Expression and regulation of the *Pseudomonas aeruginosa* hibernation promoting factor

Supplementary Information Table of Contents

Table S1. Expression reporters

Strain or plasmid	Identifier	Genotype and relevant characteristics	Source or reference
Strains			
Pseudomonas aeruginosa PAO1			
Pseudomonas aeruginosa PAO1 Δ*relA*/Δ*spoT*		*relA* Δ181 – 2019, *spoT* Δ200 – 1948	Nguyen 2013
Pseudomonas aeruginosa PAO1 Δ*hpf*			Williamson 2012
Pseudomonas aeruginosa PAO1 Δ*dksA2*		PW10380 from University of Washington *Pseudomonas aeruginosa* PAO1 transposon mutant Two-Allele Library	Jacobs 2003, Held 2012
Pseudomonas aeruginosa PAO1 Δ*rpoS*		PW7151 from University of Washington *Pseudomonas aeruginosa* PAO1 transposon mutant Two-Allele Library	Jacobs 2003, Held 2012
Plasmids			
pUC18T-mini-Tn7T-Gm-*eyfp*		Mini-Tn7 base vector with MCS, Gm^R, and *efyp*	Choi 2005
pTJ1		Mini-Tn7 base vector with MCS, P_{BAD} promoter, Tp^R, and *araC*	Damron 2013
pTNS1		Helper plasmid encoding TnsABCD transposon machinery	Choi 2005
pCR2.1		TA cloning vector	Invitrogen®
pCR-*hpf*		TA cloning vector with PCR product of PA4463 StuI 5' + PA4463 SphI 3' from PAO1 genome	This study
pCR-*rpoN*		TA cloning vector with PCR product of RpoN StuI 5' + RpoN StuI 3' from PAO1 genome	This study
pCR-$rpoN_{147}$		TA cloning vector with PCR product of RpoN StuI 5' + 3'RpoN147-StuI from PAO1 genome	This study
pCR-TSS1-*hpf-yfp*		TA cloning vector with PCR product of 5'HPFtss1-EcoRI + PA4463 SphI 3' from P_{hpf}*hpf-yfp* plasmid	This study
pCR-TSS2-*hpf-yfp*		TA cloning vector with PCR product of 5'HPFtss2-EcoRI + PA4463 SphI 3' from P_{hpf}*hpf-yfp* plasmid	This study
pCR-TSS3-*hpf-yfp*		TA cloning vector with PCR product of 5'HPFtss3-EcoRI + PA4463 SphI 3' from P_{hpf}*hpf-yfp* plasmid	This study
pCR-Up207		TA cloning vector with PCR product of 5'HPFup207-KpnI + PA4463 SphI 3' from PAO1 genome	This study
pCR-PA4463prom		TA cloning vector with PCR product of PA4463 StuI 5' + 3'PA4463prom-SphI from PAO1 genome	This study
pCR-hpf_{43}		TA cloning vector with PCR product of PA4463 StuI 5' + 3'HPF43-SphI from PAO1 genome	This study
pCR-hpf_{130}		TA cloning vector with PCR product of PA4463 StuI 5' + 3'HPF130-SphI from PAO1 genome	This study
Tn7-YFP reporters			
P_{hpf}*hpf-yfp*	A	pUC18T-mini-Tn7T-Gm-*eyfp* carrying P_{hpf} and in-frame fusion of HPF-YFP (translational reporter); StuI-SphI fragment from pCR-*hpf* ligated into pUC18T-mini-Tn7T-Gm-*eyfp*	This study
P_{rpoN}*rpoN*-P_{hpf}*hpf-yfp*	B	pUC18T-mini-Tn7T-Gm-*eyfp* carrying P_{rpoN}, *rpoN*, P_{hpf}, and in-frame fusion of HPF-YFP; StuI fragment from pCR-*rpoN* ligated into P_{hpf}*hpf-yfp*	This study
P_{rpoN}TSS1-*hpf-yfp*	C	pUC18T-mini-Tn7T-Gm-*eyfp* carrying P_{rpoN} and promoterless HPF-YFP reporter with 5'UTR starting from TSS1; StuI-KpnI fragment from pCR-*rpoN* ligated into TSS1-*hpf-yfp*	This study
P_{rpoN}*rpoN*$_{tru}$-TSS1-*hpf-yfp*	D	pUC18T-mini-Tn7T-Gm-*eyfp* carrying P_{rpoN}, 1296 nt of *rpoN*, and promoterless HPF-YFP reporter with 5'UTR starting from TSS1; StuI fragment of pCR-*rpoN* ligated into TSS1-*hpf-yfp*	This study
P_{rpoN}*rpoN*$_{147}$-P_{hpf}*hpf-yfp*	E	pUC18T-mini-Tn7T-Gm-*eyfp* carrying P_{rpoN}, 147 nt of *rpoN*, P_{hpf}, and in-frame fusion of HPF-YFP; StuI fragment of pCR-$rpoN_{147}$ ligated into P_{hpf}*hpf-yfp*	This study
TSS1-*hpf-yfp*	F	pUC18T-mini-Tn7T-Gm-*eyfp* carrying a promoterless HPF-YFP and 5'UTR starting from TSS1; KpnI-SphI fragment of pCR-TSS1-*hpf-yfp* ligated into pUC18T-mini-Tn7T-Gm-*eyfp*	This study

