

## SUPPLEMENTARY DATA

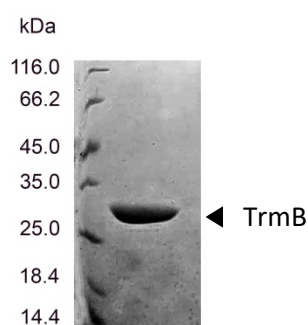
### **TrmB, a tRNA m<sup>7</sup>G46 methyltransferase, plays a role in hydrogen peroxide resistance and positively modulates the translation of *katA* and *katB* mRNAs in *Pseudomonas aeruginosa***

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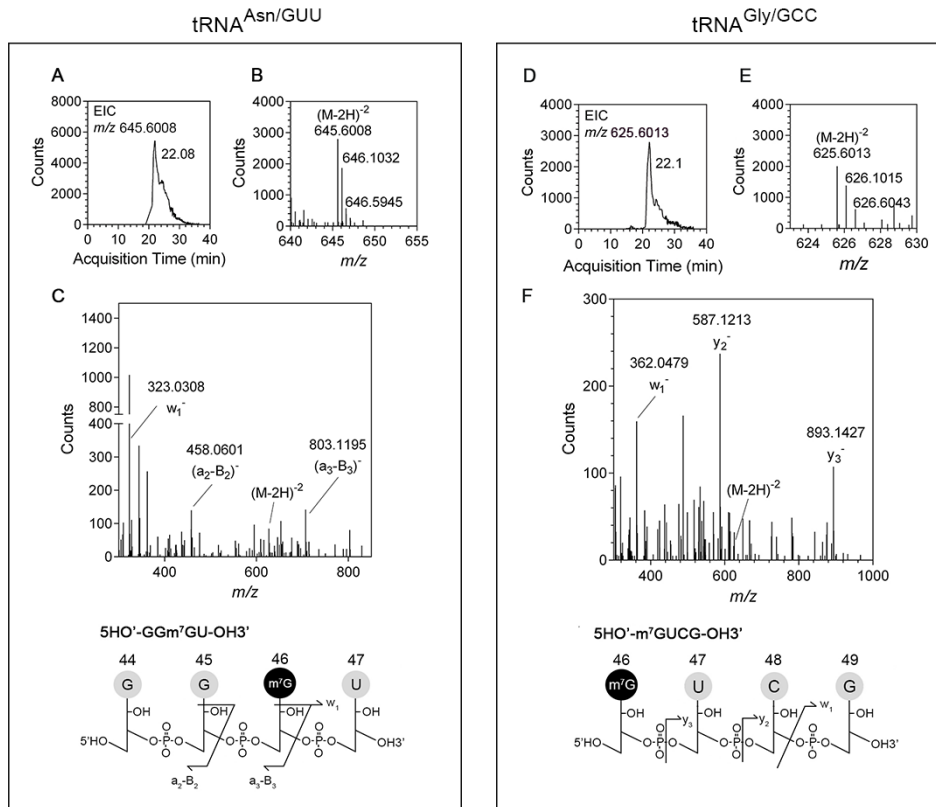
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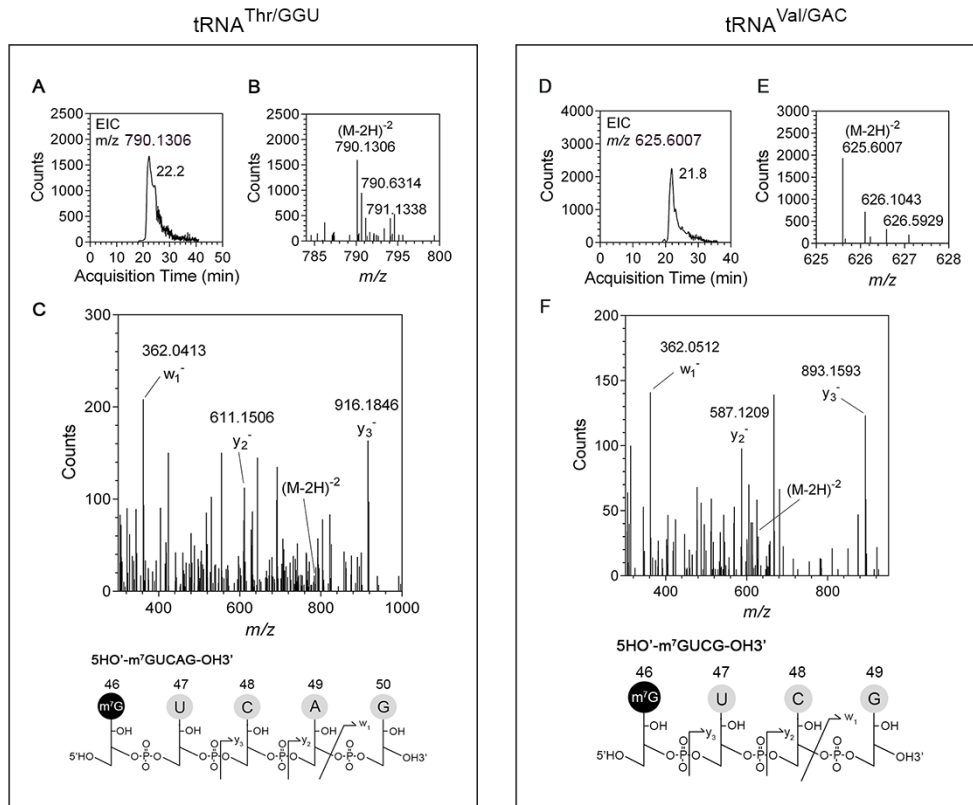
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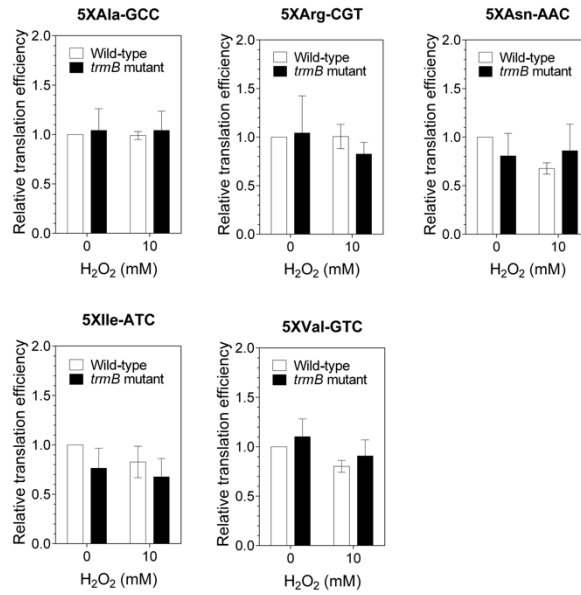
**Figure S1 SDS-PAGE analysis of the purified PaTrmB protein (27 kDa).** The purity of the PaTrmB was estimated to be >99% on a Coomassie blue stained SDS-polyacrylamide gel.



