

Figure S1:

Model I: Rho is recruited to the EC by binding to the nascent RNA via the *rut* site, translocates along the RNA, and eventually catches up the EC and dislodges it. This model predicts that RNAP-elongation and Rho-translocation have to be kinetically coupled¹.

Model II: Rho forms complex with EC prior to loading onto the RNA following which Rho gets activated to drag the mRNA through itself, and termination occurs by allosteric modification of the EC. Kinetic coupling as well as translocase activity of Rho may not be required in this model. Also *rut* site mediated recruitment of Rho is not necessary^{13,14}.

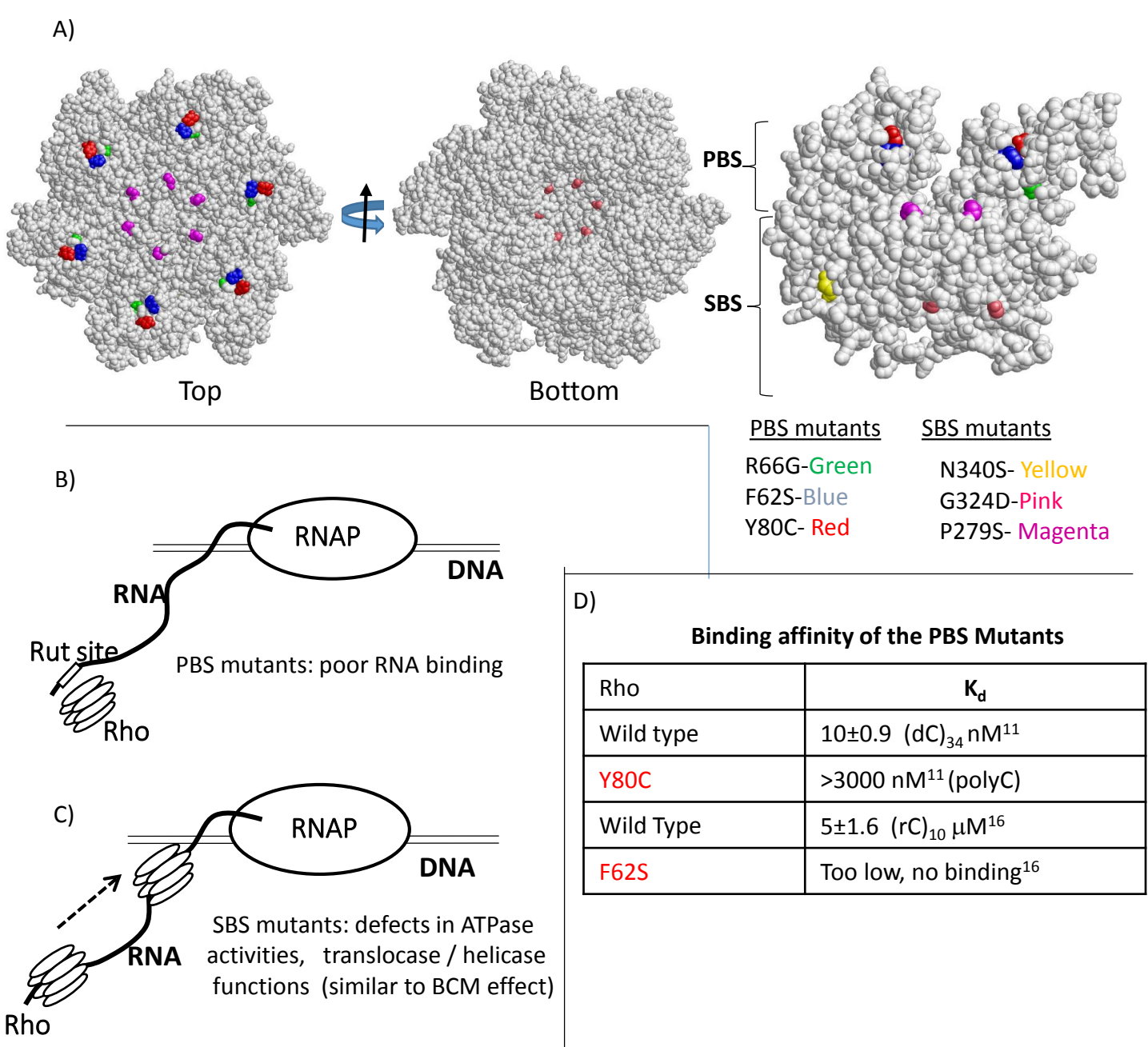


Figure S2: A) Locations of different point mutations in Rho. Mutations in primary and secondary RNA binding regions are indicated. Top view shows the primary RNA binding site, while the bottom view shows the RNA exit channel and secondary RNA binding sites. B) and C) Functional defects of PBS and SBS mutants explained through cartoons. D) Experimentally determined binding constants of WT and PBS Rho mutants for DNA and RNA molecules those are indicated in the parenthesis.

