Supplementary Information Materials and Methods

Bacterial strains and growth conditions

Bacterial strains and plasmids used in this study are listed in *SI Appendix*, Table S3. *P. aeruginosa* strains were grown in or on tryptone soy broth (TSB), lysogeny broth (LB) or Vogel-Bonner medium (VBM) at either 25°C or 37°C with the addition of agar as required. *E. coli* strains were grown in or on LB, TSB, nutrient yeast broth NYB or terrific broth (TB) at 37°C with the addition of agar as required. Media was supplemented with antibiotics where appropriate, *P. aeruginosa*: streptomycin 2000 µg/ml, carbenicillin 50-300 µg/ml, irgasan 25 µg/ml, tetracycline 50-150 µg/ml and gentamycin 40-100 µg/ml or *E. coli*: streptomycin 50 µg/ml, ampicillin 50-100 µg/ml, tetracycline 15 µg/ml and kanamycin 50 µg/ml).

DNA manipulation

DNA isolation was performed using the PureLink Genomic DNA mini kit (Life Technologies). Isolation of plasmid DNA was carried out using the QIAprep spin miniprep kit (Qiagen). Primers used are shown in *SI Appendix*, Table S4 (Sigma). DNA fragments were amplified with either KOD Hot Start DNA Polymerase (Novagen) or standard Taq polymerase (NEB) as described by the manufacturer with the inclusion of Betaine (Sigma) or DMSO (Sigma). Restriction endonucleases were used according to the manufacturer's specifications (Roche). DNA sequencing was performed by GATC Biotech. *P. aeruginosa* deletion mutants were constructed as described previously using 500 bp homologous regions and confirmed with external primers (*SI Appendix*, Table S4) (1). *amrZ* (PA14_20290) was amplified to construct a C-terminal V5 tag version (from pETDEST42 (Life Technologies)) and ligated into pMMB67HE. For protein purification *amrZ* was amplified and ligated into pET29a in frame with the sequence encoding a C-terminal His_6 tag. The DNA encoding the

C-terminus of VgrG4b (612-808 aa) was synthesised by Invitrogen GeneArt and subcloned into pET28a to construct pET28a-*vgrG4b*. For protein purification *hcp3* was amplified, digested and ligated into pET28a in frame with a His_6 tag. The coding regions of *tssB1*, *tssB2* and *tssB3* were amplified from *Pseudomonas* genomic DNA, *cfp* was amplified from pSEVA237C (de Lorenzo lab collection), 'superfolder' GFP (*sfgfp*) was amplified from mini-CTX-*gfp* (2) using KOD Hot Start DNA polymerase (Novagen). C-terminal fusions of each *tssB* gene with the respective fluorophore were generated by overlapping PCR (*cfp* and *sfgfp*) or by direct in-frame cloning (*venus*) in pME6032 (3).

Transposon mutagenesis and screening

Transposon mutagenesis was performed using pBT20 as outlined in (4). Briefly PA14*tssB2*tc::*lacZ* and SM10λ*pir* pBT20 were grown overnight on LB plates, PA14 was then incubated at 43°C for 2 hr to inactivate the restriction system. Bacteria were collected, suspended, OD_{600} measured and cells were mixed 1:2, spot plated on LB plates to enable conjugation and incubated. Cells were collected, re-suspended and diluted before being spread plated onto LB agar containing 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal) (Invitrogen), irgasan and gentamycin plates and incubated up to 4 days. Individual colonies were monitored for increased or decreased X-gal colour development twice daily. Transposon mutants of interest were isolated, grown, spot plated and confirmed via galactosidase assay essentially as previously described (5). Transposon insertion sites were mapped via arbitrary PCR (4).

T6SS competition assays

Competition assays were performed as per (6, 7) except that Top10 pRL662-*gfp* or DH5α pCR2.1 were used as the prey strains. Briefly overnight cultures were mixed 1:1 and spotted

on LB agar plates for 5 hr. LB agar contained 1 mM IPTG or 1% Arabinose if induction of pAmrZ or pJN105*tssB2-cfp* was required. Spots were recovered, suspended, serial diluted and spotted on to LB, LB X-gal, LB gentamycin and/or PIA plates respectively to enable colony counts as required.

Secretion assays

For secretion assays *P. aeruginosa* strains were grown overnight in TSB and subcultured to OD_{600} 0.1 and grown at 37°C or 25°C for 6 or 24 hr, respectively (or as otherwise indicated) with agitation. Culture supernatants were prepared as previously described using trichloroacetic acid precipitation (8). An OD_{600} equivalent of 0.1 or 1 of cell lysate and supernatant protein samples, respectively were loaded on to SDS-PAGE gels for analysis by western blotting.

Protein Purification

BL21 Star (λDE3) pAmrZ-His_6 cells were sub-cultured to an OD_{600} 0.1 in one litre of NYB and grown at 37°C to an OD_{600} 0.7 prior to induction with 1 mM IPTG for 5 hours. Cells were then harvested and frozen at -80°C. The frozen pellet was thawed on ice, suspended in 30 ml of buffer (20 mM Imidazole, 50 mM Tis, 500 mM NaCl) supplemented with cOmplete, EDTA-free protease inhibitor cocktail (Roche) and egg white lysozyme (Sigma) was added to 1 mg/ml and incubated for 45 min prior to probe sonication. The soluble fraction was separated by centrifugation at 18000g for 45 minutes at 4°C and then passed through a syringe driven 0.45 μm filter. Clarified extract was applied to Ni^{2+}–NTA columns (Hi-Trap, GE Healthcare) using an ÄKTA Prime. The column was washed with 10-15 column volumes of buffer before switching over to the elution buffer (500 mM Imidazole, 50 mM Tis, 500 mM NaCl). The sample was assessed for purity using SDS-PAGE gels and coomassie

staining and the band corresponding to AmrZ-His_6 confirmed with western blot analysis (See Fig. S10). The sample was then diluted into buffer (50 mM Tis, 500 mM NaCl) and quantification was performed using a Pierce BCA protein assay kit following the manufactures instructions and using BSA as standards. *E. coli* B834 (DE3) carrying pET28a-*vgrG4b*, along with *E .coli* BL21 (λDE3) harbouring pET28a-*hcp3* were grown in terrific broth, induced and harvested. The frozen pellet was suspended in buffer supplemented with cOmplete, EDTA-free protease inhibitor cocktail (Roche) prior to disruption by French press. Clarified extract was applied to Ni^{2+}–NTA columns (Hi-Trap, GE Healthcare) using an ÄKTA Prime for purification.

Western blot analysis

SDS-PAGE and western blotting were performed as described in (6). Briefly, proteins were resolved in 8% (VgrGs, RpoB), 12% (PldA, PldB, RpoB) or 15% (Hcps, TssBs and AmrZ) gels using the Mini-PROTEAN system (Bio-Rad) by electrophoresis and transferred to nitrocellulose membrane (GE Healthcare). Membranes were blocked in 5% milk (Sigma) in Tris-buffered saline pH 8 with 0.1% Tween-20 (TBST) prior to incubation with primary antibodies. Monoclonal antibodies were used at the following dilutions: anti-RNA polymerase (Biolegend) at 1:5000, anti-beta lactamase (Bla) (GeneTex International Corporation) at 1:1000 and anti-V5 (ThermoFisher) at 1:5000. Polyclonal primary antibodies described previously were used at a dilution of 1:1000. These include anti-Hcp1 (8), anti-Hcp2 (9), anti-TssB1 (10), anti-TssB2 (9), anti-VgrG2a and anti-VgrG2b (11). The polyclonal anti-VgrG4b and anti-Hcp3 antibodies were also used at 1.1000. Membranes were washed with TBST before incubation with HRP-conjugated secondary antibodies (Sigma) at a dilution of 1:5000. All monoclonal primary antibodies required anti-mouse secondary antibodies, whereas the polyclonal antibodies all required anti-rabbit secondary antibodies.

Signals were detect using the Novex ECL HRP Chemiluminescent substrate (ThermoFisher) or the Luminata Forte Western HRP substrate (Millipore) using a LAS-3000 Fuji Imager. ECL detection and a white light image of the ladder were taken on a Fuji LAS-3000, separately. Adobe Photoshop was used to adjust the brightness/contrast of the tiff images prior to overlaying and merging of the two layers using the multiply tool followed by merge layers.

Antibody Production

Custom Anti-VgrG4b antibodies were raised against the purified C-terminus of VgrG4b (612-808) with Eurogentec following their immunisation protocols. Antibodies against purified full-length Hcp3 protein was raised similarly.

Electrophoretic mobility shift assays

Electrophoretic mobility shift assays (EMSA) were performed as previously described (8, 9). Using the previous published controls (8, 9). Each reaction contained 40 mM NaCl, 4 mM Tris, 4 mM $MgCl_2$ (pH 8.0), 4% glycerol (W/V), 100 mg/ml BSA (non-specific protein control), 150 ng/µl poly-deoxy-inosinic-deoxy-cytidylic acid (d[(I-C)]) (Sigma) (non-specific DNA control), 5 nM ^{32}P-labeled DNA probe and purified AmrZ in the nM range . The 10 µl reactions were incubated at room temperature for 20 min prior to the addition of 2 µl of native loading dye and loading of the entire sample. Reactions were analysed on 4% (w/v) native polyacrylamide gels run at 200 V for 22 minutes prior to drying. Gels were visualised on a Typhoon FLA7000 Phosphorimager (GE Healthcare).

Real Time PCR

Overnight cultures were subcultured in TSB, grown to early exponential phase and harvested in RNAlater (Ambion). RNA was isolated using TRIzol extraction and purified using the Qiagen RNeasy Mini kit (Qiagen) followed by DNase digestion using PureLink DNase Set (Invitrogen). Purified RNA was stored at −80°C in nuclease-free water. cDNA was synthesised from 200 ng of RNA using SuperScript III Reverse Transcriptase (Invitrogen) and random hexamers (Applied Biosystems) according to the manufacturer's protocol. Real time PCR was performed with Fast SYBR Green Master Mix (Applied Biosystems) or KiCqStart SYBR Green qPCR ReadyMix (Sigma) on an Applied Biosystems StepOnePlus Real-Time PCR machine. The primers used for amplification are shown in *SI Appendix*, Table S4. Real-time analysis was performed on RNA from three independent cultures in duplicate and quantification of *rpoD* gene expression served as an internal control. The relative expression ratios were calculated using the delta-delta method relative to PA14 wild-type or PA14*rsmA* pMMB as indicated.

Microscopy Procedure

Strains for visualizing coexpression of multiple T6SS systems *in situ* were as follows: PA14*rsmA*, PA14*rsmA* H123 and PA14*rsmA* H13 A2tl (H2-) with combinations of fluorescent fusions pME6032*tssB1-venus*, pJN105*tssB2-cfp*, pJN105*tssB3-cfp* and/or pMMB67*tssB3-sfgfp* (*SI Appendix*, Table S3, S4). *P. aeruginosa* strains for microscopy were grown overnight in TSB with appropriate antibiotic(s) at 37°C, then diluted to an OD_{600} = 0.1 in 10 ml fresh TSB plus appropriate antibiotic(s) and grown to OD_{600} = 0.3 at 25°C. Plasmid expression was induced with IPTG (50 μg/ml) for strains containing pME6032 or pMMB67HE plasmids, or with arabinose (1%) for strains containing pJN105 plasmids. Cells growing exponentially (OD_{600} = 0.8-1.0) were harvested by centrifugation (8000 *g*, 3 min) and resuspended in PBS to a final OD_{600} of 10 units. One microliter of resuspended culture

was spotted on a glass-bottomed dish (Ibidi 35 mm μ-dish) and covered with a 1% PBS agarose pad. Cells were imaged using a Zeiss Axiovert 200 inverted microscope fitted with Hamamatsu Flash 4 (2048x2048 pixel) camera for fast low-light imaging, a pE4000 CoolLed Led Illumination source and a 100x 1.4 objective. A 500 nm LED was used to excite TssB1-Venus with an exposure time of 1000 ms using a YFP filter; a 435 nm LED was used to excite TssB2-CFP and TssB3-CFP with exposure times of 300 ms and 100 ms respectively using a CFP filter; and finally, a 500 nm LED was used to excite TssB3-sfGFP with an exposure time of 300 ms using a GFP filter. The LED and dichroic filter sets were selected based upon the spectral profiles for each fluorophore to ensure optimised excitation and emission settings were used for each of the relevant co-expressed fluorescent fusions.

Image analysis

Microscopy images were analysed in Fiji (10). The Bleach Correction plugin was used on time-lapse image series (15). To determine the number of cells across all fields to be analysed the background was subtracted from brightfield DIC images using a filtered Gaussian blur image with Sigma (radius) = 20 and the 'Image Calculator' Fiji tool. Huang segmentation was then used within the 'Threshold' tool to select all the cells in each field, with the tool 'Analyse Particles' finally used to quantify the number of cells in each field. The range used for 'Analyse Particles' was set at 30-infinity pixels, to ensure that small areas of background brightfield particles (i.e. not cells) were excluded. The number of cells counted by this automated method was initially manually checked on a subset of images to ensure the process was a true reflection of the number of cells present in all subsequent analyses. The number of cells containing foci was then counted manually by using the 'Multi-Point' tool in Fiji. At least 10 separate randomly selected fields of view were analysed for each fluorescent fusion

in PA14*rsmA* which contained between 185 276 and 319 440 total cells or in PA14*rsmA* H123 or PA14*rsmA* H1H3 A2tl (H2-) which contained between 31 666 and 68 208 total cells.