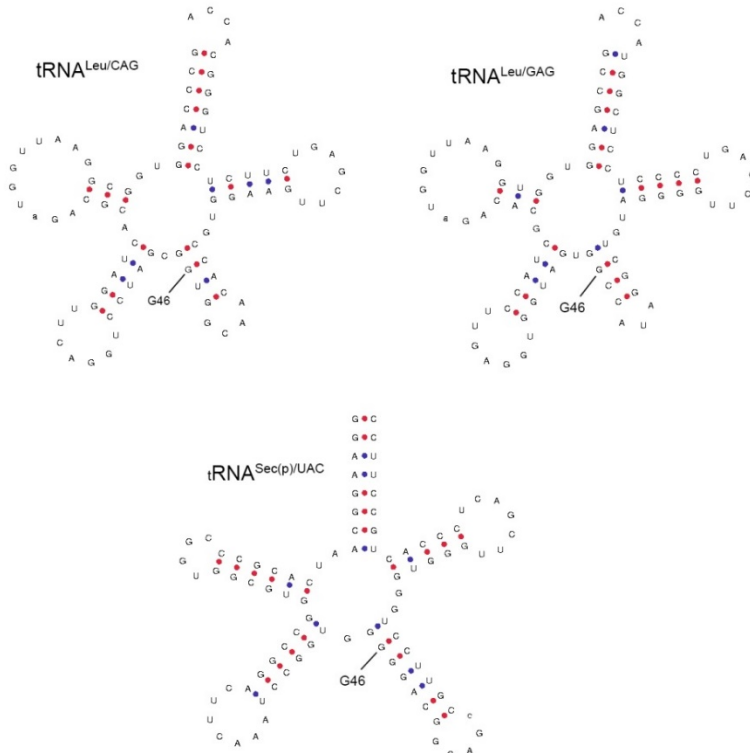
**Figure S2 The PaTrmB catalyzes the formation of m<sup>7</sup>G at position 46 in variable loop of tRNA<sup>Asn/GUU</sup> and tRNA<sup>Gly/GCC</sup> *in vitro*.** (A) Extracted ion chromatogram of GGm<sup>7</sup>GU with  $m/z$  645.6008; this fragment is an RNase A digested product of tRNA<sup>Asn/GUU</sup>. (B) The mass spectrum of GGm<sup>7</sup>GU fragment shows a doubly-charged negative ion with  $m/z$  645.6008. (C) CID spectrum and CID fragmentation pattern of  $m/z$  645.6008 with  $w_1$ ,  $a_2-B_2$ , and  $a_3-B_3$  ion products. (D) Extracted ion chromatogram of m<sup>7</sup>GUCC with  $m/z$  625.6013; this fragment is an RNase T1 digested product of tRNA<sup>Gly/GCC</sup>. (E) The mass spectrum of m<sup>7</sup>GUCC fragment shows a doubly-charged negative ion with  $m/z$  625.6013. (F) CID spectrum and CID fragmentation pattern of  $m/z$  625.6013 with  $w_1$ ,  $y_2$ , and  $y_3$  ion products.



