB-shiA
F_ryhB+30	GAAAGCACGACATTGCTCACATTGC	RT-PCR the detection of chimera products of RyhB-shiA
R_ryhB+54	GCAATGTGAGCAATGTCGTGCTTTC	RT-PCR the detection of chimera products of shiA-RyhB
F_q_t4rnl1+65	CTCAGATGATGTAAGTGCATCTGGAAG	qRT-PCR for T4 RNA ligase
R_q_t4rnl1+171	CATAATTCCACGACATTCTAGTGCATC	qRT-PCR for T4 RNA ligase
F_q_Prrf1+8	CGCGAGATCAGCCGGTAAGC	qRT-PCR for PrrF1
R_q_Prrf1+86	AAACCGTGATTAGCCTGATGAGGAG	qRT-PCR for PrrF1
F_sodB+25	CCTTACGAAAAGAACGCCCTTGAG	qRT-PCR for sodB
R_sodB+132	CGGGATCAGGTTGTTCAGGTTC	qRT-PCR for sodB
F_q_pa4880+24	CGTCCAGACCCTTCGCGAC	qRT-PCR for PA4880
R_q_PA4880+158	GCAGGAAGCAGACCAACTCGG	qRT-PCR for PA4880
F_efp-3	CGTATGAAAACCGCTCAAGAGTTCC	qRT-PCR and RT-PCR for efp
R_q_efp	CCGGTCAGCAGGTTCTTCAGC	qRT-PCR and RT-PCR for efp
F_PA3940_q+25	GATCCAGGACATCGCCGAAGC	qRT-PCR and RT-PCR for PA3940
R_PA3940+_q	GATTTCGCCATCGTTCTCCAGG	qRT-PCR and RT-PCR for PA3940
rpsL F_q_in	TGA AGG TCA CAA CCT GCA AGA GCA	qRT-PCR and RT-PCR for rpsL
rpsL R_q_in	AAC GAC CCT GCT TAC GGT CTT TGA	qRT-PCR and RT-PCR for rpsL
F_KpnI_Ptac_inf	CGACTCACTATAGGGCGAATTGGGTACCGCACGGTGCACCAATGC	clonining for pPtac-miniCTX::lacZTL
R_Fus_Ptac_inf	GCTTGGATCCCTCGAGAATTCCACACATTATACGAGCC	clonining for pPtac-miniCTX::lacZTL
F_Fus_lacZ_inf	GAATTCTCGAGGGATCCAAGCTTGCCGTCGTTTTACAACG	clonining for pPtac-miniCTX::lacZTL
R_AatII_lacZ_inf	GGTTTATGCAGCAACGAGACGTCACGGAAAATGCCGCTC	clonining for pPtac-miniCTX::lacZTL
F_ERI_sodBTSS+1	TATAATGTGTG GAATTC GCGG CACTGGGAGC CTAG	clonining for pPtac-miniCTX-sodB::lacZTL, ::6His and sodB (M2') mutant
F_ERI_4880TSS+1	TATAATGTGTG GAATTC GTAAG CAGAAAAAGC CAG	clonining for pPtac-miniCTX-PA4880::lacZTL
F_ERI_efpTSS+1	CAATATAATGTGTG GAATTC CCTGATTTTTTGTCTTTCATCCATC	clonining for pPtac-miniCTX-efp::lacZTL, and ::6His
F_ERI_pa3940TSS+1	CAATATAATGTGTG GAATTC AGCCGCGAAAACCTCTG	clonining for pPtac-miniCTX-PA3940::lacZTL
R_Hnd3_sodB+20aa	TGTAAAACGACGGCAAGCTTGGAAATGTGCGGCTCAAG	clonining for pPtac-miniCTX-sodB::lacZTL and sodB (M2') mutant
R_Hnd3_pa4880+20aa	TGTAAAACGACGGCAAGCTTGATGTTCTTGCGGGCACG	clonining for pPtac-miniCTX-PA4880::lacZTL
R_Hnd3_efp+20aa	TGTAAAACGACGGCAAGCTTCCAGGGAGCGCCATTG	clonining for pPtac-miniCTX-efp::lacZTL
R_Hnd3_pa3940+20aa	TGTAAAACGACGGCAAGCTTCTTCTGCGCGTCGATGG	clonining for pPtac-miniCTX-PA3940::lacZTL