Bioinformatics and statistical analysis

DNA sequences were retrieved from the Pseudomonas Genome Database (16). Binding motifs were investigated using Mfold (17), FUZZNUC (EMBOSS) and BPROM. DNA and amino acid sequence searches were executed using SMART, InterPsoScan, Pfam and BLAST. Statistical analysis was performed using GraphPad Prism version 5 as indicated in the text.

Additional references

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11. Miura K RC, Hiner M, Schindelin J, Rietdorf J. (2012) ImageJ Plugin CorrectBleach V2.0.2.
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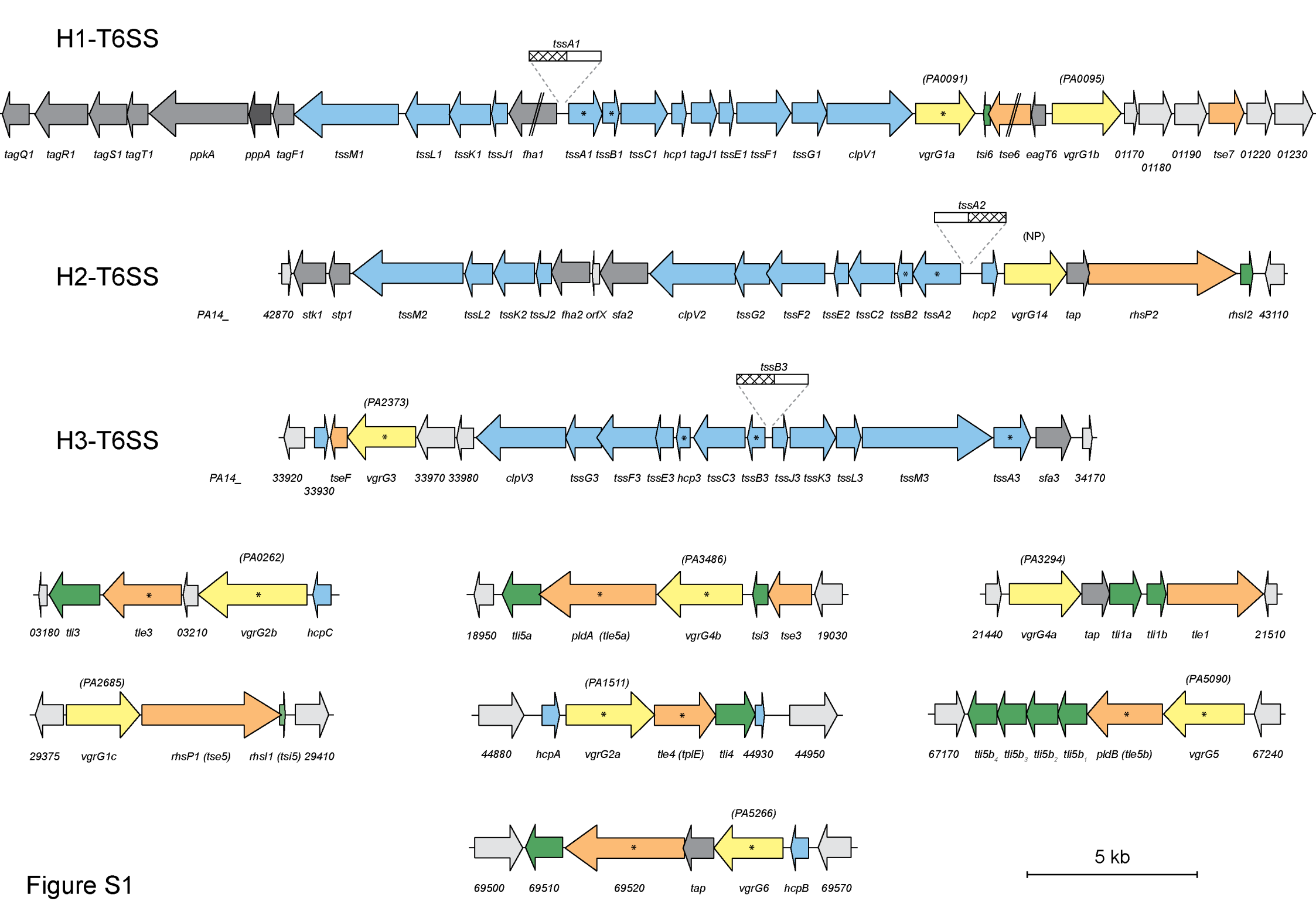
13. Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic acids research* 31(13):3406-3415.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(PA0095)
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tssA2
(NP)
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H3-T6SS
tssB3
(PA2373)
PA14_ 33920 33930 tseF vgrG3 33970 33980 clpV3 tssG3 tssF3 tssE3 hcp3 tssC3 tssB3 tssJ3 tssK3 tssL3 tssM3 tssA3 sfa3 34170
(PA0262)
03180 tli3 tle3 03210 vgrG2b hcpC
(PA3486)
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(PA3294)
21440 vgrG4a tap tli1a tli1b tle1 21510
(PA2685)
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(PA1511)
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(PA5090)
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5 kb

Figure S1

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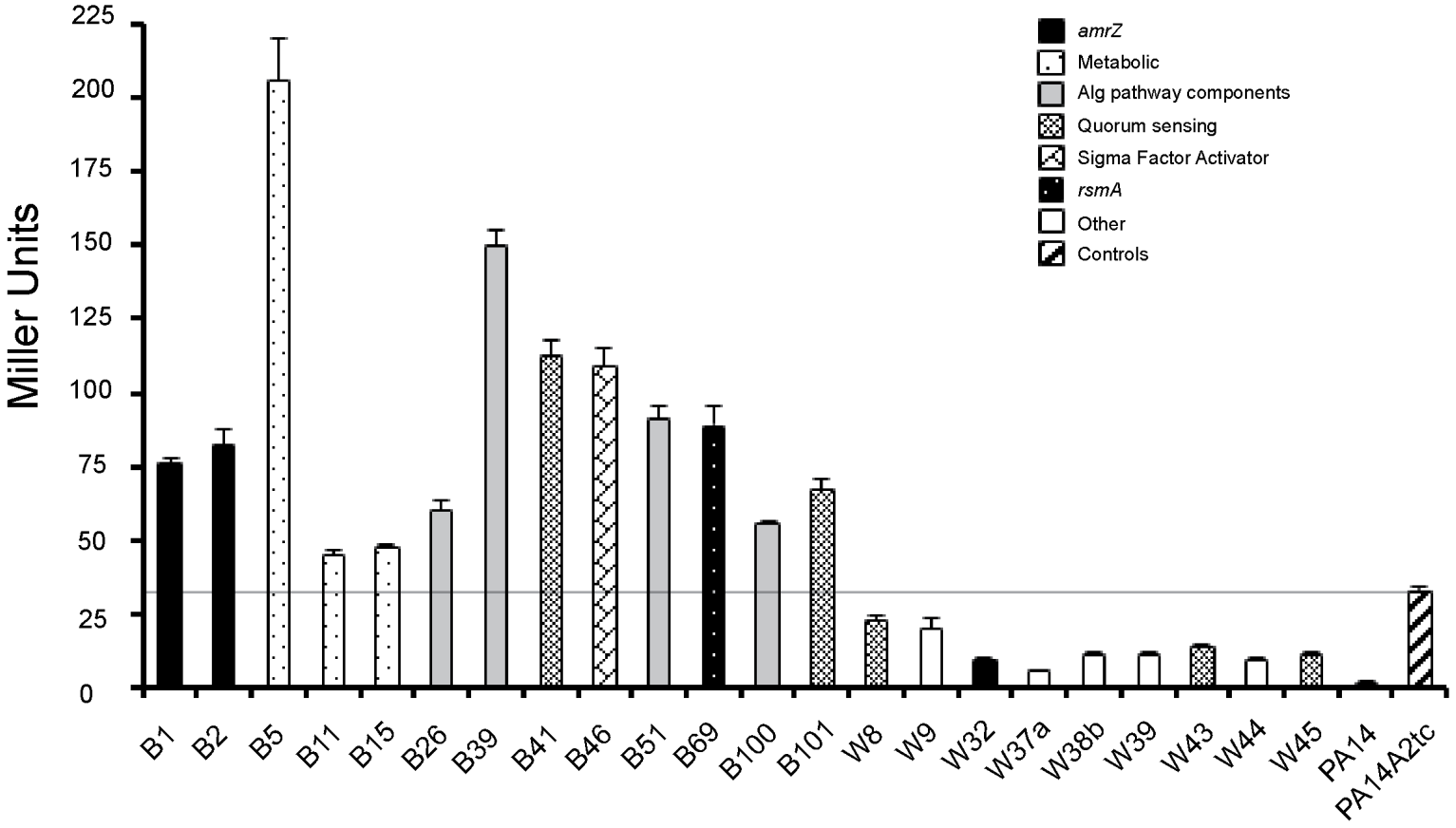
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200
175
150
125
100
75
50
25
0
amrZ
Metabolic
Alg pathway components
Quorum sensing
Sigma Factor Activator
rsmA
Other
Controls
B1
B2
B5
B11
B15
B26
B39
B41
B46
B51
B69
B100
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W44
W45
PA14
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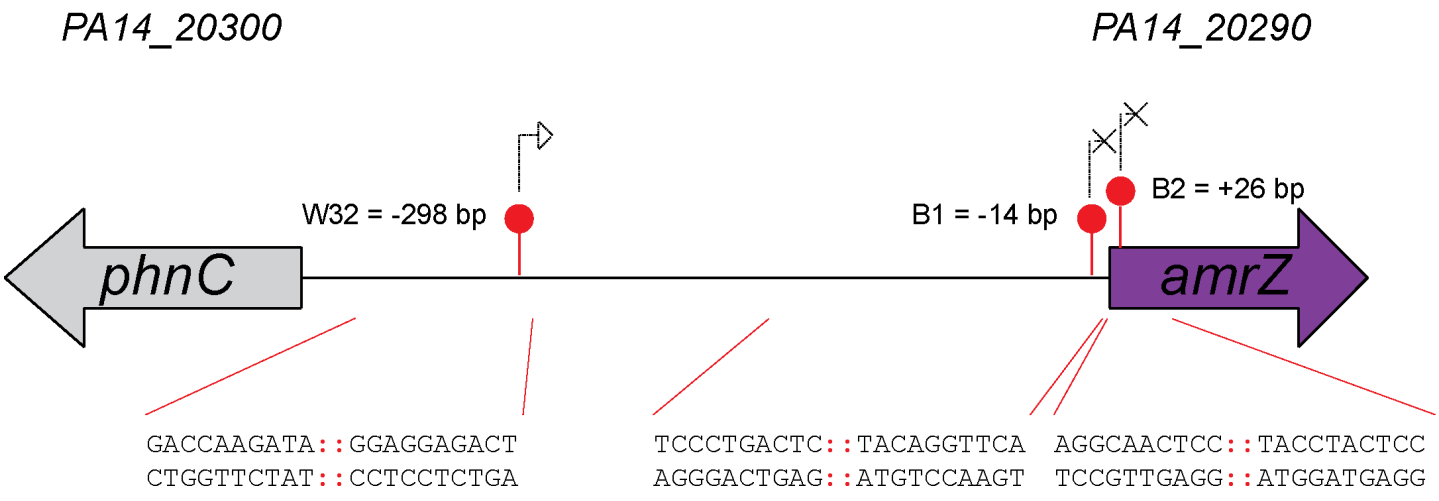
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PA14_20290
W32 = -298 bp
B1 = -14 bp
B2 = +26 bp
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amrZ
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CTGGTTCTAT::CCTCCTCTGA
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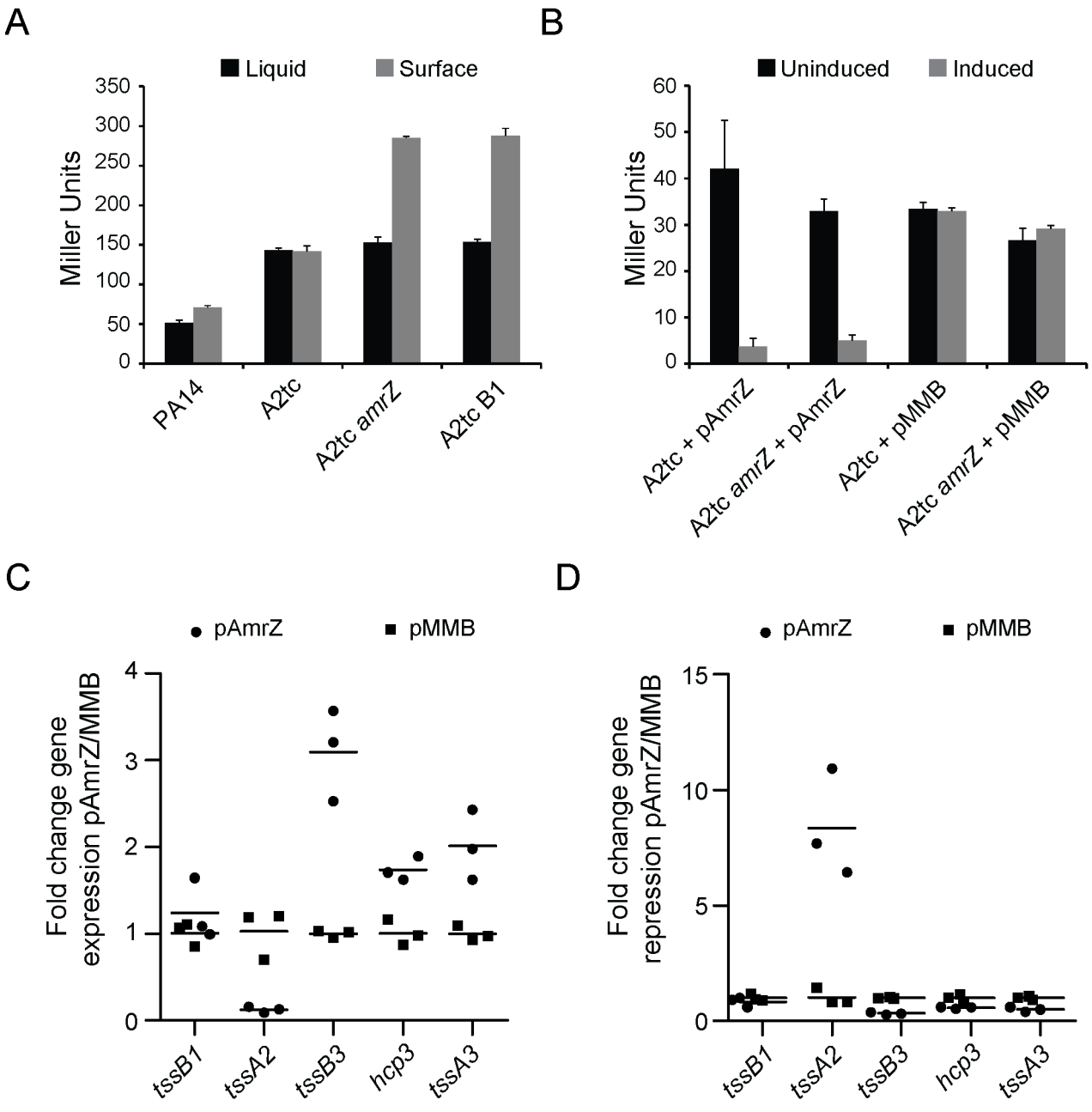
TCCGTTGAGG::ATGGATGAGG