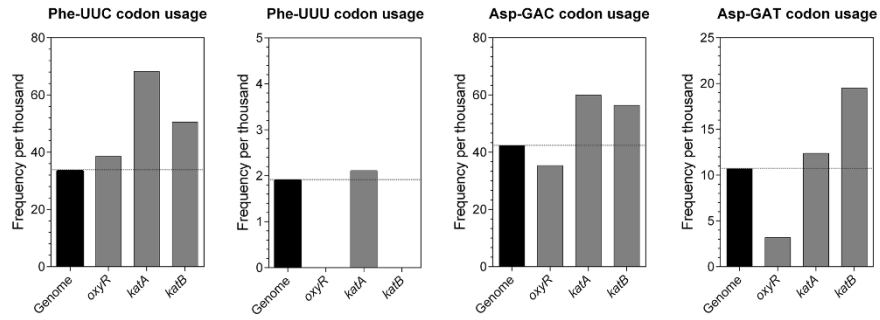
**Figure S3** The PaTrmB catalyzes the formation of  $m^7G$  at position 46 in variable loop of  $tRNA^{Thr/GGU}$ , and  $tRNA^{Val/GAC}$  *in vitro*. **(A)** Extracted ion chromatogram of  $m^7GUCAG$  with  $m/z$  790.1306; this fragment is an RNase T1 digested product of  $tRNA^{Thr/GGU}$ . **(B)** The mass spectrum of  $m^7GUCAG$  fragment shows a doubly-charged negative ion with  $m/z$  790.1306. **(C)** CID spectrum and CID fragmentation pattern of  $m/z$  790.1306 with  $w_1$ ,  $y_2$ , and  $y_3$  ion products. **(D)** Extracted ion chromatogram of  $m^7GUCG$  fragment with  $m/z$  625.6007; this fragment is an RNase T1 digested product of  $tRNA^{Val/GAC}$ . **(E)** The mass spectrum of  $m^7GUCG$  fragment shows a doubly-charged negative ion with  $m/z$  625.6007. **(F)** CID spectrum and CID fragmentation pattern of  $m/z$  625.6007 with  $w_1$ ,  $y_2$ , and  $y_3$  ion products.



**Figure S4** Relative translation efficiency of 5XAla-GCC, 5XArg-CGT, 5XAsn-AAC, 5XIle-ATC, and 5XVal-GTC reporter in the wild type and *trmB* mutant strains upon H<sub>2</sub>O<sub>2</sub> exposure. Data represent the mean ± SD for four biological replicates.



**Figure S5** Predicted secondary structure of tRNA<sup>Leu/CAG</sup>, tRNA<sup>Leu/GAG</sup>, and tRNA<sup>Sec(p)/UAC</sup>. tRNA database: <http://gtrnadb2009.ucsc.edu/>



**Figure S6** *katA* and *katB* are enriched in codons decoded by tRNA<sup>Phe/GAA</sup> and tRNA<sup>Asp/GUC</sup> compared to the full genome. **(A)** Usage of the Phe-UUC codon in H<sub>2</sub>O<sub>2</sub>-defense genes. **(B)** Usage of the Phe-UUU codon in H<sub>2</sub>O<sub>2</sub> defensive genes. **(C)** Usage of the Asp-GAC codon in H<sub>2</sub>O<sub>2</sub> defensive genes. **(D)** Usage of the Asp-GAU codon in H<sub>2</sub>O<sub>2</sub> defensive genes. Codon usage database: <https://www.kazusa.or.jp/codon/>