Early region of *rac* prophage containing the *racR/t_{rac}* terminator sequences cloned before a

lacZ reporter

In vitro termination region

RS83

CCATAAACTG CCAGGAATTG GGGATCGGAA TTCGCTTCAC TGACATATTC TCGGAACAAC ATGCCGAACG } *P_{RM}*

TCGTAATAT GACCAGTCAA TATCAGGACGAAG TTCTTCGCAC AGAACCTCAC CTCTTGTTGC ACGTTCAATT } *Untranslated region*

GCTGGACATC TCTCGGCAGG CAATTGACGT ACCCCTTTGA TCCATTGATT TACGCTTGGA GG TGATACAC

CTAAAAGCT AGCCATTGCT GATTGCCAC CGACAACAGC ACAAGCTTGC TTGAATGAAT AGTTCTCTTT

TTTCATCGAA TGAATCCAA AAACACACAG AAATATTAGG CGACGCCTAA CGCAATTGTC AATAGGCTGT

GCCTAATGCA GTAAGGGTAG GGATTGCCTA ATGTAATGCG CATAGGAGAA TATTAAGCA ATGCTTAGTG

GTAAAGACTT AGGCCGAGCG ATAGAGCAGG CCATTAACAAA AAAATCGCA TCGGGATCCG TCAAATCAAA

GGCGGAGGTC GCACGCCACT TTAAAGTCCA ACCACCATCA ATTATGACT GGATTAAGAA AGGCTCTATA

AGTAAAGATA AACTTCCAGA ATTATGGCGT TTCTTTCTG ATGTTGTTGG TCCAGAGCAT TGGGGGCTTA } *racR*

ACGAATACC CATACCAACC CCCACCAATT CAGATACAAA AAGTGAACTT TTAGATATAA ACAACCTTTA

TCAAGCAGCC TCTGATGAAA TAAGAGCGAT TGATAGCTTC CTGTTATCTG GAAATGCTAC AGAACAGAT

TGGGTTGACC ACGATGTTG CGCTACATA GCAGCGATGG AAATGAAAGT GGGTAAGTAT CTGAAAGCTC

TTGAATCTGA ACGGAAAAGC CAGAACATCA CAAAACTGG AACTTAA

ACTTATATGG TCTGACGGAA AACTCCTGGA TTCCGTTATT TAACCCCCC ATCACTTCT GCTGTCGCCA

TCACCTATTA GGTTACGCTC AAAACATTAG GCATAGCCTA TTGACAATCA ATTAGGCATT ACCTATAGTT } *Intragenic region*

CCAGCATACC ACCACCCCG CCCACAGAA CGCCGGCAA TACTTCGAGT TACCAGGCAG TGGTAAGGGG

TTAAGTAGCC AGCCGAGGC GTATGAACAT GACGGCGGGA TTCAAATTT GCAGTGCAGC AGTTAGTTCC

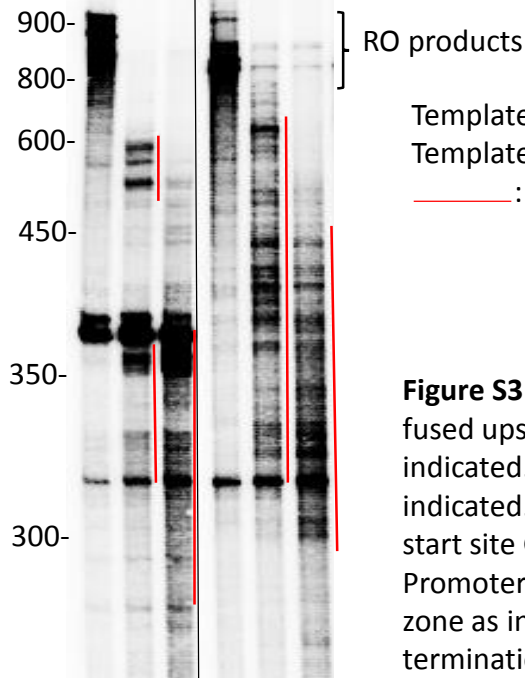
GCCACCCGCGTTAAGGGGAGAGATAAGGGATCC

TAACACTAGCGATCCGACTCACTATAGAGGGACAAACTCAAGGTCATTTCGAAGAGTGGCCTTTATGATTGACC } *LacZ*

TTCTCCGGTTAATACGACCGGGATCGAGATCCTAGGTAGGTAGGGGCGCGGCATTTAACTTTCTTTATCACACAG

GA AACAGCTATG ACCATGATTA CGGATTCCTGCGCTGTTTACAACGTCGTGACTGGGAAAAC } *RS-RK1*

Templates:	1	2
Rho:	- + +	- + +
NusG:	- + +	- + +



Template 1 made from oligo pair RS83/RK-1
 Template 2 made from oligo pair RS83/RS845.
 ——— : Termination regions

Figure S3: Sequence of the *P_{RM}-t_{rac}-racR* region of *rac* prophage fused upstream of a *lacZ* cassette. Different regions are indicated. Primers used for making the DNA templates are also indicated. In the *in vitro* transcription assays the transcription start site G, shown as a small arrow, is utilized by the RNAP. Promoter is shown in yellow, *racR* in blue highlights. Termination zone as indicated was obtained from *in vitro* and *in vivo* termination assays (see adjacent autoradiogram and figure S8).