TSS2-*hpf-yfp*	G	pUC18T-mini-Tn7T-Gm-*eyfp* carrying a promoterless HPF-YFP and 5'UTR starting from TSS2; KpnI-SphI fragment from pCR-TSS2-*hpf-yfp* ligated into pUC18T-mini-Tn7T-Gm-*eyfp*	This study
TSS3-*hpf-yfp*	H	pUC18T-mini-Tn7T-Gm-*eyfp* carrying a promoterless HPF-YFP and 5'UTR starting from TSS3; KpnI-SphI fragment from pCR-TSS3-*hpf-yfp* ligated into EcoRI-SphI site of pUC18T-mini-Tn7T-Gm-*eyfp*	This study
Up207-P_{hpf}*hpf-yfp*	I	P_{hpf}*hpf-yfp* plasmid with putative UP element and upstream sequence removed; KpnI-SphI fragment of pCR-Up207 ligated into pUC18T-mini-Tn7T-Gm-*eyfp*	This study
P_{BAD}TSS1-*hpf-yfp*	J	pTJ1 containing TSS1-*hpf-yfp* under P_{BAD}; EcoRI-SphI fragment of pCR-TSS1-*hpf-yfp* ligated into pTJ1	This study
P_{BAD}TSS2-*hpf-yfp*	K	pTJ1 containing TSS2-*hpf-yfp* under P_{BAD}; EcoRI-SphI fragment of pCR-TSS2-*hpf-yfp* ligated into pTJ1	This study
P_{BAD}TSS3-*hpf-yfp*	L	pTJ1 containing TSS2-*hpf-yfp* under P_{BAD}; EcoRI-SphI fragment of pCR-TSS2-*hpf-yfp* ligated into pTJ1	This study
P_{hpf}5'UTR-*yfp*	M	pUC18T-mini-Tn7T-Gm-*eyfp* carrying P_{hpf} and 5'UTR fused to *eyfp* (transcriptional reporter); StuI-SphI fragment from pCR-PA4463prom ligated into pUC18T-mini-Tn7T-Gm-*eyfp*	This study
P_{hpf}*yfp*	N	pUC18T-mini-Tn7T-Gm-*eyfp* carrying P_{hpf} fused to *eyfp*; StuI-KpnI digested PCR product of PA4463 StuI 5' + 3'HPFprom-KpnI ligated into RBS-*yfp*	This study
RBS-*yfp*	O	pUC18T-mini-Tn7T-Gm-*eyfp* with PA1/04/03 promoter sequence removed; Annealed oligonucleotides 5'KpnI-RBS-SphI + 3'KpnI-RBS-SphI were ligated into KpnI-SphI site of pUC18T-mini-Tn7T-Gm-*eyfp*	This study
P_{hpf}*hpf*$_{9081}$-*yfp*	P	pUC18T-mini-Tn7T-Gm-*eyfp* carrying P_{hpf} and in-frame fusion of partial HPF (9081 aa) and YFP; StuI-SphI digested PCR product of PA4463 StuI 5' + 3'HPF243-SphI from PAO1 genome ligated into StuI-SphI site of pUC18T-mini-Tn7T-Gm-*eyfp*	This study
P_{hpf}*hpf*$_{43}$-*yfp*	Q	pUC18T-mini-Tn7T-Gm-*eyfp* carrying P_{hpf} and in-frame fusion of partial HPF (43 aa) and YFP; StuI-SphI fragment from pCR-*hpf*$_{130}$ ligated into pUC18T-mini-Tn7T-Gm-*eyfp*	This study
P_{hpf}*hpf*$_{142}$-*yfp*	R	pUC18T-mini-Tn7T-Gm-*eyfp* carrying P_{hpf} and in-frame fusion of partial HPF (142 aa) and YFP (partial translational reporter); StuI-SphI fragment from pCR-*hpf*$_{43}$ ligated into pUC18T-mini-Tn7T-Gm-*eyfp*	This study
P_{BAD}*hpf-yfp*		pTJ1 containing an in-frame fusion of HPF-YFP under P_{BAD}; NcoI-NotI digested PCR production of 5'_NcoI_hpf_inframe + 3'yfp-term-NotI from P_{hpf}*hpf-yfp* ligated into NcoI-NotI site of pTJ1	This study
P_{hpf}mod1-*hpf-yfp*	S	P_{hpf}*hpf-yfp* plasmid with modification of putative HPL sequence at 5' coding sequence of *hpf* reducing the HPL stability; StuI-SphI digested fused fragments from overlap extension PCR using PA4463 StuI 5' + 3'HPLmod1 and 5'HPLmod1 + PA4463 SphI 3' ligated into StuI-SphI site of pUC18T-mini-Tn7T-Gm-*eyfp*	This study
P_{hpf}mod4-*hpf-yfp*	U	P_{hpf}*hpf-yfp* plasmid with modification of 3' end putative HPL stem at 5' UTR of *hpf* reducing the HPL stability; StuI-SphI digested fused used fragments of overlap extension PCR using PA4463 StuI 5' + 3'HPLmod4 and 5'HPLmod4 + PA4463 SphI 3' ligated into StuI-SphI site of pUC18T-mini-Tn7T-Gm-*eyfp*	This study
P_{hpf}mod5-*hpf-yfp*	V	P_{hpf}*hpf-yfp* plasmid with modification of 5' end putative HPL stem at 5' UTR of *hpf* reducing the HPL stability; StuI-SphI digested fused used fragments of overlap extension PCR using PA4463 StuI 5' + 3'HPLmod5 and 5'HPLmod5 + PA4463 SphI 3' ligated into StuI-SphI site of pUC18T-mini-Tn7T-Gm-*eyfp*	This study