**Table S1** Strains and plasmids used in this study

Strain/plasmid	Characteristic	Reference/source
<i>P. aeruginosa</i> PA14		
Wild type	<i>P. aeruginosa</i> UCBPP-PA14 wild type strain	ATCC
<i>trmB</i> mutant	<i>trmB</i> mutant::pKNOCK-Gm,Gm <sup>r</sup>	This study
<i>trmB</i> complemented	<i>trmB</i> harboring pBBR1MCS-4/ <i>trmB</i> _FL	This study
<i>E. coli</i>		
BW20767	<i>leu-63::IS10recA1creC510 hsdR17 endA1zbf-5uidA(ΔMluI)::pir<sup>+</sup>thi</i> RP4-2-tet::Mu-1kan::Tn7	Metcalf, <i>et al.</i> , 1996
DH5α	λ <sup>-</sup> φ80d <i>lacZ</i> ΔM15 <i>recA1 endA1gyrA96thi-1hsdR17(rk<sup>-</sup>mk<sup>+</sup>)</i> <i>supE44relA1deoR Δ(lacZYA-argF)U169</i>	Stratagene Inc. (USA)
DE3 (BL21)	<i>E. coli</i> B F- <i>dcm ompT hsdS</i> ( <sub>FB-MB</sub> ) <i>galA</i> (DE3)	Stratagene Inc. (USA)
Plasmids		
pKNOCK-Gm	Suicide plasmid for insertional inactivation of <i>trmB</i>	(22)
pKN- <i>trmB</i>	pKNOCK-Gm with internal 215-bp fragment of <i>trmB</i>	This study
pBBR1MCS-4	Broad-host-range plasmid for <i>trmB</i> expression	(23)
pBB- <i>trmB</i> -FL	pBBR1MCS-4 expressing PA14 TrmB	This study
pBB- <i>katB</i> -6XHis	pBBR1MCS-4 expressing His-tagged KatB	This study
pETBLUE-2	Vector for protein expression	This study
pET- <i>trmB</i> -FL	pETBLUE-2 expressing C-terminal His tagged TrmB	This study
pET- <i>katB</i> -FL	pETBLUE-2 carrying C-terminal His tagged KatB	This study
pPR9TT	<i>lacZ</i> translational fusion vector	Novagen
pPR-ATG-control	pPR9TT expressing β-galactosidase	This study
pPR-5XGln-CAA	pPR9TT expressing 5XGln fragment fused in-frame with <i>lacZ</i>	This study
pPR-5XAla-GCC	pPR9TT expressing 5XAla fragment fused in-frame with <i>lacZ</i>	This study
pPR-5XArg-CGU	pPR9TT expressing 5XArg fragment fused in-frame with <i>lacZ</i>	This study
pPR-5XAsn-AAC	pPR9TT expressing 5XAsn fragment fused in-frame with <i>lacZ</i>	This study
pPR-5XAsp-GAC	pPR9TT expressing 5XAsp fragment fused in-frame with <i>lacZ</i>	This study
pPR-5XIle-AUC	pPR9TT expressing 5XIle fragment fused in-frame with <i>lacZ</i>	This study
pPR-5XPhе-UUC	pPR9TT expressing 5XPhе fragment fused in-frame with <i>lacZ</i>	This study
pPR-5XThr-ACC	pPR9TT expressing 5XThr fragment fused in-frame with <i>lacZ</i>	This study
pPR-5XVal-GUC	pPR9TT expressing 5XVal fragment fused in-frame with <i>lacZ</i>	This study
pPR-5XPhе-UUU	pPR9TT expressing 5XPhе-UUU fragment fused in-frame with <i>lacZ</i>	This study

**Table S2** Primers and synthesized oligonucleotides used in this study

Name	Sequence	Application
BT4381_F	ACTGAGCGATGAGGTTTCAGG	Amplification of <i>trmB</i> fragment for pKN- <i>trmB</i> cloning and semi-qRT PCR fo <i>trmB</i>
BT4382_R	AGCTATACACCCGCACGTTG	
BT4767_F	TTTCCATGAGCGATACCCCG	Amplification of <i>trmB</i> full-length for pBB- <i>trmB</i> _FL clonning
BT4768_R	GGAAACGTGCAGCTTGAAGG	
BT5330_F	GGCCCATGGGCACCGTGAAGAGCT	Amplification of <i>trmB</i> full-length for pET- <i>trmB</i> _FL clonning
BT5331_R	GGTCTCGAGGTCGATGCGCTCGAA	
BT5468	GGATCCTAATACGACTCACTATAGGGGGGGCTATAGCTCAGCTGGGAGAGCG CTTGCATGGCATGCAAGAGGTCGACGGTTCGATCCCGTCTAGCTCCACCA	PCR template for the amplification of dsDNA encoded for tRNA <sup>Ala/GGC</sup>
BT6974	GGATCCTAATACGACTCACTATAGGGGGGGCCATAGCTCAGCTGGGAGAGC GCCTGCTTTGCACGCAGGAGGTCAGGAGTTCGATCCTCCTTGGCTCCACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Ala/TGC</sup>
BT5469	GGATCCTAATACGACTCACTATAGGGGGTTCGATAGCTCAGCCTGGTAGAGC AACCATCCACGAAATGGTCTGTGCGGGTTCGACTCCCGCTCGAACGT	PCR template for the amplification of dsDNA encoded fortRNA <sup>Arg/ACG</sup>
BT6948	GGATCCTAATACGACTCACTATAGGGGCATCCGTAGCTCAGCTGGATAGAGT ACTGCCCTCCGAAGGCAGGGGTGCGTGGTTCGAATCCCGCCGGATGCGCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Arg/CCG</sup>
BT6949	GGATCCTAATACGACTCACTATAGGGGTCCCGGTAGCTCAATTGGATAGAGC ATCCCCTCCTAAGGGGAAGGTTGGAGGTTGACCCCTCTCCGGGACGCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Arg/CCT</sup>
BT6950	GGATCCTAATACGACTCACTATAGGGGCGCCCGTAGCTCAGCTGGATAGAGC ATCCGCCTTCTAAGCGGATGGTGCAGGTTGAGTCTGCCGGGTGCGCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Arg/TCT</sup>
BT5470	GGATCCTAATACGACTCACTATAGGGTCCGCGATAGCTCAGTCGGTAGAGCA AATGACTGTTAATCATTGGGTCCCTGGTTCGAGTCCAGGTCGCGGAGCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Asn/GTT</sup>
BT5471	GGATCCTAATACGACTCACTATAGGGGCAGCGGTAGTTCAGTCGGTTAGAAT ACCGGCCTGTACAGCCGGGGTTCGCGGGTTCGAGTCCCGTCCGCTGCGCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Asp/GTC</sup>
BT6951	GGATCCTAATACGACTCACTATAGGGGGCTGAGTAGCAGAGTGGTTATGCAC CGGATTGCAAATCCGTGAACGCCGGTTCGATTCCGACCTCAGCCTCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Cys/GCA</sup>
BT7242	GGATCCTAATACGACTCACTATAGGGAGGGGCGTCGCCAAGCGGTAAGGCA GCAGTTTTGATCCTGCCATGCGTTGGTTCGAATCCAGCCGCCCTGCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Gln/TTG</sup>
BT5089	GGATCCTAATACGACTCACTATAGGGGTCCCCTTCGTCTAGTGGCCTAGGAC ACCGCCCTTTACGGCGGTAACAGGGGTTGAGTCCCTAGGGGACGCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Glu/TTC</sup>
BT6952	GGATCCTAATACGACTCACTATAGGGGCGGGCGTCGTATAATGGCATTACCT GAGCTTCCAAGCTCATGACGAGGTTGATTCCCTTCGCCCGCTCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Gly/CCC</sup>
BT5472	GGATCCTAATACGACTCACTATAGGGGCGGGAATAGCTCAGTTGGTAGAGCA CGACCTTGCCAAGGTCGGGGTCGCGAGTTCGAGTCTCGTTTCCCCTCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Gly/GCC</sup>
BT6953	GGATCCTAATACGACTCACTATAGGGGCGGGTATAGTTTCAAGTGGTAGAGCCT CAGCCTTCCAAGCTGATGCGGGTTCGATTCCCGCTACCCGCTCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Gly/TCC</sup>
BT7244	GGATCCTAATACGACTCACTATAGGGGTGGGCGTAGCTCAGTTGGTAGAGCA CAGGATTGTGGCTCCTGGTGTGCGTGGGTTGATTCCCATCGTCCACCCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>His/GTG</sup>