P1 TRANSDUCTION PLATES

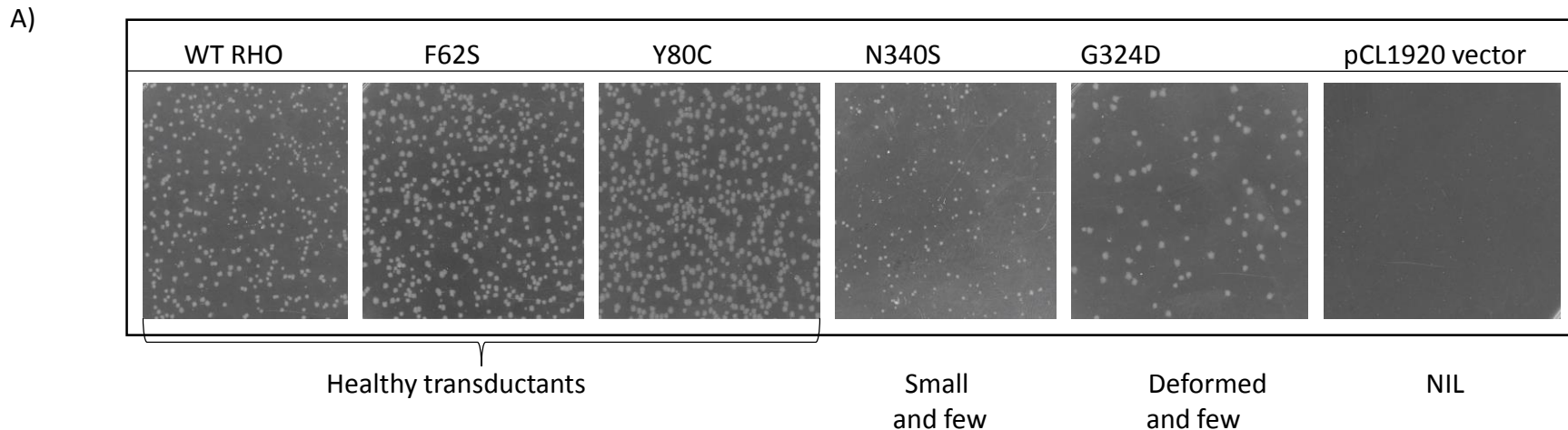


Figure S4A: *MG1655 rac⁺* strains were at first transformed with pCL1920 plasmids expressing WT and different derivatives of *rho* mutants as indicated on the top of the pictures. Resultant strains were P1 transduced with a *rho:kan^R* cassette to delete chromosomal *rho*. The pictures showed the number and nature of the transductants obtained in the presence of WT and different *rho* derivatives as indicated. In cases of WT and the two PBS mutants, Y80C and F62S, transduction efficiency was high and the transductants were healthy-looking. In case of N340S, transductants were small, whereas these were deformed when G324D was present. The transduction efficiency was also poor for these two SBS mutants. There were no transductants in the presence of empty vector. In figure 1B, these transductants were re-streaked on LB plates and incubated overnight at 37°C.