R_NP_PrrF1	GACTGCGTGGGTCTCTCAG	Northern blot for PrrF1
R_NP_5sRNA+67	CGTTCACTTCTGAGTTCGGGAAGG	Northern blot for 5S rRNA
R_NP_sodB+137	GTGCCCGGGA TCAGGTTGTT CAGG	Northern blot for sodB
R_NP_katA+69	GGTCTGCACGTTCTGGTTATCGAC	Northern blot for katA
R_NP_skatA	CATCAGTCCAGCTTCAGGCC	Northern blot for skatA
R_polyA_PrrF1	AAAAAAAAAAAAAAAAAAAGAAGAGTCCGACTGCGTGGGTC	Capturing Oligo for PrrF1
P1_R_PrrF1+45	GACTGCGTGGGTCTCTCAG	RT for PrrF1, identical to R_NP_PrrF1
P2_R_PrrF1+26	CTTACCGGCTGATCTCGCGAC	PCR after RT for PrrF1, identical to R_PrrF1+26-1
P3_F_PrrF1+62	CTCCTCATCAGGCTAATCACGG	PCR after RT for PrrF1
F_KpnI_LacIq inf	CGACTCACTATAGGGCGAATTGGGTACCCTCTTCTGCTCCCGAACGC	clonining for pPtac-miniCTX::6His
R_pKHctx3G6His-P	/5phos/GGATCCCTCGAGAATTCCACACATTATATTG	clonining for pPtac-miniCTX::6His
F_pKHctx3G6His-P	/5phos/ AAGCTTGGCCACCACCACCACCACCACTAATAATAACCGGGCAGGCCATG	clonining for pPtac-miniCTX::6His
R_speI_lacZTL_ctx_inf	GGCCGCTCTAGAACTAGTGGGAACAAAAGCTGGAAATAG	clonining for pPtac-miniCTX::6His
F_ERI_PA5491_ctZ1	CAATATAATGTGTG GAATTC GATTTTGTGTGCGCTTTATCC	clonining for pPtac-miniCTX-PA5491::lacZTL and ::6His
R_Hd3_PA5491_ctZ1	TGTAAAACGACGGCAAGCTTCGCCGCCTGCGCCGTAACG	clonining for pPtac-miniCTX-PA5491::lacZTL

F_ERI_PA4431_ctZ2	CAATATAATGTGTG GAATTC ATTTTTACTTAAAGGCCTGATTAG	clonining for pPtac-miniCTX-PA4431::lacZTL and ::6His
R_Hd3_PA4431_ctZ2	TGTAAAACGACGGCAAGCTTCGCCAACCACAGAGGTGGC	clonining for pPtac-miniCTX-PA4431::lacZTL
F_ERI_rubA1_ctZ3	CAATATAATGTGTG GAATTC GCTGAACCGTGGTAACCCG	clonining for pPtac-miniCTX-rubA1::lacZTL and ::6His
R_Hd3_rubA1_ctZ3	TGTAAAACGACGGCAAGCTTCGGCCAGCCTTTGGCCTCG	clonining for pPtac-miniCTX- rubA1::lacZTL
F_ERI_cycB_ctZ4	CAATATAATGTGTG GAATTC TTTCTGCCGGGCTTTCTTTGCC	clonining for pPtac-miniCTX-cycB::lacZTL and ::6His
R_Hd3_cycB_ctZ4	TGTAAAACGACGGCAAGCTTGGTCGTAGCCTGAGCGCTTAC	clonining for pPtac-miniCTX-cycB::lacZTL
F_ERI_sdhC_ctZ5	CAATATAATGTGTG GAATTC GACCGCAAGGGGTATTCC	clonining for pPtac-miniCTX-sdhC::lacZTL and ::6His
R_Hd3_sdhC _ctZ5	TGTAAAACGACGGCAAGCTTGAGTTTGATGGTCCTTAGGTC	clonining for pPtac-miniCTX-sdhC::lacZTL
F_ERI_iscS_ctZ6	CAATATAATGTGTG GAATTC ATTGAAGCGTCCGCCATCG	clonining for pPtac-miniCTX-iscS::lacZTL and ::6His