Figure S2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B
Uninduced
Induced
Miller Units
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50
40
30
20
10
0
A2tc + pAmrZ
A2tc amrZ + pAmrZ
A2tc + pMMB
A2tc amrZ + pMMB
C
pAmrZ
pMMB
Fold change gene expression pAmrZ/MMB
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3
2
1
0
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tssA2
tssB3
hcp3
tssA3
D
pAmrZ
pMMB
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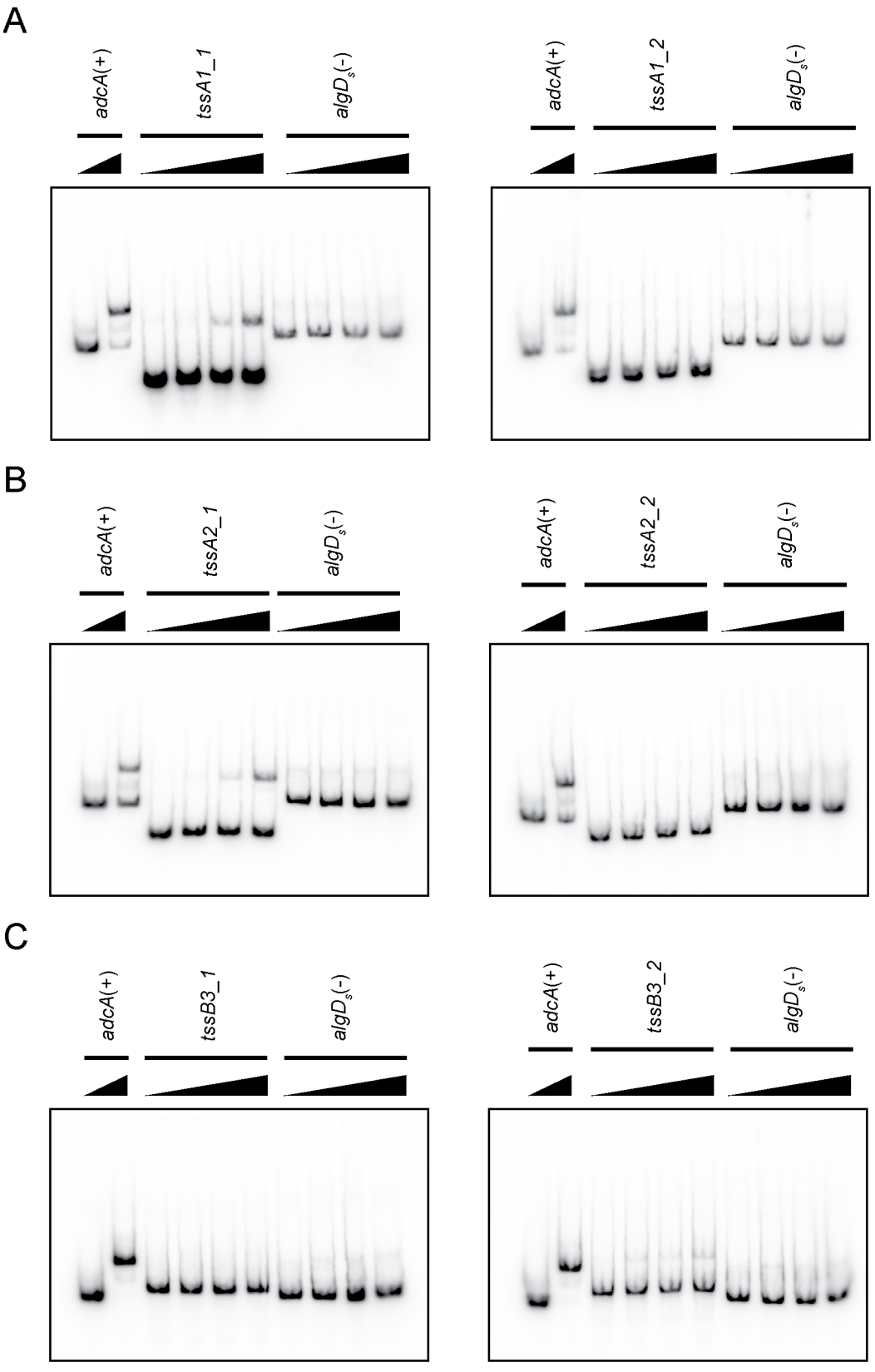
Figure S3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adcA(+)
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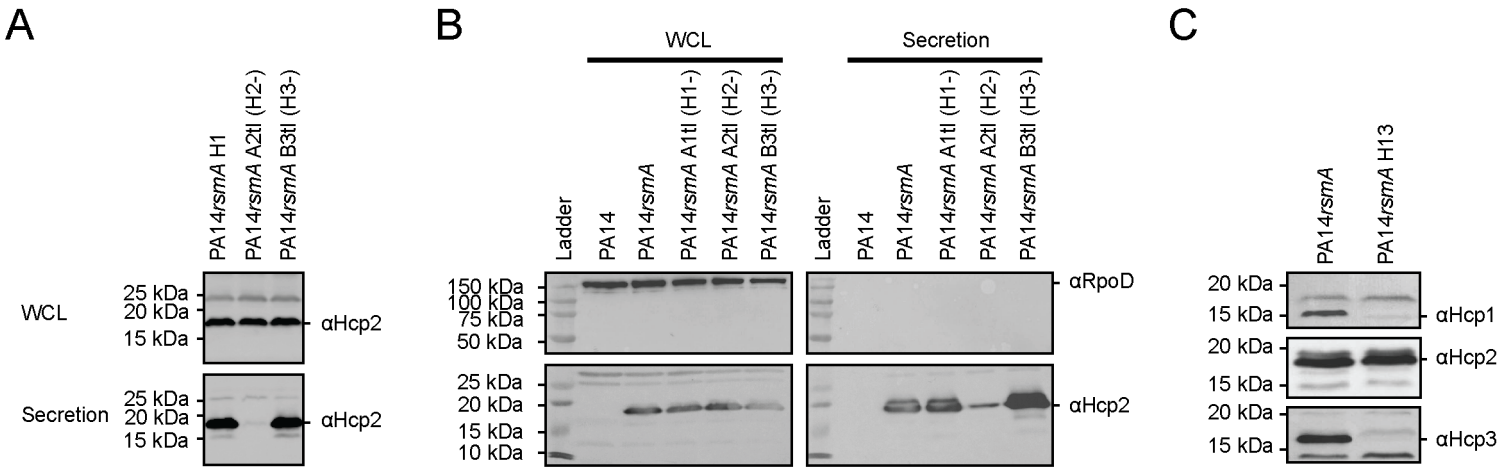
tssB3_2
algDs(-)

Figure S4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20 kDa
15 kDa
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Secretion
25 kDa
20 kDa
15 kDa
αHcp2
B
WCL
Secretion
Ladder
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PA14rsmA
PA14rsmA A1tl (H1-)
PA14rsmA A2tl (H2-)
PA14rsmA B3tl (H3-)
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100 kDa
75 kDa
50 kDa
αRpoD
25 kDa
20 kDa
15 kDa
10 kDa
αHcp2
C
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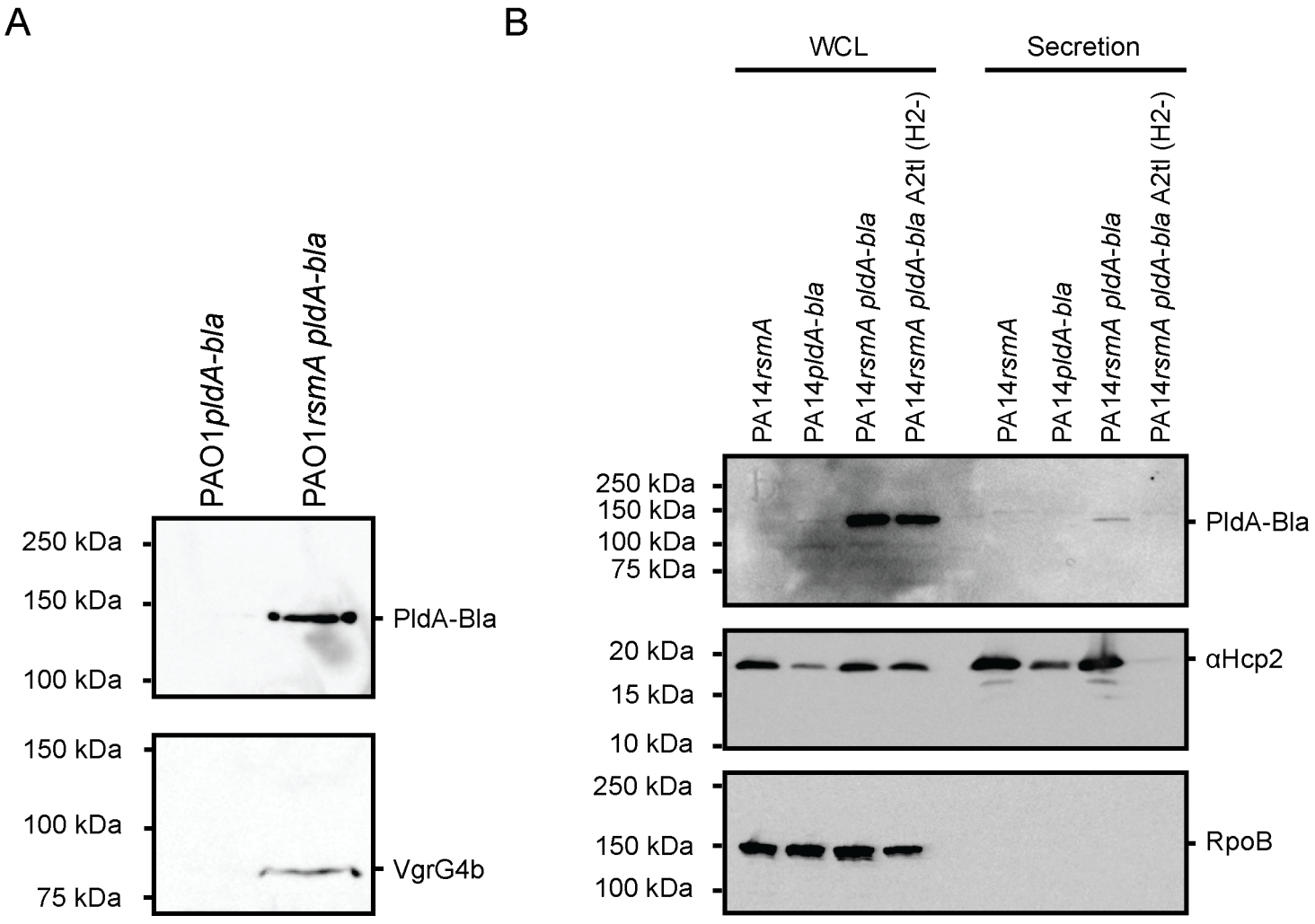
αHcp3