**Table S2** Primers and synthesized oligonucleotides used in this study (cont.)

Name	Sequence	Application
BT5473	GGATCCTAATACGACTCACTATAGGGGGTCTGTAGCTCAGTTGGTTAGAGC GCACCCCTGATAAGGGTGAGGTCCGCAGTTCGAATCTGCCAGACCCACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Ile/GAT</sup>
BT6954	GGATCCTAATACGACTCACTATAGGGGCCTCGGTGGCGGAATCGGTAGACGC GGCGGATTCAAAATCCGTTTCTGGCGACAGAGTGAGAGTTCGAGTCTCTCCC GAGGCACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Leu/CAA</sup>
BT7246	GGATCCTAATACGACTCACTATAGGGGCCAGGTGGCGGAATTGGTAGACGC ACTAGGTTCAAGTCTAGCGGTGGCAACACCGTGGAAGTTCGAGTCTTCTCC TGGGCACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Leu/CAG</sup>
BT6955	GGATCCTAATACGACTCACTATAGGGGCCAGGTGGTGGGAATTGGTAGACAC GCTACCTTGAGGTGGTAGTGGCCATAGGCTGTAGGGGTTTCGAGTCCCCTCCT CGGTACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Leu/GAG</sup>
BT7248	GGATCCTAATACGACTCACTATAGGGGCGGACGTGGTGGGAATTGGTAGACAC ACTGGATTTAGGTTCCAGCGCCGCAAGGCGTGAGAGTTCGAGTCTCTCCGTC CGCACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Leu/TAG</sup>
BT5091	GGATCCTAATACGACTCACTATAGGGGGTCTGTTAGCTCAGTCGGTAGAGCA GTTGGCTTTTAACCAATTGGTCGTAGGTTTCGAATCCTACAGACCCACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Lys/TTT</sup>
BT5092	GGATCCTAATACGACTCACTATAGGGCGCGGGATGGAGCAGTCTGGTAGCTC GTCGGGCTCATAACCCGAAGGTCGTTGGTTCAAATCCAGCTCCCGCAACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Met/CAT</sup>
BT5474	GGATCCTAATACGACTCACTATAGGGGCCAGGTAGCTCAGTTGGTAGAGCA GGGGATTGAAAATCCCGTGTCCGGCGGTTTCGATTCCGTCCTGGGCACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Phe/GAA</sup>
BT7250	GGATCCTAATACGACTCACTATAGGGCGGAGCGTAGCGCAGCTTGGTAGCGC GTCTCGTTCGGGACGAGAAGGTCGCTGGTTCGAATCCAGTCGCTCCGACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Pro/CGG</sup>
BT7252	GGATCCTAATACGACTCACTATAGGGCGGGGCGTAGCGCAGCCTGGTAGCG CACTTGCATGGGGTGCAAGGGGTCGAGTGTTTCGAATCACTCCGTCCTCCGACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Pro/GGG</sup>
BT7254	GGATCCTAATACGACTCACTATAGGGCGGGGTATAGCGCAGTCCGGTAGCGC GCCTGCTTTGGGAGCAGGATGTCGGGAGTTCGAATCTCTTACCCCGACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Pro/TGG</sup>
BT6956	GGATCCTAATACGACTCACTATAGGGGGAAGGCAATCACGCCCGGTGGCGTG GCCGGACTTCAAATCCGGTGGGGGACGGCAGCCGTTCTGGGTGGGTTTCGA CTCCACTGCCTTCC	PCR template for the amplification of dsDNA encoded fortRNA <sup>Sec(p)/TCA</sup>
BT6957	GGATCCTAATACGACTCACTATAGGGGGAGAGATGCCGGAGTGGTCCAACG GGACGGATTGCAAATCCGTTGAGTCAGCAATGGCTCCTAGGGTTCAAATCCC TATCTCTCCGCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Ser/CGA</sup>
BT6958	GGATCCTAATACGACTCACTATAGGGGGAGAGGTGGCCGAGTGGCCGAAGG CGCTCCCCTGCTAAGGAGTACACCTCAAAGGGTGTCCGGGGTTTCGAATCC CCCCCTCTCCGCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Ser/GCT</sup>