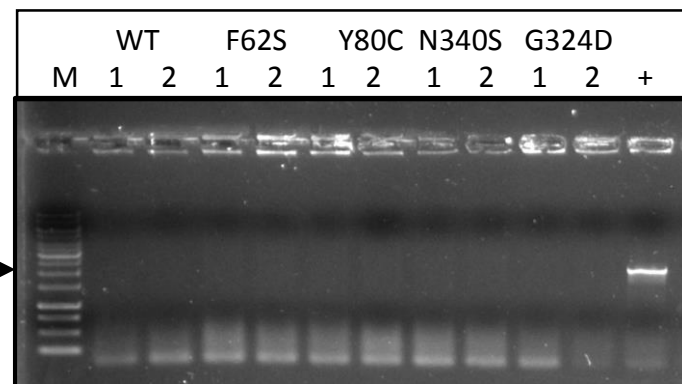
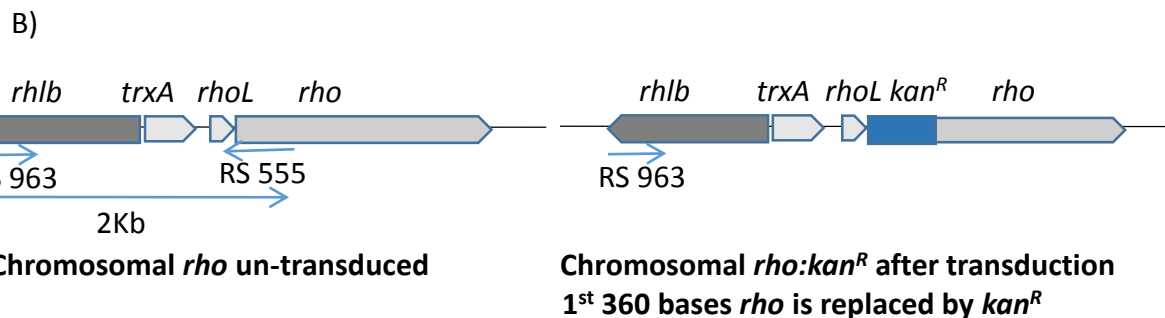
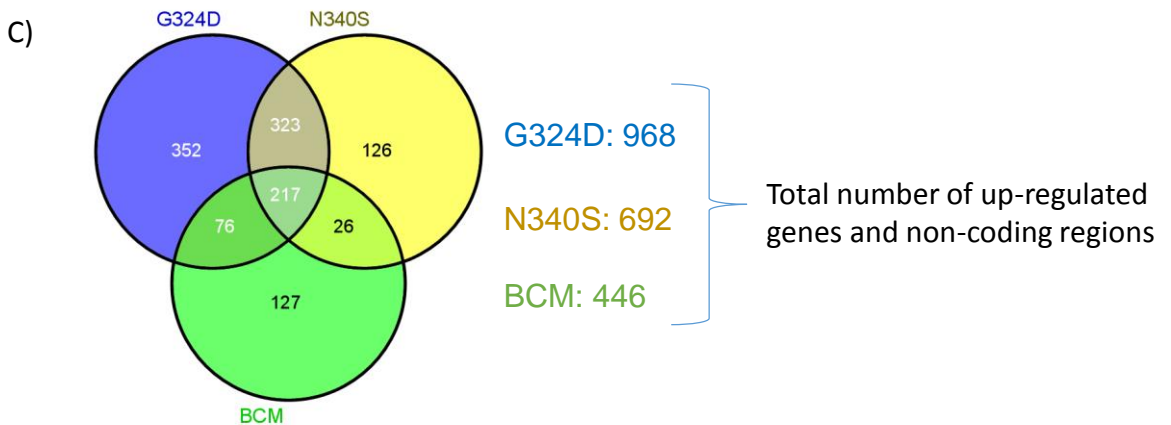
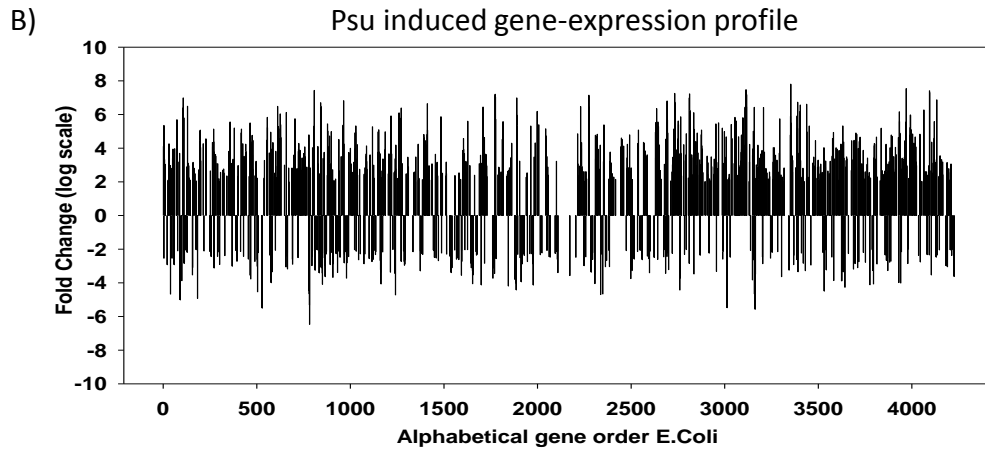
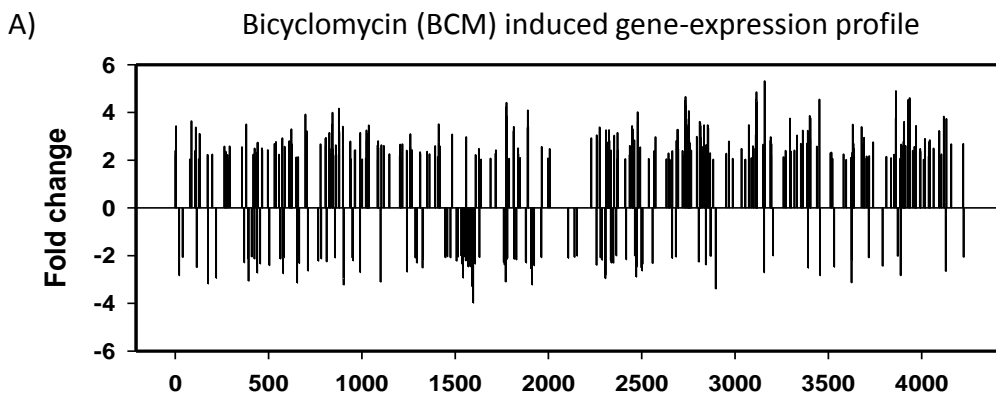


Figure 4B: RS963 and RS555 primers were designed such a way that they will produce ~2Kb PCR-product only if an intact *rho* is present in the chromosome, and thereby will detect the occurrence of the duplication of *rho*. This primer pair will not produce any PCR product neither from *kan^R:rho* nor from the pCL1920 plasmids expressing WT or mutant *rhos*, because in each case either N-terminal of *rho* or *rhlB* is absent. WT *MG1655* strain is used as the “+” control, whereas strains transformed with empty vector were used as a “-” control. Absence of the 2Kb PCR product indicates absence of *rho*-duplication. Two transductants (1, 2) for each of the *rho* mutants were tested.



D) Expression of *rac* prophage region

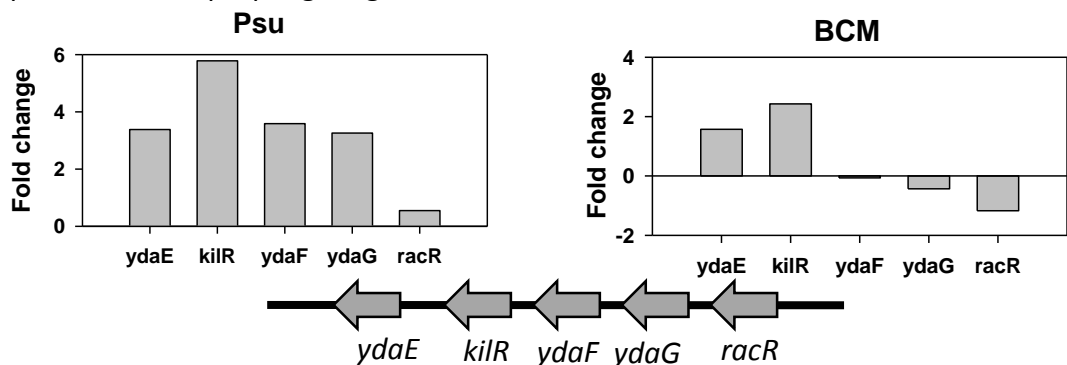


Figure S5. Microarray profiles of genome wide expression patterns of MG1655 treated with A) BCM and B) due to expression of Psu. Psu is expressed from a plasmid and has a drastic effect compared to SBS mutants or BCM. C) Venn diagram showing the overlaps between up-regulated genes of Rho mutants and those obtained after BCM treatment. This is generated using VENNY online tools from <http://bioinfogp.cnb.csic.es/tools/venny/index.html>. D) Expression profiles of the *rac* prophage genes in the presence of these two Rho-inhibitors. Expression of *kilR* is up-regulated when Rho is inhibited by Psu and BCM.