Table S2: Primer sequences used in this study

Primer Name	Sequence (5' -> 3')
molecular constructs	
PA4463 StuI 5'	GAAGGCCTGGAATGCTCGTCCACCGCGATCCG
PA4463 SphI 3'	AGGCATGCGGGCGCCTACGCCTTGCTGCCG
RpoN StuI 5'	GAAGGCCTGCTGCAGGAATTCCACATCCAC
RpoN StuI 3'	CCAGGCCTTCAGCGGTACTGACGTGACT
3'RpoN147-StuI	GAAGGCCTGCATGCGTTCGAGCATGGGATTGGATT
5'HPFtss1-EcoRI	ATGGTACCGAATTCACAGGGCATTCAAGTGGCTCGC
5'HPFtss2-EcoRI	ATGGTACCGAATTCGTTCCGGAGGCAGGTTGCTAACCTG
5'HPFtss3-EcoRI	ATGGTACCGAATTCACACACGGGCAACAAGGAGAACGC
5'HPFup207-KpnI	ATGGTACCGCCATTGAGCGACAGCAAGATC
3'PA4463prom-SphI	CAGCATGCCGCGTTCTCCTTGTTGCCCGTG
3'HPFprom-KpnI	CTAGGTACCGCCTCCAGTAAACCAGCGATCTTG
3'HPF43-SphI	GAGCATGCCGTCGGTCACATCCAGTTGATGG
3'HPF130-SphI	GAGCATGCCGACCTCCATGATCACCTGAACAT
3'HPF243-SphI	ATGCATGCTCTCTTTGTGTTTGATCAGTTGACGG
5'_NcoI_hpf_inframe	TTACCATGGAAGTCAACATCAGTGGC
3'yfp-term-NotI	ATGCGGCCGCTAATTCGATCATGCATGAGCTCG
5'HPLmod1	GCAAGTCAACATCAGTGGCCATCAGTTGGACGTAACCGACGCCCTGCGCGACTA
5'HPLmod3	AACGCGGTATGCAAGTGAACATCAGCGGCCATCAGCTAGATGTCACCGACGCCCTGCGCG
5'HPLmod4	GTTCCGGAGGCAGGTTGCTAAGGTCGGACTTACACACGGGCAACAAGGAG
5'HPLmod5	ACGCCAAGGTGTTCCGGACCGACCTTGCTAACCTGCCACTTACACACG
3'HPLmod1	TAGTCGCGCAGGGCGTCGGTTACGTCCAACTGATGGCCACTGATGTTGACTTGC
3'HPLmod3	CGCGCAGGGCGTCGGTGACATCTAGCTGATGGCCGCTGATGTTCACTTGCATACCGCGTT
3'HPLmod4	CTCCTTGTTGCCCGTGTGTAAGTCCGACCTTAGCAACCTGCCTCCGGAAC
3'HPLmod5	CGTGTGTAAGTGGCAGGTTAGCAAGGTCGGTCCGGAACACCTTGGCGT
5'KpnI-RBS-SphI	CACACATCTAGAATTAAAGAGGAGAAATTAAGCATG
3'KpnI-RBS-SphI	CTTAATTTCTCCTCTTTAATTCTAGATGTGTGGTAC
5'-RACE primers	
Poly adapter	CACCTGAGCAGAGTGACGAGGACTACGGCTTCAGCTTTTTTTTTTTTTTTTT
Poly outer adapter	CACCTGAGCAGAGTGACG
hpf	GTCGATCAGCAGGTCGATGGCCGCG
hpf nested	GTTCCGCATTGGCTACGATCTCG
RT-qPCR primers	
acp-For	ACTCGGCGTGAAGGAAGAAG
acp-Rev	CGACGGTGTCAAGGGAGT
eYFP-For	CACATGAAGCAGCACGACTT
eYFP-Rev	GGTCTTGTAGTTGCCGTCGT
UP-TSS1-F	GGGGAATGCTCGTCCACC
Down-TSS1-F	AACCTGCCACTTACACACGG
Hpf-R	CGTCGGTCACATCCAGTTGA