Figure S5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B
WCL
Secretion
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PA14pldA-bla
PA14rsmA pldA-bla
PA14rsmA pldA-bla A2tl (H2-)
PA14rsmA
PA14pldA-bla
PA14rsmA pldA-bla
PA14rsmA pldA-bla A2tl (H2-)
250 kDa
150 kDa
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αHcp2
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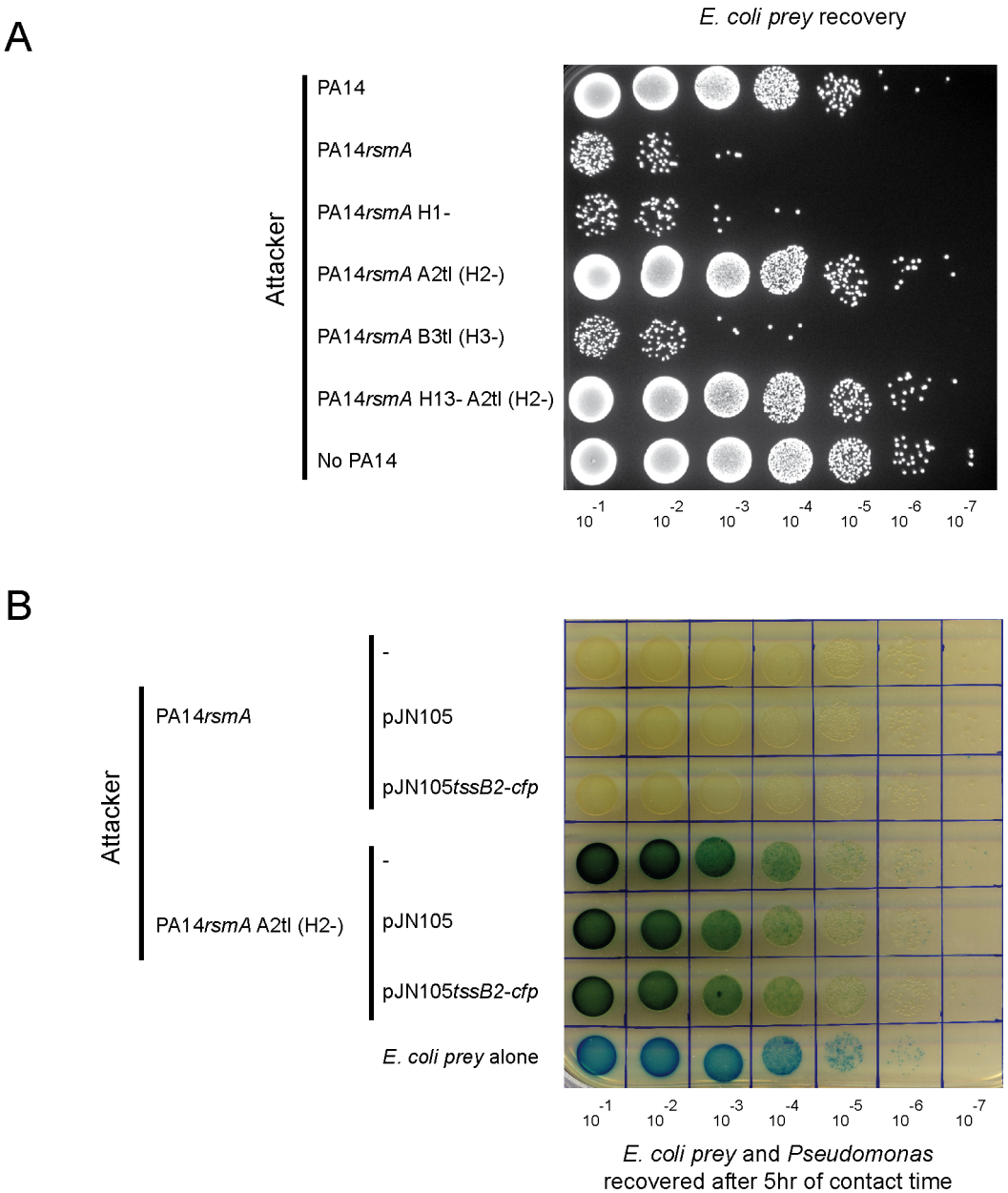
100 kDa
RpoB

Figure S6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PA14rsmA H13- A2tI (H2-)
No PA14
10-1 10-2 10-3 10-4 10-5 10-6 10-7
B
Attacker
PA14rsmA
PA14rsmA A2tI (H2-)
-
pJN105
pJN105tssB2-cfp
-
pJN105
pJN105tssB2-cfp
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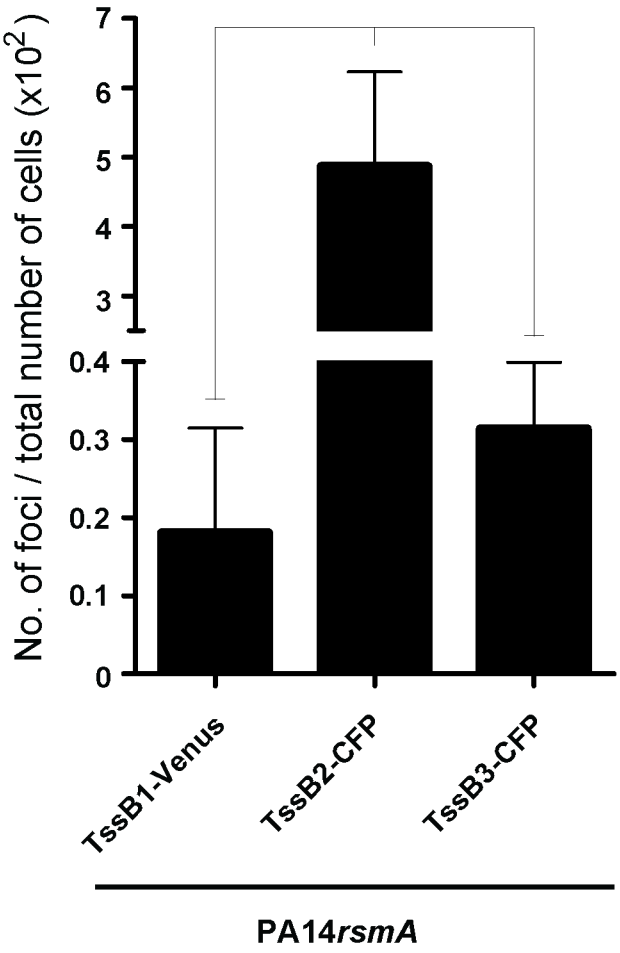
E. coli prey and Pseudomonas
recovered after 5hr of contact time

Figure S7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4
3
0.4
0.3
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TssB3-CFP
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Figure S8

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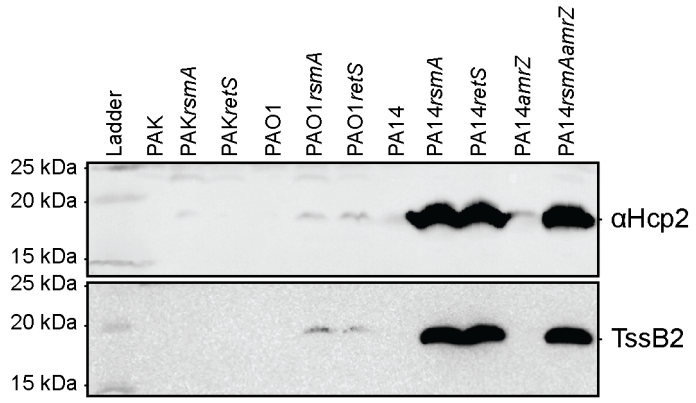
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B

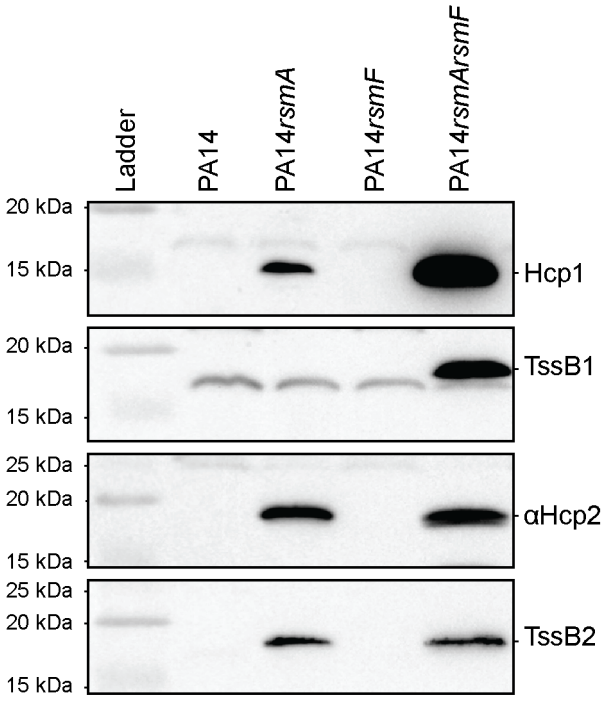
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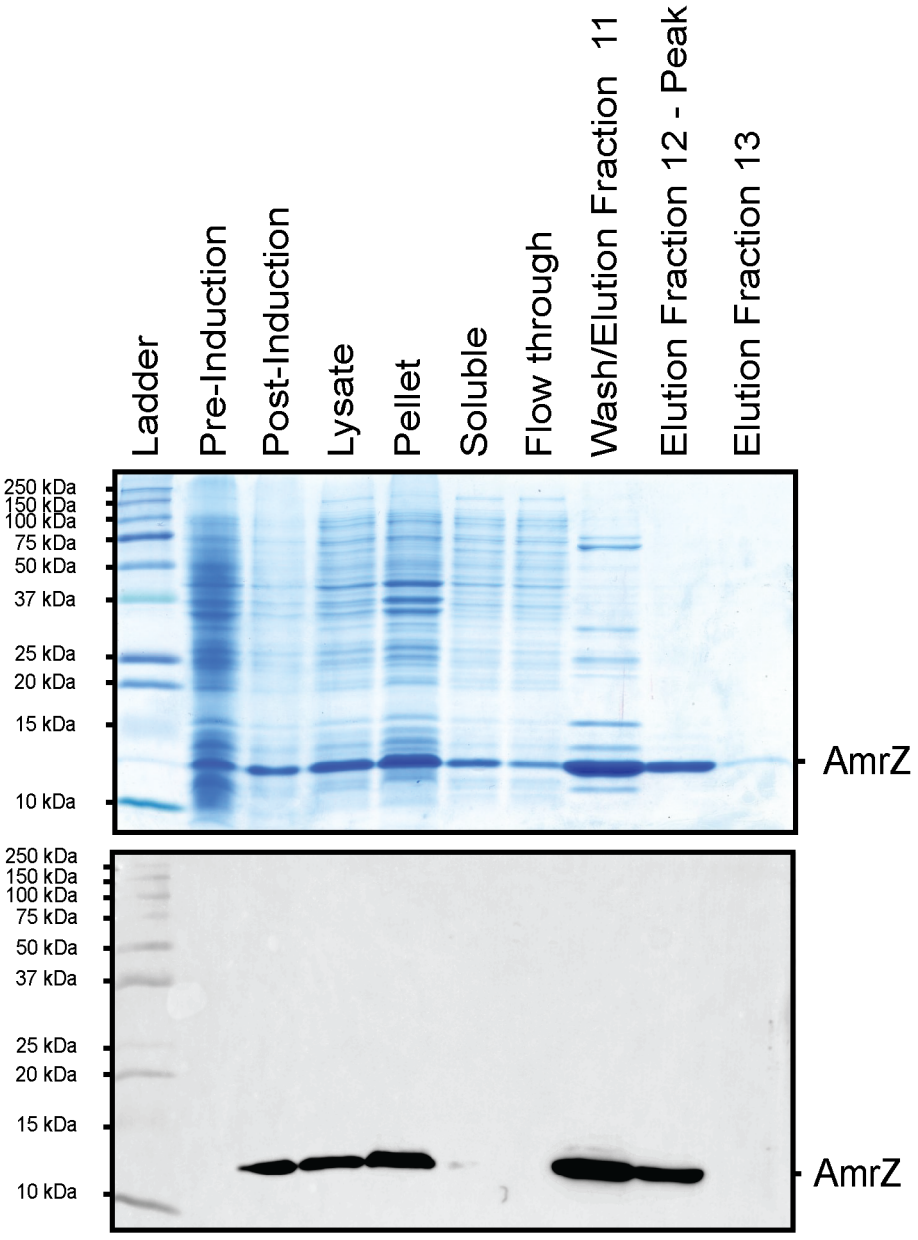
Figure S9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Soluble
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150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
AmrZ
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
AmrZ

Figure S10

Supplementary Figures Legends

Fig. S1. Schematic representation of *P. aeruginosa's* T6SS and *vgrG* clusters from PA14. Gene names or numbers are indicated below each gene cluster. PAO1 gene numbers are indicated above each cluster for the *vgrG* encoding genes for reference (NP = not present). Colored genes indicated T6SS structural components (blue), *vgrG*s (yellow), toxin/immunity pairs (orange/green), other known T6SS genes (dark grey) and not currently associated with the T6SS in light grey. Genes checked via qRT-PCR are indicated by an asterisk. Schematic of upstream regions used for AmrZ binding assays are show and hashed region indicates the sub-fragment bound by AmrZ.