**Table S2** Primers and synthesized oligonucleotides used in this study (cont.)

Name	Sequence	Application
BT6959	GGATCCTAATACGACTCACTATAGGGGGTGTAGGTGTCCGAGTGGTTGAAGGA GCACGCCTGAAAAGTGTGTATACGGGAAACCGTATCGAGGGTTCGAATCCCT CCCTCACCGCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Ser/GGA</sup>
BT7256	GGATCCTAATACGACTCACTATAGGGGGAGGTGTGGCCGAGTGGTTTAAGGC AACGGTCTTGAAAACCGTCGAAGGGGAGACTCTTCCGTGAGTTCGAATCTCA CCGCCTCCGCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Ser/TGA</sup>
BT6960	GGATCCTAATACGACTCACTATAGGGGCCGGATTAGCTCAGTCGGTAGAGCA GCTCATTCGTAATGAGAAGGTCGGGGTTCGATTCTCTATCCGGCACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Thr/CGT</sup>
BT5475	GGATCCTAATACGACTCACTATAGGGGGCTCATGTAGCTCAGTTGGTAGAGCA CACCTTGGTAAGGGTGTAGGTCAGCGGTTCAAATCCGCTCATGAGCTCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Thr/GGT</sup>
BT6961	GGATCCTAATACGACTCACTATAGGGGCCGGTATAGCTCAGCTGGTAGAGCA ACTGACTTGTAAATCAGTAGGTCCCGGGTTCGACTCCTGGTGCCGGCACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Thr/TGT</sup>
BT5090	GGATCCTAATACGACTCACTATAGGGAGGCCAGTAGCTCAATTGGCAGAGCG GCGGTCTCCAAAACCGCAGGTTGGGGTTCGATTCCCTCCTGGCCTGCCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Trp/CCA</sup>
BT6962	GGATCCTAATACGACTCACTATAGGGGGAGGGATTCCCGAGTGGCCAAAGGG ATCAGACTGTAAATCTGACGTCATAGACTTCGAAGGTTCAATCCTTCTCCCT CCACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Tyr/GTA</sup>
BT5476	GGATCCTAATACGACTCACTATAGGGAGGCACGTAGCTCAGTTGGTTAGAGC ACCACCTTGACATGGTGGGGTTCGTTGGTTCGAATCCAATCGTGCCTACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Val/GAC</sup>
BT5477	GGATCCTAATACGACTCACTATAGGGGGTGTAGGTGTCCGAGTGGTTGAAGGA TCTGCCTTACAAGCAGAGGGTTCGGCGGTTTCGATCCCGTCATCACCCACCA	PCR template for the amplification of dsDNA encoded fortRNA <sup>Val/TAC</sup>
BT5478	GGATCCTAATACGACTCACTATAGGG	Forward primer for amplification of all dsDNA templates
BT5501	TGGTGGAGCTAGACGGGATC	Reverse primer for amplification of tRNA <sup>Ala/GGC</sup>
BT6963	TGGTGGAGCCAAGGAGGATC	Reverse primer for amplification of tRNA <sup>Ala/TGC</sup>
BT5502	ACGTTTCGAGCGGGAGTCGAA	Reverse primer for amplification of tRNA <sup>Arg/ACG</sup>
BT6964	TGGCGCATCCGGCGGGATTC	Reverse primer for amplification of tRNA <sup>Arg/CCG</sup>
BT6965	TGGCGTCCCGGAGAGGGGTC	Reverse primer for amplification of tRNA <sup>Arg/CCT</sup>
BT6966	TGGCGCACCCGGCAGGACTC	Reverse primer for amplification of tRNA <sup>Arg/TCT</sup>
BT5503	TGGCTCCGCGACCTGGACTC	Reverse primer for amplification of tRNA <sup>Asn/GTT</sup>
BT5504	TGGCGCAGCGGACGGGACTC	Reverse primer for amplification of tRNA <sup>Asp/GTC</sup>
BT6967	TGGAGGCTGAGGTCGGAATC	Reverse primer for amplification of tRNA <sup>Cys/GCA</sup>
BT7243	TGGCAGGGGCGGCTGGATTC	Reverse primer for amplification of tRNA <sup>Gln/TTG</sup>
BT5093	TGGCGTCCCTAGGGGACTC	Reverse primer for amplification of tRNA <sup>Glu/TTT</sup>
BT6968	TGGAGCGGGCGAAGGGAATC	Reverse primer for amplification of tRNA <sup>Gly/CCC</sup>
BT5505	TGGAGCGGGAAACGAGACTC	Reverse primer for amplification of tRNA <sup>Gly/GCC</sup>