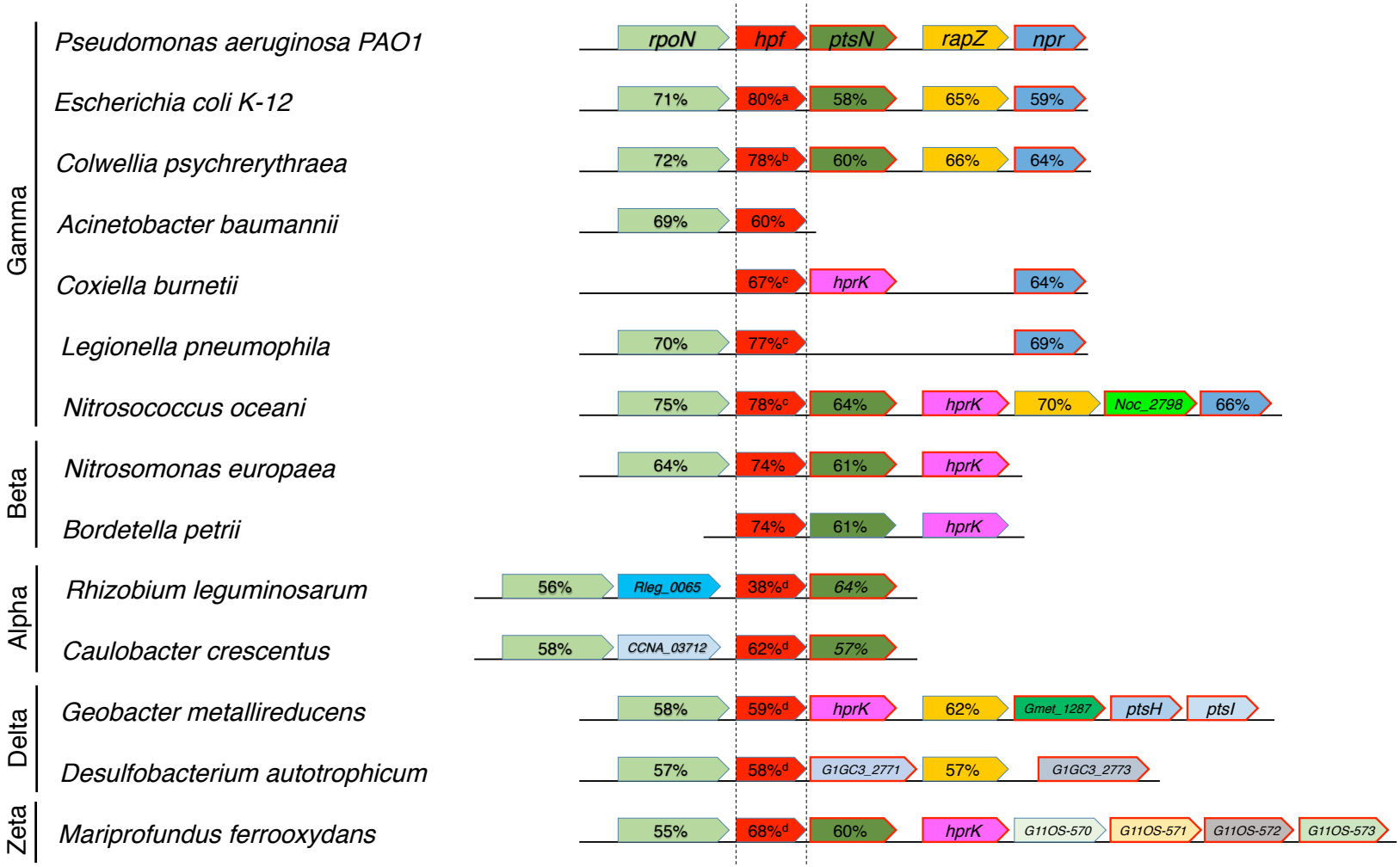


Fig S1. Schematic representation of *hpf* distribution among diverse species of bacteria, and the neighboring genes surrounding *hpf*. The percentages indicate the amino acid sequence similarity to the *Pseudomonas aeruginosa* PAO1 homologs. Letters indicate that one or more *hpf* paralogs are encoded by the representative organisms, with (a) indicating one paralog, (b) two paralogs, (c) one paralog with long HPF tail, (d) strains that contain a HPF with a long tail. Gene annotations are as follow: *rpoN*, RNA polymerase sigma-54 factor; *hpf*, hibernation promoting factor; *ptsN*, phosphotransferase system enzyme IIA(Ntr); *rapZ*, Rnase adaptor protein; *npr*, phosphorelay protein Npr; *hprK*, HPr kinase/phosphorylase; *Nco_2798*, phosphotransferase system enzyme IIA(Fru); *Rleg_0065*, hypothetical protein with 2 EamA domains; *CCNA_03712*, hypothetical protein with ExoD domain; *Gmet_1287*: phosphotransferase system, mannose-type, protein IIA; *ptsH*, phosphocarrier protein HPr; *ptsI*: phosphoenolpyruvate protein phosphotransferase.

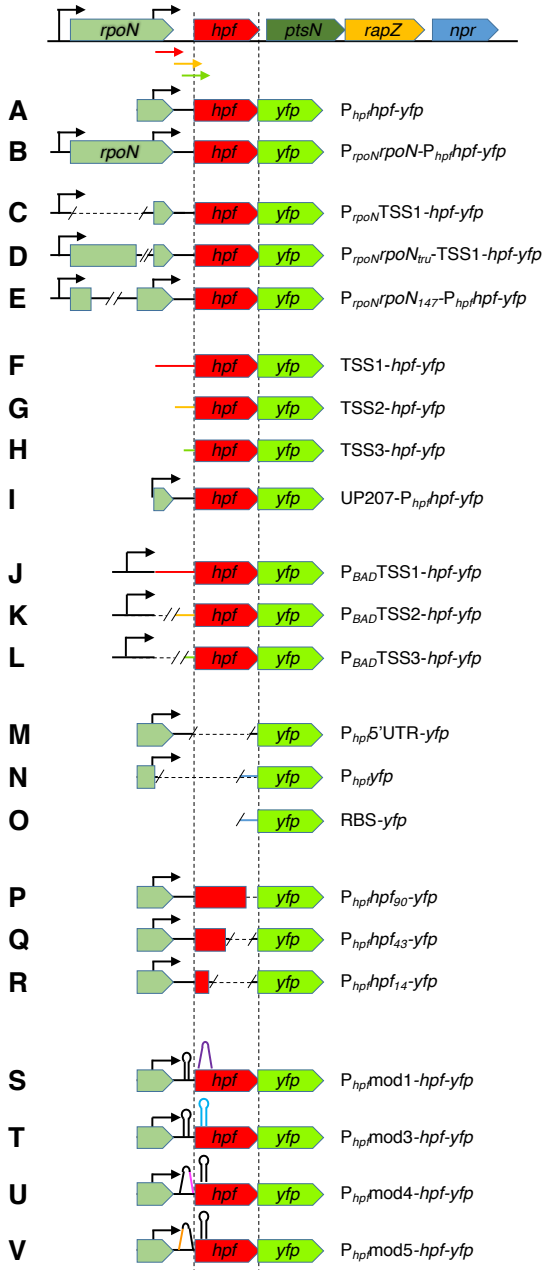


Fig S2. Schematic representation of reporter constructs used in these studies. The *hpf* gene is shown in red, and the *yfp* gene in green. Arrows indicate promoter sequences. Sites of hairpin loops are also shown in S through V.