Fig. S2. Expression of the H2-T6SS gene cluster in selected PA14 transposon mutants. (*A*) Level of β-galactosidase activity of the A2tc transcriptional fusion in selected mutants are shown. Mutants have been grouped as described in the key. (*B*) Location of transposon insertion in proximity to *amrZ* (*PA14_20290*). Insertion mutants B1 (14 bp upstream of gene) and B2 (26 bp into gene), which may prevent AmrZ expression (indicated by dotted cross), resulted in increased expression of A2tc. In contrast, insertion mutant W32 (298 bp upstream of *amrZ*) which may increase read through of *amrZ* (indicated by dotted arrow) resulted in reduced expression of A2tc.

Fig. S3. AmrZ inversely regulates the H2- and H3-T6SS. (*A*) AmrZ is a negative regulator of the H2-T6SS. Deletion of *amrZ* relieves repression of *tssA2* when grown on agar plates. Beta-galactosidase assay was performed on PA14, PA14A2tc, PA14A2tc *armZ* and PA14A2tc B1 grown in either liquid LB or on LB plates. (*B*) Beta-galactosidase assay showing that overexpression of AmrZ represses transcription of *tssA2* (A2tc) which is absent in the uninduced or vector only (pMMB67HE). Strains were grown in liquid culture. (*C*) AmrZ represses the H2-T6SS (*tssA2*) but activates the H3-T6SS (*tssB3*, *hcp3* and *tssA3*). qRT-PCR was performed on PA14*rsmA* overexpressing *amrZ* (pAmrZ) and compared to vector control (pMMB). Scatter plot of fold change gene

expression with mean (N = 3). Statistical analysis was performed on the ΔΔCT values (ANOVA Bonferroni post-test *tssB1* $P > 0.05$, $P < 0.05$ for all other genes). (*D*) Fold gene repression of data shown in C.

Fig. S4. Dissection of AmrZ binding sites. EMSA was performed using sub fragments of the DNA probes used in figure 3; (*A*) *tssA1_1, tssA1_2,* (*B*) *tssA2_1*, *tssA2_2* and (C) *tssB3_1* *tssB3_2*. In all cases AmrZ only binds to one of the sub fragments from each upstream region. Each reaction contains 5 nM ^{32}P-labelled DNA and increasing concentrations of purified AmrZ. In all cases, positive (*adcA*) or negative (*algD*$_s$) controls were used as previously published (18, 19). See also Fig. S1 for schematic of AmrZ binding fragments used in this study.

Fig. S5. The H2-T6SS is functional in a *rsmA* mutant and Hcp2 depends upon this system for secretion. (*A*) Hcp2 is only faintly detectable when the H2-T6SS is disrupted (PA14*rsmA* A2tl (H2-)) but is still secreted when either the H1-T6SS is deleted (PA14*rsmA* H1-) or the H3-T6SS is disrupted (PA14*rsmA* B3tl (H3-)) after 5 h of growth from an OD_{600} of 0.1 at 37 degrees. (*B*) Hcp2 is drastically reduced when the H2-T6SS is disrupted (PA14*rsmA* A2tl (H2-)) but is still secreted when either the H1-T6SS is deleted (PA14*rsmA* H1-) or the H3-T6SS is disrupted (PA14*rsmA* B3tl (H3-)) after 8 h of growth from an OD_{600} of 0.1 at 37 degrees. RNA polymerase (RpoB) is used as a lysis control. (*C*) Deletion of the H1-T6SS cluster including *hcp1* and the H3-T6SS cluster including *hcp3* does not diminish the level of protein detected with the anti-Hcp2 antibody.

Fig. S6. PldA and VgrG4b are expressed in a *rsmA* mutant. (*A*) Western blot analysis using anti-TEM to detect PldA-Bla expression, or anti-VgrG4b shows enhanced expression of both proteins in PAO1*rsmA* at 25°C. (*B*) Deletion of *rsmA* enables PldA secretion. Western blot analysis using anti-TEM to detect PldA-Bla expression shows expression in a PA14*rsmA* mutant at 25°C and secretion in a H2-T6SS-dependent manner (A2tl (H2-)). Anti-RNA polymerase (RpoB) is used as a lysis control and anti-Hcp2 as a control for H2-T6SS activity.

Fig. S7. *P. aeruginosa* H2-T6SS dependent killing of *E. coli.* (*A*) Serial dilution of the 1:1 bacterial mix including *E. coli* GFP+ and the PA14 attackers as listed. *E. coli* is recovered significantly less when co-incubated with PA14*rsmA* as compared to PA14 and the killing is specifically H2-T6SS dependent as it is not alleviated in either the H1- or H3-T6SS mutants. (*B*) H2-T6SS killing assays demonstrating that the H2-T6SS is as functional in PA14*rsmA* as PA14*rsmA* carrying either pJN105 or pJN105*tssB2-cfp*. *P. aeruginosa* strains were mixed individually with *E. coli* DH5α carrying pCR2.1 which expresses β-galactosidase. *Pseudomonas* strains were grown in the presence of arabinose 1% prior to the killing assay and arabinose 1% was included in the LB agar for the 5 h contact time to enable constant expression of TssB2-CFP. Recovered bacteria were diluted and spotted on X-gal plates. The level of blue color indicates survival of *E. coli* when the H2-T6SS is disabled (PA14*rsmA* A2tl).

Fig. S8. Significantly more H2-T6SS (TssB2-CFP) foci where observed then either H1-T6SS (TssB1-Venus) or H3-T6SS (TssB3-CFP) foci. Quantification of relative numbers of TssB1-Venus, TssB2-CFP and TssB3-CFP foci present in PA14*rsmA* after induction. Cells were grown at 25°C until the OD reached 0.3, at which point expression of the fluorescent fusions was induced by addition of IPTG or arabinose, with cells being harvested during mid-exponential phase and imaged directly. The mean number of foci per total PA14*rsmA* cells + SEM is represented based upon at least 40 separate fields

of view captured over two independent days. Total numbers of cells analysed were 284 985 for TssB1-Venus, 185 276 for TssB2-CFP and 319 440 for TssB3-CFP. Statistical analyses were performed using a one-way ANOVA with multiple comparisons (Tukey) comparing all 3 samples ($P < 0.005$).

Fig. S9. Differential expression of T6SSs (*A*) Western blot analysis of regulator mutants showing higher levels of expression of H2-T6SS components in PA14 mutants compared to the same mutants in PAK or PAO1. Bacteria were grown at 37°C. (*B*) Western blot analysis showing that strong expression of the H1-T6SS components Hcp1 and TssB1 requires the double *rsmArsmF* mutant. H2-T6SS components have enhanced expression in an *rsmA* mutant but no additional expression was observed in the *rsmArsmF* mutant.

Fig. S10. Representative blot for the purification of AmrZ used in EMSAs. (*A*) Coomassie stained SDS-PAGE gel and (*B*) western blot using anti-His antibody to detect AmrZ-His_6. Fraction 12 and 13 were used for EMSAs.

Table S1. Location of Transposon insertion in mutants with altered transcription of *tssA2*tc

Table S2. Identification of putative AmrZ binding sites upstream of *tssA1*, *tssA2* and *tssB3*

Table S3. Strain and Plasmid List

Table S4. Primer List

Table S1: Location of Transposon insertion in mutants with altered transcription of *tssA2*tc

Mutant code	Site of integration	Gene inserted into/near	PAO1	Gene name	Description
B1	1747943	PA14_20290/PA14_20300	PA3385/PA3384	*amrZ(algZ)/phnC*	Upstream of DNA binding protein (alginate and motility regulator Z), ABC phosphonate transporter ATP-binding protein
B2	1747903	PA14_20290	PA3385	*amrZ(algZ)*	DNA binding protein (alginate and motility regulator Z)
B5	5789115	PA14_64940/PA14_64950	PA4917/PA4918	*pncA*	NAD biosynthetic process/Nicotinamidase
B11	3920247	PA14_44030	PA1583	*sdhA*	Insertion in Succinate dehydrogenase (A subunit), effect likely on *sdhB* (B subunit)
B15	3923546	PA14_44070	PA1580	*gltA*	Citrate synthase *cisY*
B26	5143772	PA14_57760	PA4446	*algW*	AlgW protein, Alginate pathway
B39	1469468	PA14_17140	PA3649	*mucP*	Putative membrane-associated zinc metalloprotease
B41	7316	PA14_00060	PA0005	*lptA*	Putative acyltransferase (lysophosphatidic acid acyltransferase activity)
B46	3824444	PA14_42990	PA1661	*tssG2*	T6SS component of H2-T6SS, effect may be on PA14_42970 *sfa2*
B51	1470367	PA14_17140	PA3649	*mucP*	Putative membrane-associated zinc metalloprotease
B69	4660539	PA14_52570	PA0905	*rsmA*	Regulator of secondary metabolism (carbon storage regulator)
B100	4826929	PA14_54430/PA14_54450	PA0762/PA0761	*algU/nadB*	Sigma factor AlgU/AlgT/σ22 / L-aspartate oxidase
B101	4085470	PA14_45960	PA1430	*lasR*	Transcriptional regulator LasR
W8	1651112	PA14_19120	PA3477	*rhlR(sdiA)*	RhlR: sdiA acylhomoserine lactone dependent transcriptional regulator
W9	6310464	PA14_70850	PA5368	*pstC*	ABC transporter/permease
W32	1748225	PA14_20290/PA14_20300	PA3385/PA3384	*amrZ(algZ)/phnC*	Upstream of DNA binding protein (alginate and motility regulator Z), ABC phosphonate transporter ATP-binding protein
W37a	3675920	PA14_41200/PA14_41210	PA1803/PA1804	*lon/hupB*	Lon protease/DNA-binding protein HU, effect likely on *hupB*
W38b	6263767	PA14_70270	PA5322	*dut*	Deoxyuridine 5'-triphosphate nucleotidohydrolase, effect likely on downstream gene AlgC
W39	2022161	PA14_23310	PA3164	unknown	EPSP synthase/prephenate dehydrogenase
W43	4571514	PA14_51430/PA14_51440	PA0996/PA0995	*pqsA/ogt*	PqsA probable coenzyme A ligase/ methylated-DNA-protein-cysteine methyltransferase
W44	5604286	PA14_62810	PA4747	*secG*	Preprotein translocase subunit SecG, effect on downstream gene(s) encoding tRNA or transcription elongation/initiation factors
W45	1651117	PA14_19120	PA3477	*rhlR(sdiA)*	RhlR: SdiA acylhomoserine lactone dependent transcriptional regulator

Table S2: Identification of putative AmrZ binding sites upstream of *tssA1*, *tssA2* and *tssB3*

Gene	Predicted AmrZ site?	Bp from ideal	3' end relative to ATG (bp)	Strand relative to gene	Location	Putative Site CAAATTGCCATCA	In Sub fragment
tssA1	Yes	5	-156	-ve		CACAACGCCACTA	*tssA1_1*
tssA1	Yes	5	-110	+ve	Overlapping predicted-35	AATTTTGCCAACC	*tssA1_2*
tssA1	Yes	5	-86	+ve	Overlapping predicted -10	CCGATATTCATCA	*tssA1_2*
tssA2	Yes	1	-265	+ve	Close to predicted -35	CAAATGGCCATCA	*tssA2_1*
tssA2	Yes	4	-243	-ve	Overlapping predicted -10	GCTATAGCCATCA	*tssA2_2*
tssB3	Yes	4	-436	-ve		CCAATCGCGAGCA	*tssA3_1*
tssB3	Yes	5	-231	+ve	Close to predicted -35	AGAATTTCCGACA	*tssA3_2*

Note: Fragment in red text is bound by AmrZ in *SI appendix*, Fig. S4.