**Table S2** Primers and synthesized oligonucleotides used in this study (cont.)

Name	Sequence	Application
BT6969	TGGAGCGGGTAGCGGGAATC	Reverse primer for amplification of tRNA <sup>Gly/TCC</sup>
BT7245	TGGGGTGGACGATGGGAATC	Reverse primer for amplification of tRNA <sup>His/GTG</sup>
BT5506	TGGTGGGTCTGGGCAGATTC	Reverse primer for amplification of tRNA <sup>Ile/GAT</sup>
BT6970	TGGTGCCTCGGGAGAGACTC	Reverse primer for amplification of tRNA <sup>Leu/CAA</sup>
BT7247	TGGTGCCAGGAGAAGACTC	Reverse primer for amplification of tRNA <sup>Leu/CAG</sup>
BT6971	TGGTACCGAGGAGGGGACTC	Reverse primer for amplification of tRNA <sup>Leu/GAG</sup>
BT7249	TGGTGC GGACGGAGAGACTC	Reverse primer for amplification of tRNA <sup>Leu/TAG</sup>
BT5095	TGGTGGGTCTGTAGGATTCG	Reverse primer for amplification of tRNA <sup>Lys/TTT</sup>
BT5096	TGGTTGCGGGAGCTGGATTT	Reverse primer for amplification of tRNA <sup>Met/CAT</sup>
BT5507	TGGTGCCAGGGACGGAATC	Reverse primer for amplification of tRNA <sup>Phe/GAA</sup>
BT7251	TGGTCCGAGCGACTGGATTC	Reverse primer for amplification of tRNA <sup>Pro/CGG</sup>
BT7253	TGGTCCGGACGGAGTGATTC	Reverse primer for amplification of tRNA <sup>Pro/CGG</sup>
BT7255	TGGTCCGGGTAGAGAGATTC	Reverse primer for amplification of tRNA <sup>Pro/TGG</sup>
BT6972	GGAAGGCAGTGGGAGTCGAA	Reverse primer for amplification of tRNA <sup>Sec(p)/TCA</sup>
BT6973	TGGCGGAGAGATAGGGATTT	Reverse primer for amplification of tRNA <sup>Ser/CGA</sup>
BT6974	TGGCGGAGAGGGGGGATTC	Reverse primer for amplification of tRNA <sup>Ser/GCT</sup>
BT6975	TGGCGGTGAGGGAGGGATTC	Reverse primer for amplification of tRNA <sup>Ser/GGA</sup>
BT7257	TGGCGGAGGCGGTGAGATTC	Reverse primer for amplification of tRNA <sup>Ser/TGA</sup>
BT6976	TGGTGCCGGATAGAGGAATC	Reverse primer for amplification of tRNA <sup>Thr/CGT</sup>
BT5508	TGGAGCTCATGAGCGGATTT	Reverse primer for amplification of tRNA <sup>Thr/GGT</sup>
BT6977	TGGTGCCGGCACCAGGATC	Reverse primer for amplification of tRNA <sup>Thr/TGT</sup>
BT5094	TGGCAGGCCAGGAGGGAATC	Reverse primer for amplification of tRNA <sup>Trp/CCA</sup>
BT6978	TGGTGGAGGGAGAAGGATTC	Reverse primer for amplification of tRNA <sup>Tyr/GTA</sup>
BT5509	TGGTAGGCACGATTGGATTC	Reverse primer for amplification of tRNA <sup>Val/GAC</sup>
BT5510	TGGTGGGTGATGACGGGATC	Reverse primer for amplification of tRNA <sup>Val/TAC</sup>
BT7258	ATCCCCCGGGCTGCAGGAATTCGATATCAACATCTCGAGACTC	Control fragment without codon bias
BT7259	GAGTCTCGAGATGTTGATATCGAATTCCTGCAGCCCGGGGGAT	
BT7260	ATCCCCCGGGCTGCATTGTTGTTGTTGTTGCATCTCGAGACTC	Experimental fragment with 5XGln-CAA
BT7261	GAGTCTCGAGATGCAACAACAACAATGCAGCCCGGGGGAT	
BT5970	ATCCCCCGGGCTGCAGGCGGCGGGCGGGCCATCTCGAGACTC	Experimental fragment with 5XAla-GCC
BT5971	GAGTCTCGAGATGGCCGCGCCCGCCCTGCAGCCCGGGGGAT	
BT5972	ATCCCCCGGGCTGCAACGACGACGACGACGCATCTCGAGACTC	Experimental fragment with 5XArg-CGU
BT5973	GAGTCTCGAGATGCGTCGTCGTCGTTGCAGCCCGGGGGAT	
BT5974	ATCCCCCGGGCTGCAGTTGTTGTTGTTGTTTCATCTCGAGACTC	Experimental fragment with 5XAsn-AAC
BT5975	GAGTCTCGAGATGAACAACAACAACACTGCAGCCCGGGGGAT	
BT5976	ATCCCCCGGGCTGCAGTCGTCGTCGTCGTCATCTCGAGACTC	Experimental fragment with 5XAsp-GAC
BT5977	GAGTCTCGAGATGGACGACGACGACTGCAGCCCGGGGGAT	