R_Hd3_iscS_ctZ6	TGTAAAACGACGGCAAGCTTGGCGACGCGGGGATCGACG	clonining for pPtac-miniCTX-iscS::lacZTL
F_ERI_cysI_ctZ7	CAATATAATGTGTG GAATTC ACTCCGCGTGGAGCGCGTAG	clonining for pPtac-miniCTX-cysI::lacZTL and ::6His
R_Hd3_cysI_ctZ7	TGTAAAACGACGGCAAGCTTGCGGAACTGCTTGACGCGGTC	clonining for pPtac-miniCTX-cysI::lacZTL
F_ERI_fdxA_ctZ8	CAATATAATGTGTG GAATTC GTTATATGCATGGAAGATGC	clonining for pPtac-miniCTX-fdxA::lacZTL and ::6His
R_Hd3_fdxA_ctZ8	TGTAAAACGACGGCAAGCTTGACTTCCACGCAGTCGGTG	clonining for pPtac-miniCTX-fdxA::lacZTL
F_ERI_fadD1_ctZ9	CAATATAATGTGTG GAATTC GCCTAATAGCCCTGGGTCCAG	clonining for pPtac-miniCTX-fadD1::lacZTL and ::6His
R_Hd3_fadD1_ctZ9	TGTAAAACGACGGCAAGCTTGGGATTGATCTCGGCAGC	clonining for pPtac-miniCTX-fadD1::lacZTL
F_ERI_tagQ1_ctZ10	CAATATAATGTGTG GAATTC AGCAATTGGACGGAATCATTG	clonining for pPtac-miniCTX-tagQ1::lacZTL
R_Hd3_tagQ1_ctZ10	TGTAAAACGACGGCAAGCTTCAACAGGCAGAGCGCGTAGC	clonining for pPtac-miniCTX-tagQ1::lacZTL
F_ERI_gloA1_ctZ11	CAATATAATGTGTG GAATTC CTTTGACCGTGAAGCTCG	clonining for pPtac-miniCTX-gloA1::lacZTL and ::6His
R_Hd3_gloA1_ctZ11	TGTAAAACGACGGCAAGCTTATAGAAATCGATCGAGCGATC	clonining for pPtac-miniCTX-gloA1::lacZTL and M1.5' mutant
F_ERI_katA_ctZ12	CAATATAATGTGTG GAATTC CTTTTACGAAAAGCTACGTTCGG	clonining for pPtac-miniCTX-katA::lacZTL and ::6His
R_Hd3_katA_ctZ12	TGTAAAACGACGGCAAGCTTGTTCTGGTTATCGACCACC	clonining for pPtac-miniCTX-katA::lacZTL
R_Hn3_PA5491_ctH1	GTGGTGGCCAAGCTTCTTCTTCACCATCCACTGGATG	clonining for pPtac-miniCTX-PA5491::6His
R_Hn3_PA4431_ctH2	GTGGTGGCCAAGCTTGGCTTTCTCCTGGTCCACG	clonining for pPtac-miniCTX-PA4431::6His
R_Hn3_rubA1_ctH3	GTGGTGGCCAAGCTTGCCGATTTCGATCATCTCGAAG	clonining for pPtac-miniCTX-rubA1::6His
R_Hn3_cycB_ctH4	GTGGTGGCCAAGCTTCAGACCGGACATATGCTTG	clonining for pPtac-miniCTX-cycB::6His
R_Hn3_sdhC _ctH5	GTGGTGGCCAAGCTTCCATACCCATACCCCCGCCAGAAC	clonining for pPtac-miniCTX-sdhC::6His
R_Hn3_iscS_ctH6	GTGGTGGCCAAGCTTGTGCGCCTGCCATTCGACCTG	clonining for pPtac-miniCTX-iscS::6His
R_Hn3_cysI_ctH7	GTGGTGGCCAAGCTTATGATTCGCTGCATATACG	clonining for pPtac-miniCTX-cysI::6His
R_Hn3_fdxA_ctH8	GTGGTGGCCAAGCTTGCGCTCCAGATGCTGGAGCTTG	clonining for pPtac-miniCTX-fdxA::6His
R_Hn3_fadD1_ctH9	GTGGTGGCCAAGCTTCTTCTGGCCCGCTTTCTTCAG	clonining for pPtac-miniCTX-fadD1::6His