Expression of GENES those are less affected by

PBS mutants

Genes *Fold change in gene expression level*
(Grouped values)

	<i>PBS mutants</i>	<i>SBS mutants</i>
aaeA	0.8560	3.7106
aaeB	1.9583	3.7579
aaeX	0.6806	4.5353
allB	0.2170	3.0600
acpZ	-0.1951	3.8572
asIB	1.2368	3.5999
bcsA	0.2331	3.3926
cadA	2.2939	5.4593
cadB	1.2461	3.1624
caiC	0.3784	3.1889
caiD	0.2293	3.4299
cbrA	1.3545	3.2240
cbrB	0.8678	3.1717
ecpD	1.0164	3.3764
etp	2.7829	5.0305
eutD	1.8017	3.3614
eutG	0.5982	3.3481
flu	1.8894	3.4883
fryC	1.9004	3.0495
gfcC	2.9529	6.0432
mdtA	0.8001	3.2401
mutL	2.0040	3.5415
phoH	1.8294	3.3983
pitB	7.6934e-3	3.0954
rhsB	1.2866	3.1663
sfmH	-0.3877	3.1238
sgcX	3.6982	3.5332
thiC	1.5139	4.8160
thiE	1.6363	4.2424
thiF	1.6704	4.0786
thiG	0.8281	3.8829
thiH	0.8979	3.5300
thiS	0.9938	3.9311
uhpT	2.3552	4.4967
yagK	1.8767	4.1309
yagL	1.1385	4.7311
yagM	1.4124	3.2965
ybcK	1.0373	4.4540
ybcL	-0.0450	3.7694
ybcN	1.4722	3.7652
ybeF	0.8198	3.1497
ybfB	0.5121	3.2270
ybfD	1.1082	4.3139
ybfQ	0.9176	5.2946
yciW	3.6497	3.1442
ydcC	0.8906	4.4863
ydeO	0.3647	3.1981
ydeP	1.8224	3.2871
ydeQ	2.1027	4.8034
ydeS	1.1530	3.8827
ydfU	1.0241	3.2317
ydhS	2.6417	7.0625
ydiO	0.1592	3.1277
yeeR	2.7713	3.8542
yeeS	2.7728	3.2037
yeeU	1.1379	4.0239
yegR	-0.0215	6.0768
yfbN	1.0878	4.2325
yfhR	2.6596	4.3419
ygiZ	1.0328	3.6525
yhcA	0.6425	3.3130
yhhH	1.6212	3.1063
yhhI	1.8361	4.3626
yhiD	-0.2105	3.0049
yhjH	1.9629	3.5160
yibV	0.7608	5.0041

CP4-6

DLP12

Qin

CP-44

Genes

Fold change in gene expression level
PBS mutants *SBS mutants*

yjfJ	0.5340	3.5846
yjfK	0.4294	3.7685
yjfM	-0.1782	3.0266
yigZ	2.8882	3.3361
yjjB	0.6138	3.3658
yjjP	0.4738	3.9590
ykgB	0.4550	3.4725
ylcH	0.6785	3.9251
ypdF	1.6239	3.3379

Non-CODING regions those affected less by PBS mutants
(gene expression in anti-sense direction)

Co-ordinates *Fold change in gene expression level*
(Grouped values)

	<i>PBS mutants</i>	<i>SBS mutants</i>
357915-358022	1.2001	3.4466
499198-499348	1.6630	4.1688
527176-527883	-0.6973	3.6324
527864-528354	-0.1204	4.3537
528721-528816	-0.8110	3.2165
2167717-2169751	2.2526	4.9453
2663267-2663456	2.1295	4.1394
2807516-2807638	2.0076	4.2569
2995714-2996850	2.9151	5.3990
3621910-3622250	0.7082	3.1326
3766662-3766913	0.5788	3.8835
3766915-3767279	0.0850	3.6723
3767971-3768169	0.4993	3.5761
3826689-3826967	0.0577	3.0610
3951437-3951500	0.4482	3.2183
3955844-3955992	1.2378	3.6199
4028995-4029183	2.0502	3.6462
4425446-4425716	1.5729	3.6507
4425717-4426118	0.4648	3.2787
4506699-4506965	1.3087	3.9089
4506966-4507826	1.5441	3.5148
4571942-4574878	1.3672	3.0425
4600882-4601499	-0.5985	3.8964
4602226-4602332	2.0464	5.4412

Figure S6. List of genes and non-coding regions less affected by PBS mutants compared to that obtained for SBS mutants.

Genes belong to the prophages (CP4-6, DLP12, Qin, CP-44) are indicated in colors. Similar to rac prophage, these prophage genes are also not much affected by PBS mutants. Numbers are fold changes in gene expression w.r.t. WT.