Table S3: Strain and Plasmid list

Strain/Plasmid	Relevant Characteristics
Strain	
E. coli	
DH5α	*F⁻ endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20* ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, *hsdR17* ($r_K^- m_K^+$), λ⁻
Top10	*F⁻ mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80*lacZ*ΔM15 Δ*lacX74 nupG recA*1 *araD139* Δ(*ara-leu*)7697 *galE15 galK16 rpsL* (Str^R) *endA1* λ⁻
CC118λ*pir*	Host strain for pKNG101 replication; Δ(ara leu) *araD ΔlacX*74 *galE galK-phoA* 20 thi-1 *rpsE rpoB argE* (Am) *recA* 1 Rfr λ*pir*
SM10λ*pir*	Host strain for pKNG101 replication; *thi-1 thr leu tonA lacY supE recA*::RP4-2-Tc::Mu λ*pir*, KmR
BL21 Star (λDE3)	*F⁻ ompT hsdS*$_B$(r_B- m_B-) *gal dcm rne* 131 (λDE3)
B834 (λDE3)	*F⁻ ompT hsdS*$_B$(r_B⁻ m_B⁻) *gal dcm met* (λDE3)
1047	Host strain for pRK2013
P. aeruginosa	
PA14	Wild type
PAO1	Wild type
PAK	Wild type
PA14 A1tl	PA14*tssA1*::*lacZ* tl (*tssA1 lacZ* translational fusion (PA14_00990))
PA14 A2tc	PA14*tssA2*::*lacZ* tc (*tssA2 lacZ* transcriptional fusion (PA14_43050))
PA14 A2tl	PA14*tssA2*::*lacZ* tl (*tssA2 lacZ* translational fusion (PA14_43050))
PA14 B3tl	PA14*tssB3*::*lacZ* tl (*tssB3 lacZ* translational fusion (PA14_34070))
PA14 A1tl *rsmA*	PA14*tssA1*::*lacZ* tl *rsmA* deletion mutant (Also refered to as PA14rsmA A1tl (H1-))
PA14 A2tl *rsmA*	PA14tssA2::*lacZ* tl *rsmA* deletion mutant (Also refered to as PA14*rsmA* A2tl (H2-))
PA14 B3tl *rsmA*	PA14*tssB3*::*lacZ* tl rsmA deletion mutant (Also refered to as PA14*rsmA* B3tl (H3-))
PA14 A2tc *amrZ*	PA14*tssA2*::*lacZ* tc *amrZ* deletion mutant
PA14*rsmA*	PA14*rsmA* (PA14_52570) deletion mutant
PA14*amrZ*	PA14*amrZ* (PA14_20290) deletion mutant
PA14*rsmAamrZ*	PA14*rsmA* (PA14_52570) and *amrZ* (PA14_20290) deletion mutant
PA14*rsmF*	PA14*rsmF* (PA14_68470) deletion mutant
PA14*rsmArsmF*	PA14*rsmA* and *rsmF* deletion mutant
PA14*rsmA* H1-	PA14*rsmA* and H1-T6SS (*tagQ1-vgrG1b*) deletion mutant
PA14*rsmA* H13-	PA14*rsmA,* deletion of H1-T6SS (*tagQ1 -vgrG1b*) and H3-T6SS (*tssB3 -clpV3*)
PA14*rsmA* H13- A2tl (H2-)	PA14 *rsmA* A2tl, deletion of H1-T6SS (*tagQ1 -vgrG1b*) and H3-T6SS (*tssB3 -clpV3*)
PA14*rsmA* H123-	PA14*rsmA,* deletion of H1-T6SS (*tagQ1 -vgrG1 b*), H2-T6SS (*tssA2-clpV2*) and H3-T6SS (*tssB3 -clpV3*)
PA14 *hcp3v5*	PA14 *hcp3v5* (PA14_34030)
PA14*rsmA hcp3v5*	PA14*rsmA hcp3v5*
PA14*rsmA hcp3v5* B3tl (H3-)	PA14*rsmA* B3tl (H3-) *hcp3v5*
PA14*pldB-bla*	PA14 *pldB* fusion with Bla Beta Lactamase
PA14*rsmA pldB-bla*	PA14*rsmA* deletion mutant, *pldB* fusion with Bla Beta Lactamase
PA14 *pldA-bla*	PA14 *pldA* fusion with Bla Beta Lactamase
PA14*rsmA pldA-bla*	PA14*rsmA pldA* fusion with Bla Beta Lactamase
PA14*rsmA pldA-bla* A2tl (H2-)	PA14*rsmA pldA* fusion with Bla Beta Lactamase
PAO1*rsmA*	PAO1*rsmA* deletion mutant
PAO1*rsmA tssB2* (H2-)	PAO1*rsmA tssB2* (H2-) deletion mutant
PAO1 *pldA-bla*	PAO1 *pldA* fusion with Bla Beta Lactamase
PAO1*rsmA pldA-bla*	PAO1*rsmA pldA* fusion with Bla Beta Lactamase
PAO1*rsmA pldA-bla tssB2* (H2-)	PAO1*rsmA tssB2* (H2-) *pldA* fusion with Bla Beta Lactamase
PAO1*rsmA pldA-bla vgrG4b*	PAO1*rsmA pldA* fusion with Bla Beta Lactamase, deletion of *vgrG4b* (PA3486)
PAK*rsmA*	PAK*rsmA* deletion mutant
PAK*retS*	PAK*retS* deletion mutant
PAO1*rsmA*	PAO1*rsmA* deletion mutant
PAO1*retS*	PAO1*retS* deletion mutant
PA14*retS*	PA14*rsmA* deletion mutant
Plasmids	
pCR-BluntII-TOPO	Blunt cloning vector, ZeoR/KmR
pKNG101	Suicide vector, *sacB* , StrR
pRK2013	Tra+, Mob+, KmR
pRL662-*gfp*	Broad host range vector derived from pBBR1MCS-5 expressing GFP, GmR
pBT20	For mariner transposon mutagenesis, GmR/AmpR
pKNG101-*tssA1*::*lacZ* tl	*tssA1*::*lacZ* tl (PA14_00990) translational fusion, StrR
pKNG101-*tssA2*::*lacZ* tc	*tssA2*::*lacZ* tc (PA14_43050) transcriptional fusion, StrR
pKNG101-*tssA2*::*lacZ* tl	*tssA2*::*lacZ* tl (PA14_43050) translational fusion, StrR
pKNG101-*tssB3*::*lacZ* tl	*tssB3*::*lacZ* tl (PA14_34070) translational fusion, StrR
pKNG101-*rsmA*	*rsmA* (PA14_52570) mutator, StrR
pKNG101-*amrZ*	*amrZ* (PA14_20290) mutator, StrR
pKNG101-*pldA-bla*	*pldA* fusion with Bla Beta Lactamase, StrR
pKNG101-*pldB-bla*	*pldB* fusion with Bla Beta Lactamase, StrR
pKNG101-H1-T6SS	H1-T6SS (PA0070-PA0095) mutator, StrR
pKNG101-H2-T6SS	H2-T6SS (PA1656-PA1662) mutator, StrR
pKNG101-*tssB2*	*tssB2* (PA14_43040) mutator, StrR
pKNG101-H3-T6SS	H3-T6SS (PA2365-PA2371) mutator, StrR
pKNG101-*retS*	*retS* (PA14_64230) mutator, StrR
pKNG101-*hcp3v5*	*hcp3* (PA14_34030) mutator for addtion of v5 tag, StrR
pKNG101-*vgrG4b*	*vgrG4b* (PA3486) mutator, StrR
pET28a	Expression vector, KmR
pET28a-*vgrG4b* (612-808)	Sequence encoding the C-terminus of *vgrG4b* (PA3486) was synthesised by Invitrogen GeneArt and subclone pET28a, KmR
pET28a-*hcp3*	pET28a-*hcp3* (PA2367) cloned in frame with a His6 tag, KmR
pMMB67HE	Broad host range expression vector with *tac* promoter (MCS HindIII-EcoRI), AmpR
pAmrZv5	pMME67HE-*amrZ* (PA14_20290) amplified with a V5 tag, AmpR
pET29a	Expression vector, KmR
pAmrZhis	pET29a-*amrZ* (PA14_20290) cloned in frame with a His_6 tag, KmR
pME6032::VenusC	Broad host range expression vector, for fusion of Venus at C-terminus IPTG induction, TetR
pME6032*tssB1-venus*	Strain expressing TssB1-Venus (PA0083) under IPTG induction, TetR
pJN105	Broad host range expression vector, araC-PBAD cassette cloned in pBBR1MCS-5, GmR
pJN105*tssB2-cfp*	Strain expressing TssB2-CFP (PA1657) under arabinose induction, GmR
pJN105*tssB3-cfp*	Strain expressing TssB3-CFP (PA2365) under arabinose induction, GmR
pMMB67HE*tssB3-sfgfp*	Strain expressing TssB3-GFP (PA2365) under IPTG induction, AmpR
pSEVA237C	Cloning vector with pBBR1 ORI containing *cfp* coding region, KmR
mini-CTX-*gfp*	Plasmid for integration of *gfp* fusions into the *att* site of the *P. aeruginosa* chromosome, TetR
pCR2.1	Constituative expression of β-Galactosidase, self ligated, AmpR/KmR