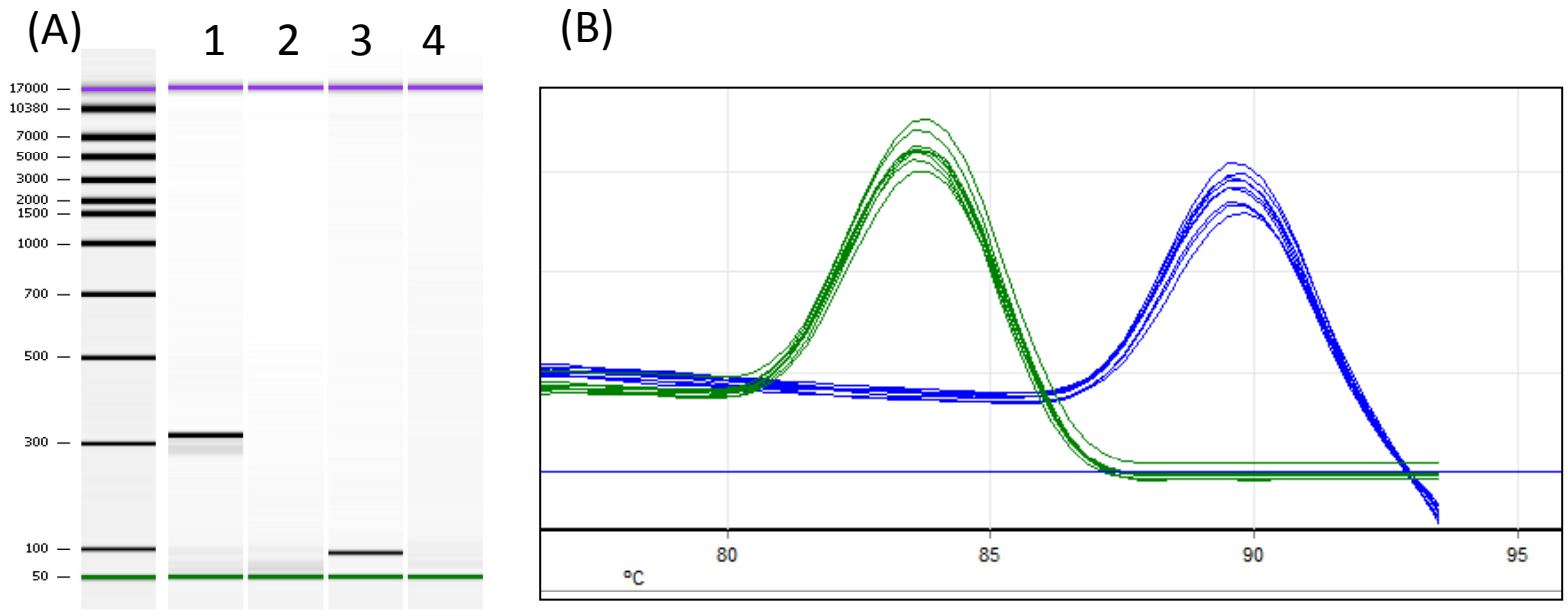


Fig S3. (A) Bioanalyzer Gel image of PCR products produced from primer upstream of P_{hpf} but downstream of P_{rpoN} (lanes 1, 2), showing transcriptional read-through from P_{rpoN} to *hpf*. Lane 2 was a control lacking reverse transcriptase. Bioanalyzer gel image of PCR product produced from a primer downstream of P_{hpf} (lane 3,4). Lane 4 lacked reverse transcriptase. (B) Melt curve analyses of PCR products from P_{hpf} promoter (green) and from P_{rpoN} promoter (blue). Each RT-qPCR experiment was performed with three biological replicates, and three technical replicates per biological replicate.