A)

Genes affected significantly by PBS mutants**Coding genes**

Name **Fold change in gene expression level (\log_2)**

PBS **SBS**

cysA	3.402	3.89
cysC	4.516	2.126
cysD	4.457	3.162
cysH	4.624	3.837
cysI	4.878	3.961
cysJ	4.361	3.409
cysN	3.613	2.83
cysP	4.102	2.44
cysU	4.123	2.87
cysW	3.300	2.77
fhuF	3.555	4.421
gfcB	3.333	4.536
gfcD	3.012	5.182
pgaB	3.287	3.417
pgaC	3.203	3.893
rho	3.490	2.00
sgcB	3.809	3.40
sgcX	3.698	3.293
yafW	4.481	3.075
yafX	3.535	3.204
yahM	3.078	1.13
yciW	3.650	2.97
ydjN	3.216	2.311
yedK	3.022	2.92
yeeD	3.171	3.737
yeeE	3.506	3.495
yjeF	3.999	5.07
yjhP	3.244	2.84
yjhQ	3.031	2.76
yjjZ	4.297	1.4
ykfG	4.506	2.623
ykfH	3.980	2.501
ykfl	5.350	3.17
ypjJ	4.238	1.506
yqaA	3.314	4.14
yqaB	4.576	3.65

Non-coding regions

Region (nt) **Fold change in gene expression level (\log_2)**

1285750-1285931	4.462
1922994-1923131	3.060
2008514-2008623	3.392
261981-262373	3.170
262374-262436	5.773
262437-262551	4.130
264768-264843	3.393
266000-266191	3.405
2815526-2816982	3.366
4529675-4530072	3.305
4530073-4530333	3.355
4603687-4603826	6.537
497044-497278	3.635
688237-688565	3.485

B)

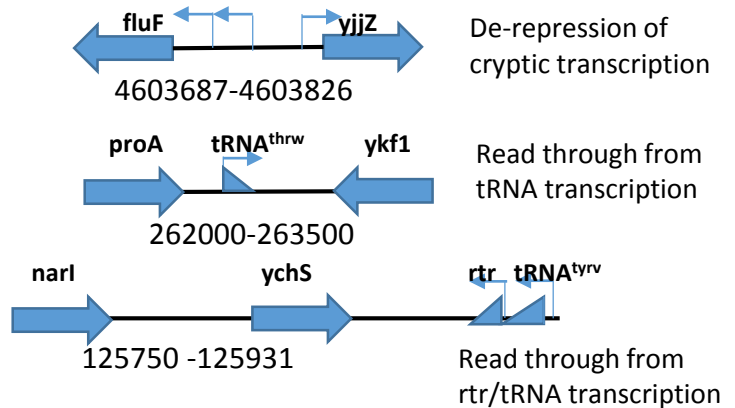
Flanking genes of some of the non-coding regions upregulated by PBS mutants

Figure S7. Genes and non-coding regions those are severely affected by PBS mutants.

A)

ybcK region

GTTGATTGTG	GCCGGTGTGT	TGATTATTAA	TTTATTGTCA	CGAAGCACAC	CACATTAAAA	TAATTTGTTT	C7AAACGACT
AAAATATGGA	GGCTCTTATA	TTTATATGAG	CCTCGTTTTA	TGCTTTTTTGT	TAATGTCTTT	ATTTTTTATG	TATTCTTTTG
G CTTTCAAG	ATTATGGCGT	AAGAAAATTG	CAATACGATT	ATTGTTGTAT	ATTCAAGATA	A TGTGACCTT	AATTGTCTTT
TTAAATAAAA	AATAAACAAA	AATTATATCC	CACCACTAAG	GTTTATAAAA	GCATACGTTA	GCAGGTGTCA	CC ATG AAAAA
AGCCATAGCA	TATATGCGAT	TATCATCACC	AGGTCAGATG	TCTGGCGACT	CATTAAACCG	ACAGAGAAGA	CTTATTGCTG
AATGGTTAAA	GGTAAATAGT	GATTATTATC	TTGATACCAT	AACATATGAA	GATTTAGGAT	TAAGTGCATT	CAAAGGAAAG
CATGCACAAT	CAGGAGCTTT	TTCGGAATTT	TTAGATGCTA	TAGAGCATGG	TTATATATATG	CCAGGAACTA	CATTGTTAGT
TGAAAGTCTG	GACAGACTTT	CAAGAGAAAA	AGTCGGTGAA	GCGATTGAAC	GCTCTGAAAT	GATTTTGAAT	CACGGTATTG
ATGTTATAAC	TCTTTGCGAC	AATACAGTCT	ATAATATTGA	CTCTTTGAAT	GAGCCATATT	CATTAATAAA	AGCCATACTT
ATAGCACAAA	GGGCAAAATGA	AGAAAGCGAG	ATAAAGTCAA	GTCGGGTAA	ATTATCATGG	AAGAAAAAAC	GGCAGGATGC
ACTGGAATCA	GGTACGATTA	TGACGGCGTC	TTGTCCGAGA	TGGCTCTCCT	TAGATGACAA	AAGAACGGCT	TTTGTTCAG
ACCCCGACAG	GGTGAAACT	ATTGAGCTAA	TTTTTAACT	CAGGATGGAA	AGGCGCTCAT	TGAATGCAAT	AGCCAAGTAT
TTAAATGATC	ATGCTGTAAA	GAATTTCTCA	GGAAAAGAAA	GTGCATGGGG	ACCTTCTGTA	ATTGAAAAAT	TATTAGCGAA
TAAAGCTCTG	ATAGGTATTT	CGGTACCTTC	ATATCGTGCA	AGAGGGAAAG	GGATAAGTGA	AATCGCTGGC	TATTATCCCA
GAGTCATATC	AGATGATTTG	TTTACGCTG	TACAGGAAAT	TCGGTTGGCA	CCTTTTGGTA	TTAGCAATAG	TAGCAAGAAT
CCTATGCTAA	TAAATCTACT	TGGAACAGTT	ATGAAGTGTG	AGGCTTGTGG	TAATACCATG	ATTGTTTATG	CGGTATCTGG
AAGTTTGCAT	GGCTATTATG	TTTGTCCGAT	GAGAAGATTA	CATCGATGTG	ACAGGCCATC	AATAAAAAGA	GATTTGGTTG
ATTATAATAT	CATTAATGAA	TTGCTTTTTA	ATTGTAGCAA	AATTCAACCA	GTTGAAAACA	AGAAAGATGC	TAATGAACT
TTAGAGTTAA	AAATTATTGA	GCTTCAGATG	AAAATTAATA	ATTTAATCCG	TGCATTGTCT	GTCGCCCTG	AAGTTACCGC
RS848 TATAGCAGAG	AAAATAAGAC	TATTAGATAA	GGAATTACGA	AGGGCTTCGG	TATCATTGAA	AACTTTGAAG	AGTAAAGGTG
TAAATTCATT	CAGTGATTTT	TATGCTATTG	ACTTAA CC AG	TAAAAATGGA	CGAGAGTTAT	GCCGTACACT	TGCCTATAAA
ACATTCGAAA	AAATCATAAT	TAATACGGAT	AATAAAACCT	GTGATATCTA	TTTTATGAAT	GGCATTGTTT	TTAAACACTA
TCCTTTAATG	AAAGTAATAT	CCGCCAGCA	GGCGATAAGT	GCTCTCAAAT	ATATGGTTGA	TGGTGAGATT	TATTCTTAAA
TAATGATCTC	GGATTTTAA	TTATGCTATG	GTGATAAAGT	GCAAGACAGA	ATTAATTATC	TTTGACGAAA	CTTAATGGGT