Table S4: Primer List

Primer list	Name	Oligonucleotide Sequence (5'-3')	Description
Fusions H1			
OAL3541	tssA1_Left_screening	TGAGGTAGTACACCCCGTCGCG	tssA1_Left_screening (PA14_00990)
OAL3542	tssA1_F_Right F	TGGTCTGGTGTCAAAAATAACTAGCGGCCGTCCGGGCCAACG	Right pool amplification of tssA1 (PA14_00990) transcriptional/translational fusion
OAL3543	tssA1_R_Right R	GCGCGCCCGGGCGGGTCCTTGATCGCCTTCATG	Right pool amplification of tssA1 (PA14_00990) transcriptional/translational fusion
OAL3544	tssA1_right_screening	TGGTCTGCTCGGTGGTGGTCTG	tssA1_right_screening (PA14_00990)
OAL3545	tssA1_F_Left_tl	GCGCGGGATCCGTCCGGCAGGACCCAGTCGTTG	Right pool amplification of tssA1 (PA14_00990) translational fusion
OAL3546	tssA1_R_Left_tl	GACGGCCAGTGAATCCGTAATCATGGTCACGGTGACGATCTCCCTATCATCG	Right pool amplification of tssA1 (PA14_00990) translational fusion
Fusions H2			
OAL1574	tssA2_F_Left_tc	GCGCGGGATCCATGGACGCAGACGCCTGACGTTTGG	Left pool of 500 bp for tssA2 (PA14_43050) transcriptional fusion: BamH1 site for pKNG101
OAL1575	tssA2_R_Left_RBS_tc	TCATTATTAATACCCTCTAGCTAGACAGCAATCCCAGTCCGGCCAGCAAA	Left pool of 500 bp for tssA2 (PA14_43050) replacement LacZ with 21 bases from the RBS region pQF50
OAL1576	tssA2_Left_screening_tc	TACGCTCTAAATCAGCTGTTGG	tssA2_Left_screening_transcriptional fusion (PA14_43050)
OAL1549	tssA2_F_Right	TGGTCTGGTGTCAAAAATAAGGCTTCGCGCCCTCAATTCTCAAGGAGAAA	Right pool of 500 bp for tssA2 (PA14_43050) replacement with LacZ
OAL1550	tssA2_R_Right	GCGCGGGGCCCTATCTGCTTCCTTGGCTAGGGGTG	Right pool of 500 bp for tssA2 (PA14_43050) replacement with LacZ: Smal site
OAL1539	tssA2_Right_screening	CGATGATGCTGTCGAGAATG	tssA2_Right_screening (PA14_43050)
OAL1546	tssA2_F_Left	GCGCGGGATCCTCCATGTCTCGTTTTGAATGA	Left pool of 500 bp for tssA2 (PA14_43050) replacement with LacZ: BamHI site
OAL1548	tssA2_R_Left	GACGGCCAGTGAATCCGTAATCATGGTCATCCTAACCCTTCAATGCACAC	Left pool of 500 bp for tssA2 (PA14_43050) replacement with LacZ
OAL1538	tssA2_Left_screening	CCTAACAATTCTCATGCCAGTG	tssA2_Left_screening (PA14_43050)
Fusions H3			
OAL1556	tssB3_F_Right	CATTACCAGTTGGTCTGGTGTCAAAAATAACCCTCTTCCCGGAGAAGCCGCC	Right pool of 500 bp for tssB3 (PA14_34070) replacement with LacZ
OAL1557	tssB3_R_Right	GCGCGGGGCCCTCGGTGAAGCTGTTCATGTCGA	Right pool of 500 bp for tssB3 (PA14_34070) replacement with LacZ: Smal site
OAL1552	tssB3_Right_screening	CCCAGAGGTACTTGGAGTGG	tssB3_Right_screening (PA14_34070)
OAL1553	tssB3_F_Left	GCGCGGGATCCATCGTTGACCAGTCGCATTTCC	Left pool of 500 bp for tssB3 (PA14_34070) replacement with LacZ: BamHI site
OAL1555	tssB3_R_Left	GACGGCCAGTGAATCCGTAATCATGGTCATGGCGGCTGACTCCGATGCAA	Left pool of 500 bp for tssB3 (PA14_34070) replacement with LacZ
OAL1551	tssB3_Left_screening	AATCCCTGCAACTGGAAATGCTG	tssB3_Left_screening (PA14_34070)
LacZ			
OAL1544	LacZ_ATG_F	ATGACCATGATTACGGATTCAC	Amplification of entire lacZ gene from mini-CTX-lacZ
OAL1573	LacZ_ATG_F_RBSpQF50	TCTAGCTAGAGGGTATTAATAATGACCATGATTACGGATTCAC	Amplification of entire lacZ gene from mini-CTX-lacZ with RBSpQF50
OAL1545	LacZ_Stop_R	TTATTTTTGACACCAGACC	Amplification of entire lacZ gene from mini-CTX-lacZ
OAL1540	lacZ_R_screening	GTGCTGCAAGGCGATTAAGT	lacZR_screening
OAL1541	lacZATG+100-3	GGGGGATGTGCTGCAAGGCGATTAAG	lacZATG+100-3 lacZR screening
OAL1542	lacZ_F_screening	ACGGTTTCCATATGGGGATT	lacZF_screening
Identification of Tn			
OAL1795	Rnd1-ARB1-Pa: 5'-	GGCCACGCGTCGACTAGTACNNNNNNNNNNAGAG	Round 1 arbitrary primers transposon identification
OAL1796	Rnd1-ARB2-Pa: 5'-	GGCCACGCGTCGACTAGTACNNNNNNNNNNACGCC	Round 1 arbitrary primers transposon identification
OAL1797	Rnd1-ARB3-Pa: 5'-	GGCCACGCGTCGACTAGTACNNNNNNNNNNGATAT	Round 1 arbitrary primers transposon identification
OAL1798	Rnd1-ARB4-Pa: 5'-	GGCCACGCGTCGACTAGTACSNNNNNNNNSNSSSGCG	Round 1 arbitrary primers transposon identification
OAL1799	Rnd1-TnM20	TATAATGTGTGGAATTGTGAGCGG	BT20 transposon specific primer: Rnd1-TnM20 round 1 arbitrary primers
OAL1800	Rnd2-ARB	GGCCACGCGTCGACTAGTAC	Round 2 common primer which hybridizes in to round 1 round 2 arbitrary primers
OAL1801	Rnd2-TnM20	ACAGGAAACAGGACTCTAGAGG	For pBT20 transposon
Gene Deletion rsmA			
OAL2276	Primer 1 rsmA_F_Left	ATATAGGGCCCCTTCAAGATCCTCGGGCCGATC	Primer 1 rsmA deletion mutant
OAL2277	Primer 2 rsmA_R_Left	TGACGCCGAAATCAGCATTCCTTTCTC	Primer 2 rsmA deletion mutant
OAL2278	Primer 3 rsmA_F_Right	ATGCTGATTTCGGCGTCAACGCGCCGA	Primer 3 rsmA deletion mutant
OAL2279	Primer 4 rsmA_R_Right	ATATACCCGGGGCAACTGTCGATCCTTCGTCCG	Primer 4 rsmA deletion mutant
OAL2280	Primer 5 rsmA_F_Screen	GTCGAGTTCGCCGGCAAGTACAAC	Primer 5 screening for deletion mutant of rsmA
OAL2281	Primer 6 rsmA_R_Screen	CTTTCGGTATGGCGCACTCAGG	Primer 6 screening for deletion mutant of rsmA
Gene Deletion amrZ			
OAL2335	Primer 1 amrZ_F_Left	ATATAGGGCCCGGCGGTTGACCAGGTTGAACTG	Primer 1 amrZ/algZ (PA14_20290) deletion mutant
OAL2336	Primer 2 amrZ_R_Left	TCAGGCCTGTGGGCGCATACATTGAAC	Primer 2 amrZ/algZ (PA14_20290) deletion mutant
OAL2337	Primer 3 amrZ_F_Right	ATGCGCCCACAGGCCTGAGCGCCGAAG	Primer 3 amrZ/algZ (PA14_20290) deletion mutant
OAL2338	Primer 4 amrZ_R_Right	ATATACCCGGGGGCGCAGCACTATCCAGATACG	Primer 4 amrZ/algZ (PA14_20290) deletion mutant
OAL2339	Primer 5 amrZ_F_Screen	CTGGGTCAGGGCGCGGGCGATG	Primer 5 screening for deletion mutant of amrZ/algZ (PA14_20290)
OAL2340	Primer 6 amrZ_R_Screen	CCGTAGGCCGAGAGGTGTGTGG	Primer 6 screening for deletion mutant of amrZ/algZ (PA14_20290)
Gene Deletion rsmF			
OAL3239	Primer 1 rsmF	ATGATGGGCCCGCTCCAGGTTGAGCTGATTGAGGC	Primer 1 rsmF gene deletion
OAL3240	Primer 2 rsmF	CAACTCGTCGAAACCCATGTTCCGCGT	Primer 2 rsmF gene deletion
OAL3241	Primer 3 rsmF	ATGGGTTTCGACGAGTTGAAGACGGCACCG	Primer 3 rsmF gene deletion
OAL3242	Primer 4 rsmF	ATCATGGATCCTAATCGCGTTCGGCCTGCTG	Primer 4 rsmF gene deletion
OAL3243	Primer 5 rsmF	CGGCCCAGTGACAGAGATCG	Primer 5 screening for deletion mutant of rsmF
OAL3244	Primer 6 rsmF	TGATGGTCCGGCCTTTGTGC	Primer 6 screening for deletion mutant of rsmF
Gene Deletion H1-			
OAL862	Primer 1 H1-T6SS	ATGGTCAACGACATGGAGCTGGAG	Primer 1 deletion of H1-T6SS cluster (tagQ1-vgrG1b)
OAL863	Primer 2 H1-T6SS	TCAGTTCTGAAGGCCTGATCGGCCTCG	Primer 2 deletion of H1-T6SS cluster (tagQ1-vgrG1b)
OAL864	Primer 3 H1-T6SS	TCAGGCCTTCAGAACTGAAGCGGCGCA	Primer 3 deletion of H1-T6SS cluster (tagQ1-vgrG1b)
OAL865	Primer 4 H1-T6SS	GGTGGCGTTCAACAGTTCCATGTC	Primer 4 deletion of H1-T6SS cluster (tagQ1-vgrG1b)
OAL866	Primer 5 H1-T6SS	ATCAGCCTGACCACCCTCGACGAC	Primer 5 screening for deletion mutant of H1-T6SS cluster (tagQ1-vgrG1b)
OAL867	Primer 6 H1-T6SS	AGAACGGCTTGAAGGGAACGAACT	Primer 6 screening for deletion mutant of H1-T6SS cluster (tagQ1-vgrG1b)
Gene Deletion H2-			
OAL996	Primer 1 H2-T6SS	GACTGGTTGAAAATCCTGGAAAAC	Primer 1 deletion of H2-T6SS cluster (tssA2-clpV2)
OAL997	Primer 2 H2-T6SS	TCAGGCGAACGGCCTCCTGCTGGGCGC	Primer 1 deletion of H2-T6SS cluster (tssA2-clpV2)
OAL998	Primer 3 H2-T6SS	AGGAGGCCGTTCGCCTGAGGTGGGTGC	Primer 1 deletion of H2-T6SS cluster (tssA2-clpV2)
OAL999	Primer 4 H2-T6SS	CAACACGGTATAGGGGTTGTG	Primer 1 deletion of H2-T6SS cluster (tssA2-clpV2)
OAL1000	Primer 5 H2-T6SS	GAATTGTTAAGATATTCATTGGCGCAC	Primer 5 screening for deletion mutant of H2-T6SS cluster (tssA2-clpV2)
OAL1001	Primer 6 H2-T6SS	TCGAGCAGCAGGGTTCCGCCATCCGCG	Primer 5 screening for deletion mutant of H2-T6SS cluster (tssA2-clpV2)
Gene Deletion H3-			
OAL1002	Primer 1 H3-T6SS	ATTTCCGACATATGGTGAAACATC	Primer 1 deletion of H3-T6SS cluster (tssB3-clpV3)
OAL1003	Primer 2 H3-T6SS	TGCTGATCAGAAGCGCAGCTCGACGTT	Primer 2 deletion of H3-T6SS cluster (tssB3-clpV3)
OAL1004	Primer 3 H3-T6SS	CTGCGCTTCTGATCAGCATCAACCTCT	Primer 3 deletion of H3-T6SS cluster (tssB3-clpV3)
OAL1005	Primer 4 H3-T6SS	GTGAATGGCACGAATAAATAGTTCATA	Primer 4 deletion of H3-T6SS cluster (tssB3-clpV3)
OAL1006	Primer 5 H3-T6SS	AACTGCTGCCGGTAGTCGCGGCGGTAC	Primer 5 screening for deletion mutant of H3-T6SS (tssB3-clpV3)
OAL1007	Primer 6 H3-T6SS	CTGACAGTTCCCCTGCCGTTAACGCCG	Primer 6 screening for deletion mutant of H3-T6SS (tssB3-clpV3)
hcp3 V5 fusion			
OAL2784	Primer 1 Hcp3	GTCAGATGCGATGGATGCGATCATTCTCGATTTCGGCAGCGACAT	Primer 1 V5 insertion to Hcp3 in PA14
OAL2785	Primer 2 Hcp3	GAGACCGAGGAGAGGGTTAGGGATAGGCTTACCCTTGACCAACTGGTTGGCGG	Primer 2 V5 insertion to Hcp3 in PA14
OAL2786	Primer 3 Hcp3	TATCCCTAACCCTCTCCTCGGTCTCGATTCTACGTAGCCGGGCGCCGACATGTC	Primer 3 V5 insertion to Hcp3 in PA14
OAL2787	Primer 4 Hcp3	ATGGCGCACCTCGAGGCCATCGTTGCCCTGTTCGATGACGAATAGCGCAGGCATCAG	Primer 4 V5 insertion to Hcp3 in PA14
OAL2788	Primer 5 Hcp3	AGGAACTCAGCGCGTCGATC	Primer 5 screening for V5 insertion to Hcp3 in PA14
OAL2789	Primer 6 Hcp3	AACCTTCCAGCAGACGCTCGAC	Primer 6 screening for V5 insertion to Hcp3 in PA14
Gene Deletion tssB2			
OAL1596	Primer 1 tssB2_F_Left	GCGCGGGATCCGGATCAGCGTCCATGTCATG	Primer 1 tssB2 gene deletion
OAL1597	Primer 2 tssB2_R_Left	TCAGGCGTCTTTGGCCATGGCTTTTTC	Primer 2 tssB2 gene deletion
OAL1598	Primer 3 tssB2_F_Right	ATGGCCAAAGACGCCTGAGCCACCCCT	Primer 3 tssB2 gene deletion
OAL1599	Primer 4 tssB2_R_Right	GCGCGGGGCCCTGGAGACGTATTGCATCAGC	Primer 4 tssB2 gene deletion
OAL1594	Primer 5 tssB2_screening	CAGGCGATGCGGGAAGTCGAAA	Primer 5 screening for deletion mutant of tssB2
OAL1595	Primer 6 tssB2_screening	TCTGCCACTTGGCGAACTGC	Primer 6 screening for deletion mutant of tssB2
pldA-bla fusion			
OAL2618	pldA 5' up	ACCGAACAGATCTACGTGCA	External of pldA-Bla shuttle up
OAL2613	Linker 5' up	AAGCATCGCACAGCGGCCAGCCT	PldA w/o STOP + overhang vgrG2b-linker, F
OAL2614	Linker 3' up	AGGCTGGCCGCTGTGCGATG	PldA w/o STOP + overhang vgrG2b-linker, R
OAL2615	bla up 5'	CCCTTTTTACCAATGCTTAATCAGTG	bla + overhang downstream of pldA, R
OAL2616	bla up 3'	CATTGGTAAAAAGGGTTTTGATGG	bla + overhang downstream of pldA, F
OAL2760	bla down 3'	TCATGTGCGATGCTTTCCTTACCAATG	bla & RBS of tli5a, R
OAL2761	bla down 5'	ATCGCACATGAAAAGGGTTTTGAT	bla & RBS of tli5a, F
OAL2899	tli5a down 3'	TACCTTCGCAGTTTGGCATG	Cloning whole tli5A after pldA-Bla shuttle
OAL2900	tli5a external down 3'	CAGGATAAAGCCGATGGTGC	External downstream of pldA-Bla shuttle
pldB-bla fusion			
OAL3206	Primer 1	CAAGATCGAGGCGCTCGAAG	Primer 1 PldB-Bla fusion
OAL3212	Primer 2	GTCAAAATCTTACGGTGGACGCGGCCAGCCTGGAAG	Primer 2 PldB-Bla fusion
OAL3213	Primer 3	GCATCGCTCAGTCCACCGTTACCAATGCTTAATCAG	Primer 3 PldB-Bla fusion
OAL3209	Primer 4	ACAGAGCACGGCCCAAAGTC	Primer 4 PldB-Bla fusion
OAL3210	Primer 5	ATGACTTGCGGGAAAAGCGG	Primer 5 PldB-Bla fusion
OAL3211	Primer 6	ACGGCGGCAGATGGTCTTTT	Primer 6 PldB-Bla fusion
OAL3247	Primer 7 pldB-bla PA14	CTTCCAGGCTGGCCGCGTCCACCGTAAGATTTTGAC	Primer 7 Primer to amplify upsteam region for pldB-bla PA14
OAL3248	Primer 8 pldB-bla PA14	CTGATTAAGCATTGGTAACGGTGGACTGAGCGATGC	Primer 8 Primer to amplify downsteam region for pldB-bla PA14
Gene Deletion vgrG4b			
OAL800	Primer 1 VgrG4bF	CCACGCCAACTGGAGGTC	Primer 1 vgrG4b gene deletion
OAL801	Primer 2 VgrG4bR	CGCAGCAACGTTGAACATCCGCTGTCC	Primer 2 vgrG4b gene deletion
OAL802	Primer 3 VgrG4bF	ATGTTCAACGTTGCTGCGAAGGCGACC	Primer 3 vgrG4b gene deletion
OAL803	Primer 4 VgrG4bR	ATAGCCGATGCGGTTGTC	Primer 4 vgrG4b gene deletion
OAL804	Primer 5 VgrG4bF	GCCTACGCCACCTTCCTCGA	Primer 5 screening for deletion mutant of vgrG4b
OAL805	Primer 6 VgrG4bR	GCGCATGCAGCGTCAGGCTG	Primer 6 screening for deletion mutant of vgrG4b
Gene Deletion of retS			
OAL1893	Primer 1 retSLeftF	GCGCGACTAGTGCCTGATGATCGACAAGAGCGG	Primer 1 retS gene deletion
OAL1894	Primer 2 retSLeftR	TCAGGAGGGCCGTACCACGGCGAAGTCC	Primer 2 retS gene deletion
OAL1895	Primer 3 retSRightF	GTGGTACGGCCCTCCTGAGGGCAGCGAC	Primer 3 retS gene deletion
OAL1896	Primer 4 retSRightR	CGCGCACTAGTGCCATGCGCTTGCGGTCG	Primer 4 retS gene deletion
OAL1897	Primer 5 retS Screening F	GAGGAGGCCAGCTTCATCGTCATG	Primer 5 screening for deletion mutant of retS
OAL1898	Primer 6 retS Screening R	TGCTGCACGTTGTCGCTCTG	Primer 6 screening for deletion mutant of retS
AmrZvS			
OAL2649	amrZ_F_pMM	GCTAATCTAGATAACAGGAGGAATTAACCATGCGCCCACTGAAACAGGCAACTCCTACCT	Forward primer RBS with XbaI site for cloning into pMMB67HE
OAL2650	amrZ_R_pMM	GCTAAGGATCCTCACGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACCGGCCTGGGCCAGCTCCGCA	Reverse primer V5 tag with BamHI site
AmrZhis			
OAL2807	AmrZ_F_his	GTCAGTCATATGCGCCCACTGAAACAGGCAACTCCTACCT	Primer to amplify AmrZ and cut with NdeI for cloning into pET29a
OAL2808	AmrZ_R_his	ACTGACCTCGAGTGCGGCCGCGGCCTGGGCCAGCTCCGCA	Primer to amplify AmrZ and cut with XhoI for cloning into pET29a
Hcp3his			
OAL3577	hcp3_F_his	GAGCATATGGATGCGATCATTCTCGATTTC	Primer to amplify hcp3 and cut with NdeI for cloning
OAL3578	hcp3_R_his	AAAGGATCCTCACTTGACCAACTGGTTG	Primer to amplify hcp3 and cut with BamHI for cloning
Primers for EMSA			
OAL2979	algD111_EMSA	CAAACGGCCGGAACTTCCCT	$algD_1$ negative control AmrZ binding assay
OAL2980	algD112_EMSA	TAGTTCGGTCCATAGAATTCAAG	$algD_1$ negative control AmrZ binding assay
OAL2981	adcA_FAM_F_EMSA	CGTAGTCCGTCGCACAAAG	PA4843 positive control AmrZ binding assay
OAL2982	adcA_R_EMSA	GCGCTTCTTTCGTGGTCTTC	PA4843 positive control AmrZ binding assay
OAL2985	tssA1_F	TCTTTTAAAGATTTGTCCGATCTGC	tssA1F promoter region to test AmrZ binding
OAL2986	tssA1_R	CTCACCTTCGCTTGTAAGATCC	tssA1F promoter region to test AmrZ binding
OAL2988	tssA2_F	GGAATCCACTGTTGTCTGCCTTAT	tssA2F promoter region to test AmrZ binding