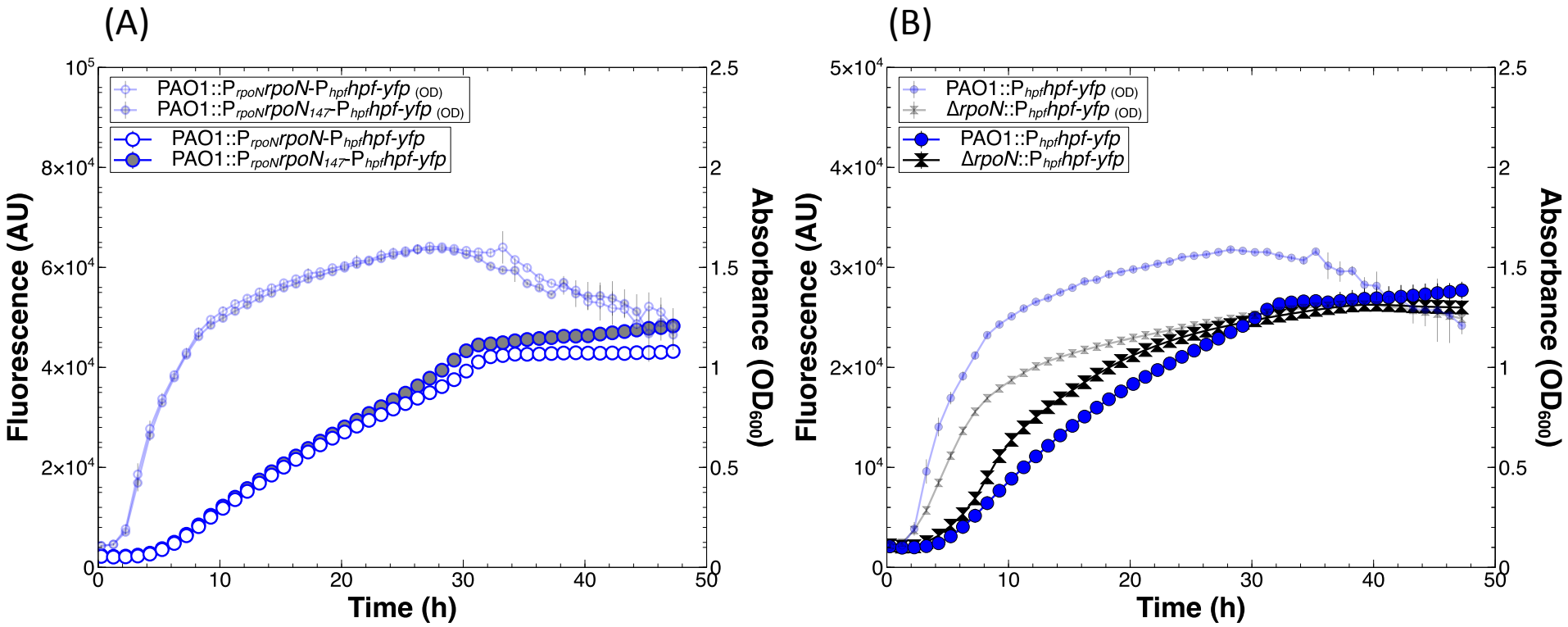


Fig S4. Effect of RpoN on expression of *hpf*. (A) Expression patterns (large symbols) and growth curves (small symbols) of HPF-YFP reporter constructs containg both the P_{rpoN} and P_{hpf} promoters with the reporter construct containing an intact *rpoN* gene (open circles) and with the *rpoN* gene truncated (closed circles) (constructs B and E from Fig S2) . (B) Growth curves (small symbols) and expression of *hpf* (large symbols) from P_{hpf} in the wild-type strain (closed circles) and in a *rpoN* deletion mutant (hourglass) (construct A from Fig S2). The results show that RpoN is not required for expression from P*hpf*. Data show the average and standard deviations from three biological replicates.

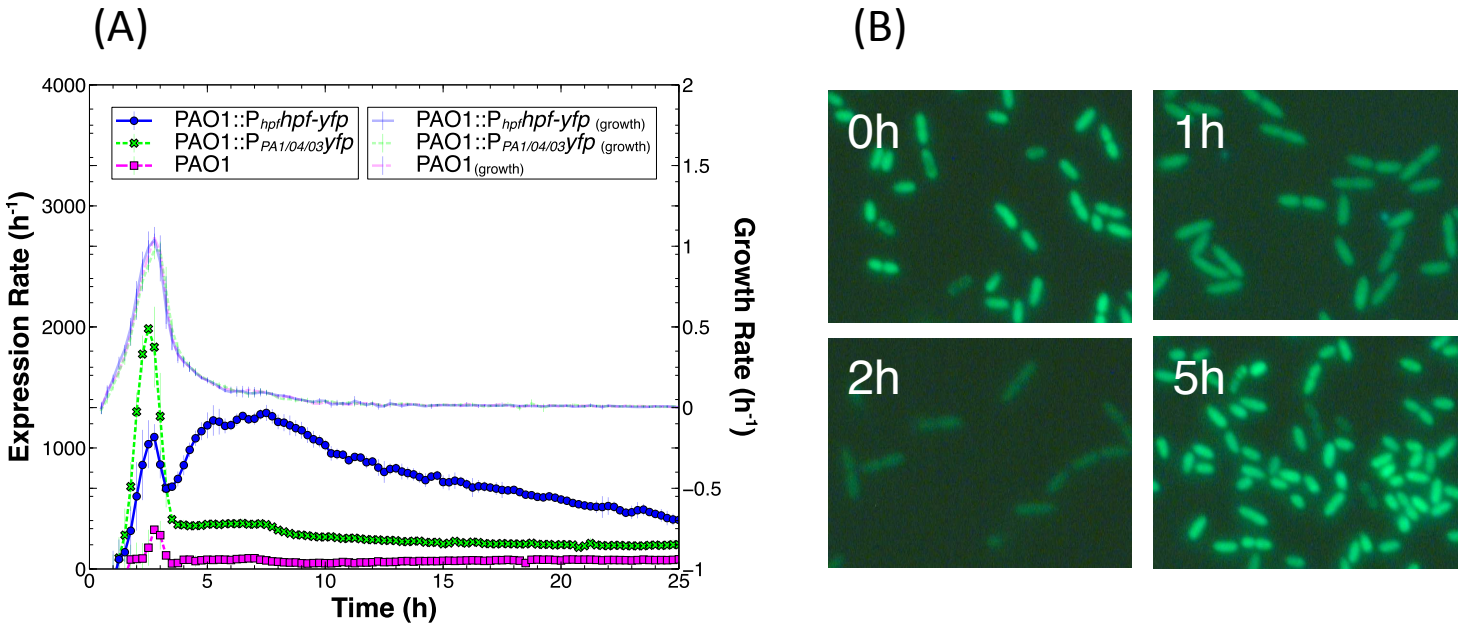
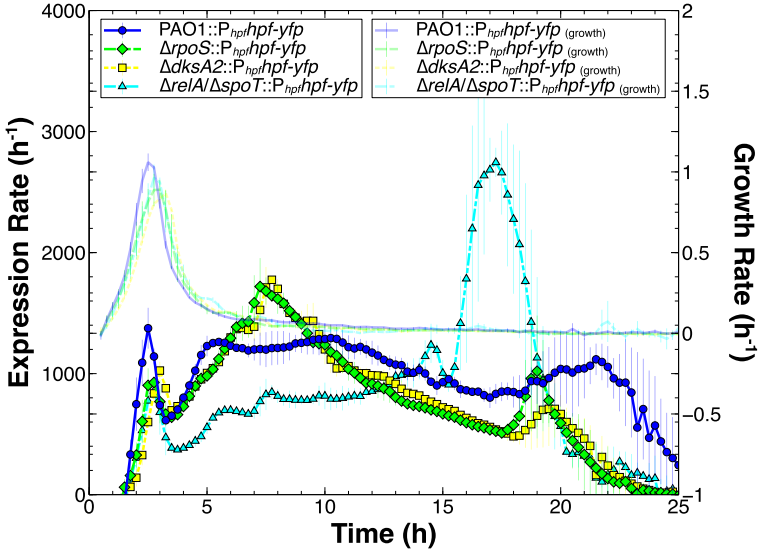


Fig S5. Growth phase dependence expression of *hpf*. (A) Expression rate (dark blue) and growth rate (light blue) were calculated based on the increases in optical density and fluorescence from the P$_{hpf}$-*hpf-yfp* reporter. (B) Epifluorescence micrographs showing fluorescence of individual cells containing the *P$_{hpf}$-hpf-yfp* reporter over time.