 σ^{70} promoter*ybcK*

Figure 8A. Different elements of *ybcK* are indicated. Untranslated region is shown in yellow. Transcription start sites are shown by bent arrows. Primer pair RS848/849 was used for RT-PCR reactions to measure *in vivo* transcriptions described in figure 3E. It is likely that the untranslated region contains the Rho-loading site(s) and the termination occurs inside the gene.

yagN-M region

TTACGAAAAAT	GGCACGAGAA	AATTGAGACA	TGGATCTTAA	ATGAAGCAGG	TATTACCATA	AAAAACAACG	TTGATATGCG
TTGATTCCAT	TAAAAATCAA	CATATTACAA	AATATC ATCA	ACT A TTGATC	AAGATAGATT	TTCATGTATC	GTAATACACA
GTTTAGTCAA	TGATACAGCA	ACTACACAGG	AGATAAGCCA	ATG GCAACCC	CAGCAACTGT	ATCCATAGAA	CCCACTCTGG
CAGCTATCAG	AGCTCGCTGG	TGTATTAATT	CAAGTAAAC	AACTCAATCC	TTTAACGATC	CTGCGTCCAT	GGAAGAGGTT
GTCGAGTATC	TCAAAGGAAC	ATACTCAGCT	CTTCGCAAGT	CTGTGCGATG	CGCCAACTG	AAAATTTTAC	ATCTTAAACA
AAGAAATGCAA	AATGCTACTA	ACTTTCTCGC	GCGTCTGATG	TCATGTAAAA	ATCAGGCATC	CAGATCGCAT	CACAGTACGG
CTAAATCAGC	TAAAAGTGCC	TTATCATCAG	ATTGAGGTGA	TGGTAGTGAC	CCCGACCCCG	AGCCCGAAAC	GTTTCCTTCT
GCCTTCATTA	CTACCCCTAC	TAATTCATAA	ATGCTTAAAG	CTTTCTTTGC	CAATATCTCA	ATCACTGAGG	TGGCAAAATG
A GCGCATTCA	AACTCCCGGA	TACATCTCAA	TCACAGTCA	TTTCAACAGC	TGAGTTAGT	AAAATCATA	GCTACAAATC
TCAAACCAT	CGTAAATGGC	TTGTGTCAGG	CAAATTGCCT	GAGGGCTAC	CTCGCCAAA	ACAAATCAAT	GGCCGCCATT
ACTGTTTACG	TAAAGATGTC	CTCGATTTTA	TAGATACATT	TTCTGTACGA	GAAAGTCTGT	AATAAATTAC	AGATTTAATT
TTATTGATTT	ATAGCGATGT	TGCCCCGAGA	AAAATGGGGC	AACACTGAGA	AATTTGAGAT	AGTAGTTTTA	TATTGAGATA
ACAAAGAGGT	TTCTTAAAA	ATG TCTAATA	GTGTTACTAA	TTTTGAGATG	AGCAGCGTTC	TACCAGGAAA	AAAACCTTGT
CA AGCAAAA	ACA ATGAGTC	AC AGGTA AG TA	CAGACTACTC	CCATAAAAA	ACACTCAGTC	ACGTTCAAAA	ATCAATCTTC
ATTAGGAGTA	ATTGATCATT	ATGCCAGACT	AACAAATAAA	TCTCACTCTT	CCGTAATAGC	GGAAGTTGTG	GATTTGGCTA
TCCCTATATT	AGAAAAATGC	AATCGTCATA	ACTGGTCAAT	AAATGAAATA	AAAAATGACC	TGTTAAAGTT	CTCTATAAAA
GAAAGCATCA	ATCGAAGCCG	AGGTAAAACA	GAAGTAACTC	TGGAAGAGTA	CTGTTCTGTTA	ATCTGGAAAA	CGAACATCAT
GAGTCCATTA	AAAATCCCCA	TTGCAGATTA	CTTTCAACTG	AACGGTAATG	ATGAATTCAT	GGGGAAAGAT	GAAAAACAGC
TTATACGTGA	AAGGCTATCC	TCGTAAGGG	AAAATTACGA	TATGGAAAA	GCCATTTACA	TTTACAATCA	AAGACATTTT
GATGTAAAGC	ATCAAAAGTGT	CTCAGGATAT	TCAAACATTA	TTCTTATTTCA	TAGAACAACC	TTTGAGGGTT	ATTACTTTGA
TGCCGGGACG	GCCTACTCT	TGTCAACATC	CCAATTGATT	ATATTCGGGA	TAAATGAAGT	TCTTAGAAGA	AAGGGGATTG
TTATGCCTTA	TCCGGTTGTT	TGTT G ATTG	ATATTTACCA	TGTCAATGAA	ATGGTGGTTA	TGCTGCCAGT	GCTCCGCAAA
ACAGATGTTT	CCAACCGTGT	TAATGTACCG	GATGACATCA	TTATAAACC	ATACT C ACAA	GAGAGCAGAA	CCTAA RS847