OAL2989	tssA2_R	CCCTCTTTGGGAATTTTCCTATTC	tssA2R promoter region to test AmrZ binding
OAL3088	tssB3_F	CGACTCGATGAACTGCCCCGGG	tssB3F promoter region to test AmrZ binding
OAL2991	tssB3_R	CCTCTGGTCCGTCACTGGCGAC	tssB3R promoter region to test AmrZ binding
OAL3091	algB68	CGCATGCCAGCCTTTCTGA	algB negative control for AmrZ binding assay longer fragment
OAL3092	algB69	TTCACTGCCATTCGGCTTCTG	algB negative control for AmrZ binding assay longer fragment
OAL3093	algD71	ACCGTTCGTCTGCAAGTCATG	algD positive control for AmrZ binding assay longer fragment
OAL3094	algD73	ACCAACTTGATGGCCTTTCCG	algD positive control for AmrZ binding assay longer fragment
OAL4016	tssA1amrZ75R	TGCCGCAAGGCTTGTCAA	Use with OAL2985 to amplify a 75 bp fragment with 1 putative amrZ binding site
OAL4017	tssA1amrZ93F	TGCCACAAGAATTTTGCCAAC	Use with OAL2986 to amplify a 94 bp fragment with 2 putative amrZ binding site
OAL4022	tssA2amrZ82R	GACATTGATGGCCATTTGGAA	Use with OAL2988 to amplify a 82 bp fragment with 1 putative amrZ binding site
OAL4023	tssA2amrZ110F	GTTATGATGGCTATAGCCAAGC	Use with OAL2989 to amplify a 110 bp fragment with 1 putative amrZ binding site
OAL4024	tssB3amrZ200R	TGGATGTCGCCGCGCGCGCCA	Use with OAL3088 to amplify a 200 bp fragment with 1 putative amrZ binding site
OAL4025	tssB3amrZ200F	GGGTCAGCCGCTGCAACTCGAC	Use with OAL2991 to amplify a 200 bp fragment with 1 putative amrZ binding site
RT PCR			
OAL2134	vgrG2a F	TTCGACACCGTTCGATGA	qRT-PCR (PA14_44900/PA1511)
OAL2135	vgrG2a R	GTCATCAGTTTCACCACACC	qRT-PCR (PA14_44900/PA1511)
OAL536	vgrG2b F	GGAGCCGGGAAAGACGTT	qRT-PCR vgrG2b (PA14_03220/PA0262)
OAL537	vgrG2b R	AGGCTTCCCCGAACTCGTT	qRT-PCR vgrG2b (PA14_03220/PA0262)
OAL3042	vgrG6 F	GGCATTGCTGCAGAAGAAAC	qRT-PCR vgrG6 (PA14_69550)
OAL3043	vgrG6 R	TCAGTGCGCATCGGTTTT	qRT-PCR vgrG6 (PA14_69550)
OAL3030	vgrG5 F	AGCCATCGCCCAGAAGAT	qRT-PCR vgrG5 (PA14_67230/PA5090)
OAL3031	vgrG5 R	CGTAGAGCCGATACGGTTG	qRT-PCR vgrG5 (PA14_67230/PA5090)
OAL3034	vgrG4b F	GTCCAGGGTAAGGAGCACAA	qRT-PCR vgrG4b (PA14_18985/PA3486)
OAL3035	vgrG4b R	TTCGATAGCTGACGCTGTTG	qRT-PCR vgrG4b (PA14_18985/PA3486)
OAL2136	tle4(tplE) F	CAATCTGGTTTGTCCGAAGA	qRT-PCR tle4 (PA14_44910/PA1510)
OAL2137	tle4(tplE) R	GCTGACGATGTTATCCAGTC	qRT-PCR tle4 (PA14_44910/PA1510)
OAL3038	tle3 F	TCTACTACCGGCGCAAGTTC	qRT-PCR tle3 (PA14_03200/PA0260)
OAL3039	tle3 R	CCGCGGTCTTGTTGATGTAT	qRT-PCR tle3 (PA14_03200/PA0260)
OAL3044	PA14_69520 F	CGAATACCGCATCGACAAG	qRT-PCR putative toxin next to vgrG6 in PA14 (PA14_69520)
OAL3045	PA14_69520 R	GGTACGGGGAAAGACCAGTT	qRT-PCR putative toxin next to vgrG6 in PA14 (PA14_69520)
OAL3032	pldB(tle5b) F	ACATCAAGCCGCTGATCAAT	qRT-PCR pldB (PA14_67220/PA5089)
OAL3033	pldB(tle5b) R	AAGCCCCAGGTGATGATGT	qRT-PCR pldB (PA14_67220/PA5089)
OAL3036	pldA(tle5a) F	CCTCCACGAAAAGGAACTCA	qRT-PCR pldA(tle5a) (PA14_18970/PA3487)
OAL3037	pldA(tle5a) R	AAGAGGTTGGACCCCTTGAT	qRT-PCR pldA(tle5a) (PA14_18970/PA3487)
OAL1029	tssB1 F	GCCTTCAACGTACCGAACGT	qRT-PCR tssB1 (PA14_01010/PA0083)
OAL1030	tssB1 R	GAAGTCGTCCATGCTCTCGAA	qRT-PCR tssB1 (PA14_01010/PA0083)
OAL511	tssA2 F	GGTTGACCTGGGCCCTCTAC	qRT-PCR tssA2 PA14_43050/PA1656)
OAL512	tssA2 R	GATGGATCTCGACCCAATGC	qRT-PCR tssA2 PA14_43050/PA1656)
OAL3048	tssB3 F	GTACGCAGCACAAGCTGGAC	qRT-PCR tssB3 (PA14_34070/PA2365)
OAL3049	tssB3 R	GCAGCTCCTTCTTCTCGATG	qRT-PCR tssB3 (PA14_34070/PA2365)
OAL3028	hcp3 F	GATCATGCCCTTCATCGTCT	qRT-PCR hcp3 (PA14_34030/PA2367)
OAL3029	hcp3 R	TTGGTGAAGTTCAGCGACAG	qRT-PCR hcp3 (PA14_34030/PA2367)
OAL3046	tssA3 F	TGGAAGTGCTGCTCGAACC	qRT-PCR tssA3 (PA14_34140/PA2360)
OAL3047	tssA3 R	GCAGGCTGCTGTCGTCCT	qRT-PCR tssA3 (PA14_34140/PA2360)
OAL4035	tssA1 F	CTGCTGCAGAGCAACGTC	qRT-PCR tssA1 (PA0082/PA14_00990) 100 bp
OAL4036	tssA1 R	GATAGACGCCGTCCCAGTA	qRT-PCR tssA1 (PA0082/PA14_00990) 100 bp
OAL4037	tssB2 F	GCCAAAGAAGGCTCGGTAG	qRT-PCR tssA1 (PA1656/PA14_43050) 96 bp
OAL4038	tssB2 R	CTTCAACGGCAGTTCGATTT	qRT-PCR tssA1 (PA1656/PA14_43050) 96 bp
OAL3022	vgrG3 F	GCGACAGTCTGGATGTGAAG	qRT-PCR vgrG3 (PA2373/PA14_33960) 114 bp
OAL3023	vgrG3 R	CCACCGTCAGCTCGAAGT	qRT-PCR vgrG3 (PA2373/PA14_33960) 114 bp
OAL820	rpoD F	AGGCCGTGAGCAGGGATAC	qRT-PCR rpoD (housekeeping gene)
OAL821	rpoD R	TCCCCATGTCGTTGATCATG	qRT-PCR rpoD (housekeeping gene)
Microscopy plasmids			
pME6032tssB1-venus			
OAL2946	5' tssB1	GCGAGCTCATGGGAAGCACTACCAGC	Cloning tssB1 into pME6032-Cterm-venus
OAL2947	3' tssB1	GGGGTACCCGCGCCTGCGGCTCGTCGTC	Cloning tssB1 into pME6032-Cterm-venus
pJN105tssB2-CFP			
OAL3403	5' tssB2	GGCGCCCCGGGTCAATTCTCAAGGAGAAAAAGCC	To amplify tssB2 (PA1657) with a linker to generate overlapping product with CFP linker
OAL3404	3' tssB2	TCCTCCTCCTGCTGCTGCGGCGTCCTGGGAGGGG	To amplify tssB2 (PA1657) with a linker to generate overlapping product with CFP linker
pJN105tssB3-CFP			
OAL3405	5' tssB3	GCGCCCCGGGTTGCATCGGAGTCAGCCGCC	To amplify tssB3 (PA2365) with a linker to generate overlapping product with CFP linker
OAL3406	3' tssB3	TCCTCCTCCTGCTGCTGCGGCCGGCTGGTCGGCC	To amplify tssB3 (PA2365) with a linker to generate overlapping product with CFP linker
OAL3439	CFP_linker_F_new	GCAGCAGCAGGAGGAGGAATGGTGAGCAAGGGCGA	Generation of C-term CFP fusion with linker using pSEVA237C as template
OAL3440	CFP_linker_R_new	GCGCGTCTAGATTACTTATACAGCTCGTCCATGCC	Generation of C-term CFP fusion with linker using pSEVA237C as template
pMMB67HEtssB3-GFP			
OAL3260	tssB3_sfGFP_F	GCATCGGAGTCAGCCGCC	5' tssB3 coding region to fuse with sfGFP
OAL1686	5'sfGFP	GCAGCAGCAGGAGGAGGAATGCGTAAAGGCGAAGAA	5' primer sfGFP with linker
OAL1687	3'sfGFP	CCTAGTTCATTACTTATACAGCTC	3' primer sfGFP