FigS6. Growth rate (lines) and expression rate of P_{hpf} (symbols) in mutants strains impaired in stationary phase factors, *rpoS* (green diamonds) *dksA2* (yellow squares) and ppGpp production (blue triangles). The P_{hfp} expression rate in the wild-type strain is shown (blue circles) for comparison. Expression rate of P_{hpf} is moderately inhibited during stationary phase of the *rpoS* and *dksA2* mutants.

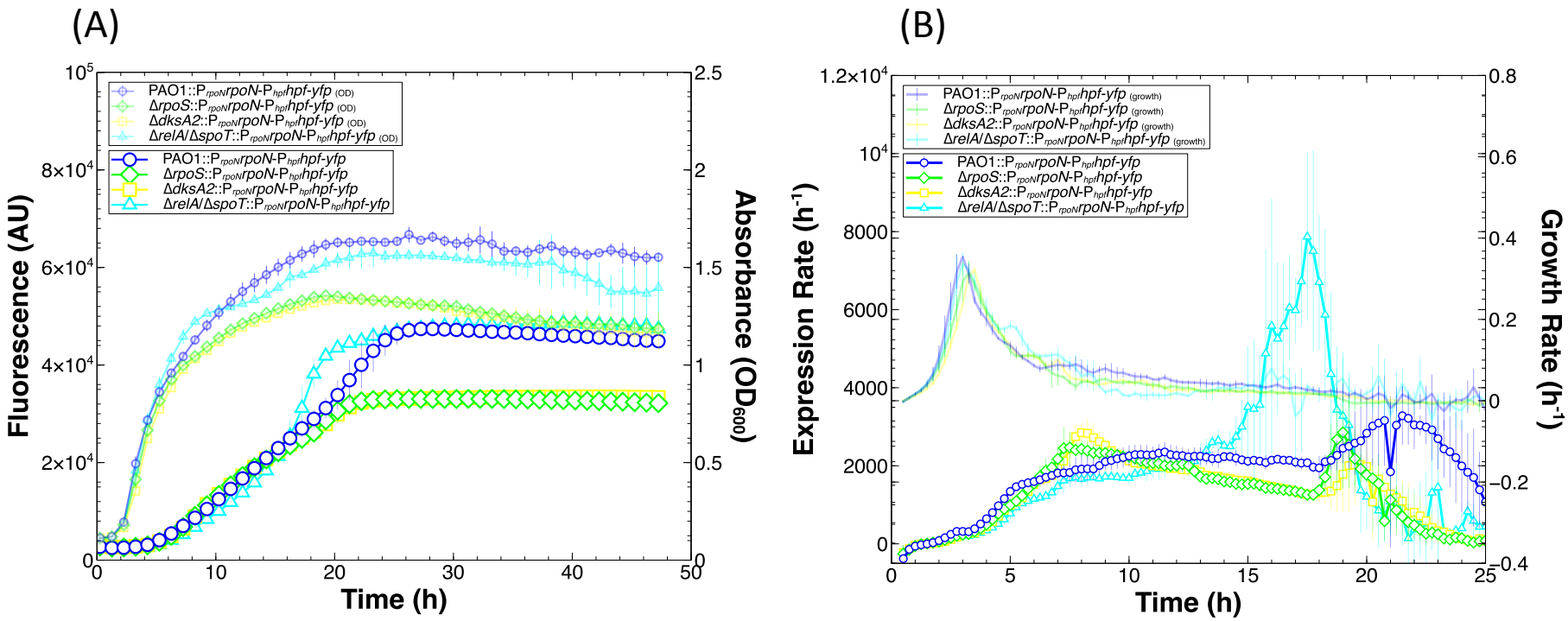
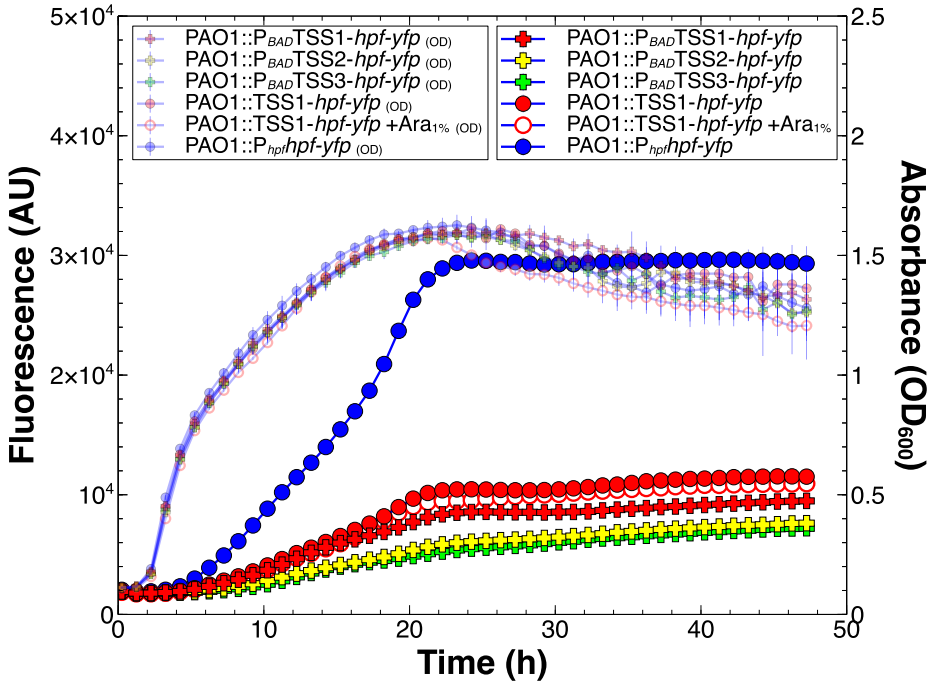