**Table S3** Ribonucleosides identified by LC-MS/MS analysis of *P. aeruginosa* PA14 tRNA

rN Name	Precursor ion (m/z)	Product ion (m/z)	Fragment (V)	Collision (V)	Retention time (min)	Signal intensity normalized to $\Sigma$ AUCG					
						Wild type		<i>trmB</i> mutant		<i>trmB</i> complemented	
						Mean	SD	Mean	SD	Mean	SD
Cm	258	112	380	8	5.1	0.004845966	0.0002401	0.005756626	0.0002153	0.006072198	0.000335
m <sup>3</sup> C	258	126	380	8	2.2	-	-	-	-	-	-
m <sup>5</sup> C	258	126	380	8	2.7	1.90826E-05	1.294E-06	2.04589E-05	4.996E-06	2.72853E-05	1.58E-05
Um	259	113	380	4	9.1	8.39385E-05	8.333E-05	0.000204017	6.115E-05	0.000155449	4.409E-05
m <sup>5</sup> U	259	127	380	8	6.3	0.010569546	0.0007471	0.008096794	0.0006416	0.007937585	0.0003169
s <sup>2</sup> U	261	129	380	8	6.3	5.70115E-05	3.272E-06	4.35724E-05	3.862E-06	4.28974E-05	1.043E-06
s <sup>4</sup> U	261	129	380	8	7.9	0.001973445	0.0003525	0.002042688	0.000128	0.001758821	0.0001094
l	269	137	380	12	6.2	0.002223452	2.898E-05	0.037350303	0.0011602	0.036179178	0.0011242
mo <sup>5</sup> U	275	143	380	4	7.3	1.70892E-06	3.513E-07	9.76926E-07	1.841E-07	9.98527E-07	2.321E-07
Am	282	136	380	16	12.4	0.000851609	0.0002882	0.000748527	0.0002474	0.000797983	0.0001581
m <sup>1</sup> A	282	150	380	16	2.9	3.51548E-05	3.796E-05	1.60471E-05	1.578E-06	1.43217E-05	7.197E-06
m <sup>2</sup> A	282	150	380	16	11.5	0.03199429	0.00093568	0.033155675	0.0019178	0.029919588	0.0034706
m <sup>6</sup> A	282	150	380	16	16.2	0.011258614	0.0007696	0.009094374	0.0002814	0.007364891	0.001638
mnm <sup>5</sup> U	288	156	380	10	1.6	3.21294E-05	1.801E-06	3.09328E-05	4.002E-06	3.01965E-05	3.889E-06
m <sup>6</sup> 2A	296	164	380	16	20.4	6.7303E-05	1.306E-05	4.94945E-05	6.018E-06	6.52538E-05	5.463E-05
Gm	298	152	380	8	15	0.003787192	0.0002225	0.003011863	0.0002998	0.003073118	0.0004603
<b>m<sup>7</sup>G</b>	<b>298</b>	<b>166</b>	<b>380</b>	<b>12</b>	<b>5.1</b>	<b>0.038565559</b>	<b>0.0003676</b>	<b>0.001360776</b>	<b>0.0005876</b>	<b>0.018155591</b>	<b>0.0018724</b>
m <sup>1</sup> G	298	166	380	12	15	0.009522496	0.0014462	0.006761964	0.0008228	0.007100262	0.0016468
m <sup>2</sup> <sub>2</sub> G	312	180	380	8	19.5	-	-	-	-	-	-
cmo <sup>5</sup> U	319	187	380	8	6.5	-	-	-	-	-	-
cmnm <sup>5</sup> U	332	125	380	18	1.6	6.62787E-06	2.861E-06	4.31408E-06	3.235E-07	1.08442E-05	4.697E-06
i <sup>6</sup> A	336	204	380	16	22.4	0.000653115	0.0001341	0.000476001	4.662E-05	0.000548744	3.849E-05
cmnm <sup>5</sup> s <sup>2</sup> U	348	216	380	20	3.7	-	-	-	-	-	-
t <sup>6</sup> A	413	136	380	8	21.9	0.004719937	0.0001347	0.002769214	0.000313	0.002728713	0.0002978