R_Hn3_gloA1_ctH11	GTGGTGGCCAAGCTTGGAAGACTTCTGGATCAGTTC	clonining for pPtac-miniCTX-gloA1::6His, and M1.5' mutant
R_Hn3_katA_ctH12	GTGGTGGCCAAGCTTGTCCAGCTTCAGGCCGAGG	clonining for pPtac-miniCTX-katA::6His
F_BHI_tagQ1_6H	CAGGTCGACTCTAGA GGATCC CTACATGGAGAAGCAGAAGC	clonining for pEXG-tagQ1-6H
R_tagQ1_6H_fus	GGCCTTGGCGCAGCTCGGC	clonining for pEXG-tagQ1-6H
F_tagQ1_6H_fus	CCGAGCTGCGCCAAGGCCCACCACCACCACCACCACTGATCGGCCTCGTTACCGGCAC	clonining for pEXG-tagQ1-6H
R_ERI_tagQ1_6H	GAAATTAATTAAGGTACC GAATTC CGCTGTTCGAGGAATGGC	clonining for pEXG-tagQ1-6H
F_tagQ1_6H_veri	CTGCAACAGCCCGGACATGG	Validation of tagQ-6His integration on chromosome
R_tagQ1_6H_veri	GTTCGTGCCGGTAACGAGG	Validation of tagQ-6His integration on chromosome
R_Hn3_sodB_ctHctrl	GTGGTGGCCAAGCTTGGCAGCGAAATTCTTCGCTAC	clonining for pPtac-miniCTX-sodB::6His, and M2' mutant
R_Hn3_efp_ctHctrl	GTGGTGGCCAAGCTTGGCCTTGACGCGGGACTTG	clonining for pPtac-miniCTX-efp::6His
F_sodB_M2_RVT_fus	GCTAGCCGCGCCAGTCGTGACCTGAGGAAGAATAG	clonining for pPtac-miniCTX-sodB (M2')::lacZTL and sodB (M2')::6His
R_sodB_M2_RVT_fus	CTATTCTTCCTCAGGTCACGACTGGCGCGGCTAGC	clonining for pPtac-miniCTX-sodB (M2')::lacZTL and sodB (M2')::6His
F_gloA1_M1.5_RVT_ERI	CAATATAATGTGTG GAATTC CTTTGACCCTGAAGCTCGAGGCAATG	clonining for pPtac-miniCTX-gloA1 (M1.5')::lacZTL and gloA1(M1.5')::6His
NEBNext Index 5 Primer for Illumina	CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	Library 1 for GRIL-Seq: ACAGTG
NEBNext Index 6 Primer for Illumina	CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	Library 2 for GRIL-Seq: GCCATT
NEBNext Index 7 Primer for Illumina	CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	Library 1 (Vec.) for RNA-Seq: CAGATC
NEBNext Index 8 Primer for Illumina	CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	Library 2 (Vec.) for RNA-Seq: ACTTGA
NEBNext Index 9 Primer for Illumina	CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	Library 3 (PrrF1++) for RNA-Seq: GATCAG
NEBNext Index 10 Primer for Illumina	CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	Library 4 (PrrF1++) for RNA-Seq: TAGCTT
NEBNext Universal PCR Primer for Illumina	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	Libraries for GRIL-Seq and RNA-seq

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