Fig 7S. Effect of stationary phase factors on expression (large symbols) from P_{rpoN}-*rpoN*-P_{hpf} *hpf-yfp* reporter. (A) Expression from both P_{rpoN} and P_{hpf} in the *rpoS* mutant (green circles) and *dksA2* mutant (yellow squares) compared to the wild-type strain (blue circles). The mutant defective in ppGpp production (blue triangles) had a similar pattern of expression as in the reporter construct lacking P_{rpoN}. (B) Growth rate (lines) and expression rates (symbols) for the *rpoS*, *dksA2*, and *relA*/*spoT* mutants calculated from the growth and expression data in (A).



FigS8. Growth (small symbols) and expression of *hpf-yfp* reporter with the P_{hpf} promoter replaced by the P_{BAD} promoter, in the absence of arabinose induction. The P_{BAD} promoter was placed in orientation for expression from TSS1 (red symbols), TSS2 (yellow symbols), and TSS3 (green symbols). Expression from P_{hpf} (blue circles) is shown as a positive control.

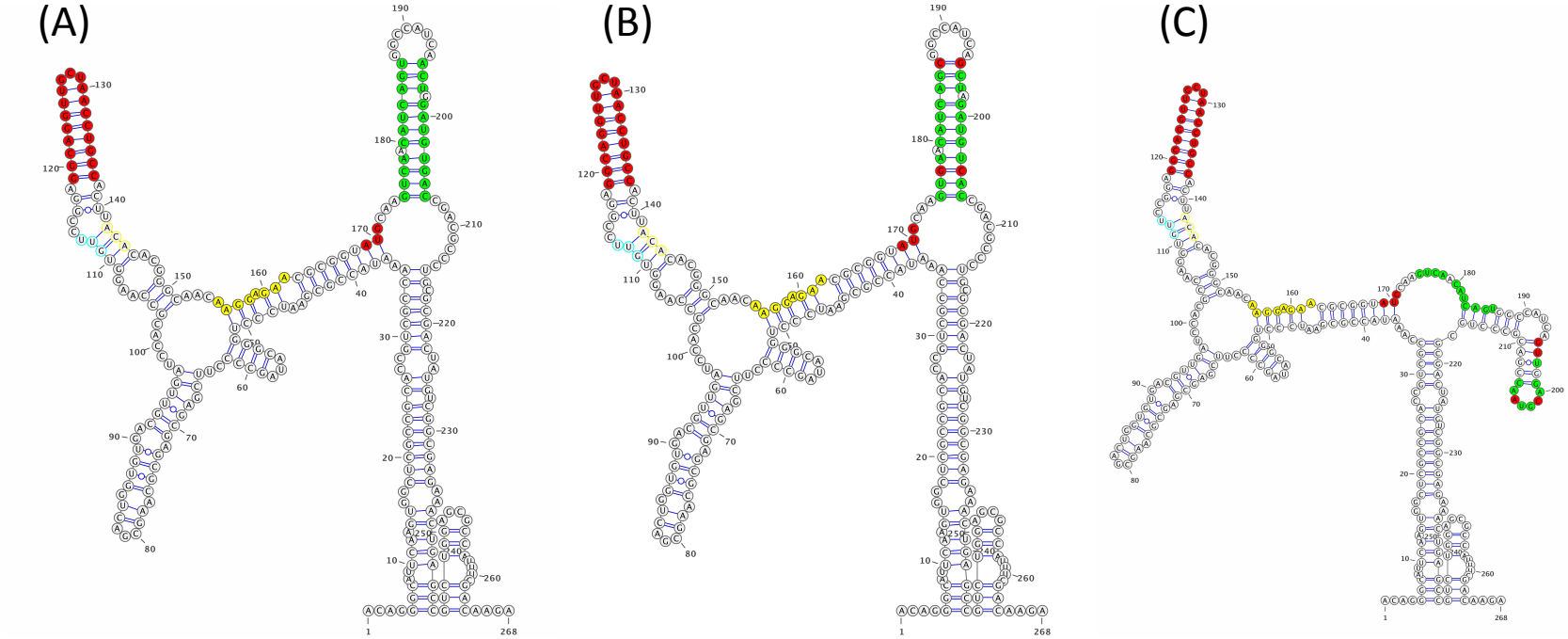


Fig S9. Secondary structure predictions of the *hpf* mRNA wild-type sequence, starting from TSS1 through the first 100 nucleotides of the *hpf* structural gene, as predicted by RNAfold server and visualized using VARNA (Lorenz et al., 2011). Shown in red is the stable hairpin loop in the 5' UTR. Also shown in red is the *hpf* start codon. Shown in blue is the site of TSS2 and yellow is the site of TSS3. The hairpin loop (HPL) shown in green is downstream of the start codon, and is the HPL modified by mod 1 and mod3. (B) The HPL structure predicted by RNAfold after mod1, creating an open 5' end of the *hpf* mRNA. (C) The HPL predicted by RNAfold after mod3, which creates a tighter HPL structure.