 σ^{70} promoter*yagN*

Intergenic region

yagM

Figure 8B. Different elements of *yagN-M* operons are indicated. Untranslated region is shown in yellow. transcription start sites are shown by bent arrows. Primer pairs RS1083/1084 and RS846/847 were used to make DNA templates for *in vitro* transcription by PCR and for qPCR reactions, respectively.

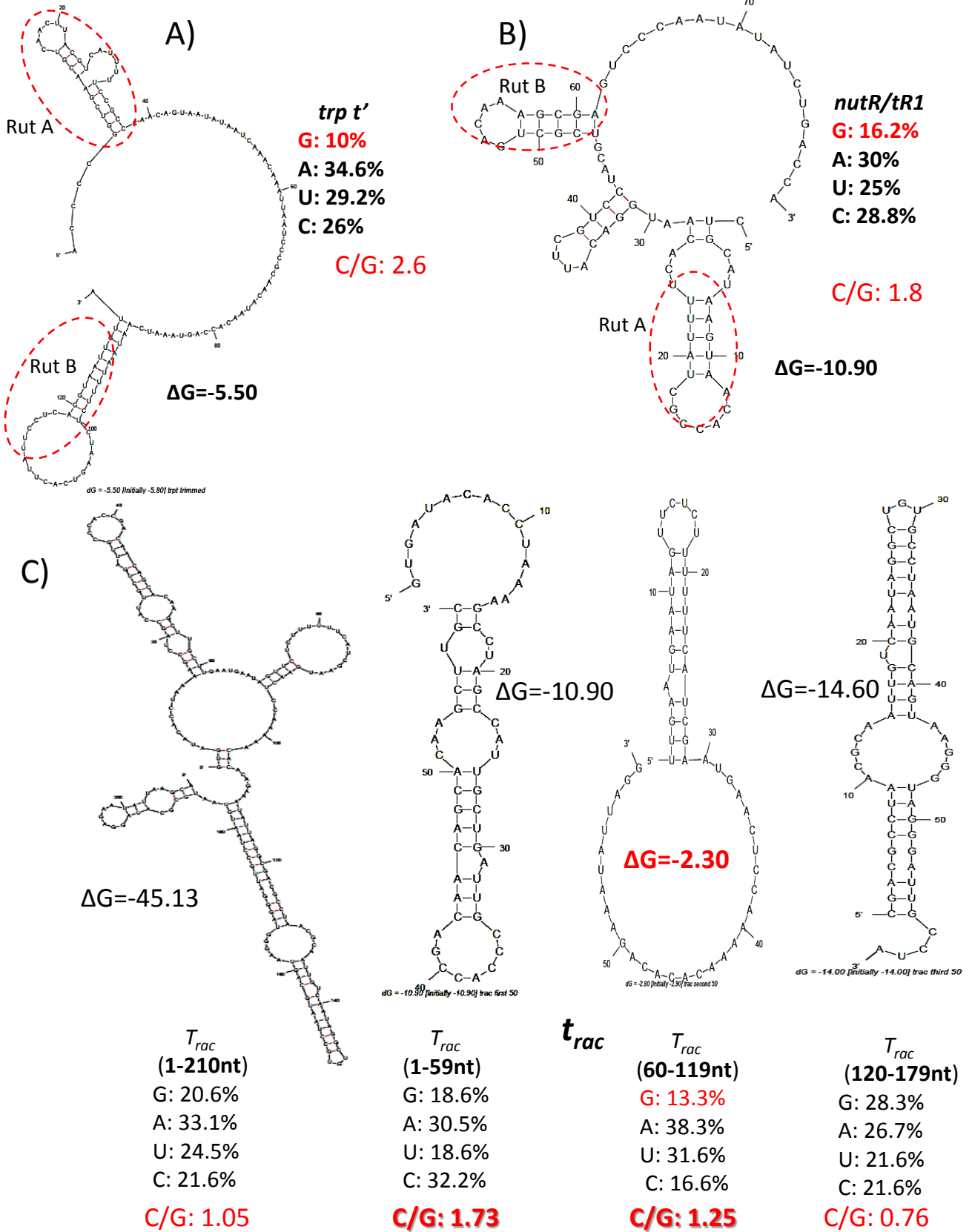


Figure S9: The predicted secondary RNA structures of rut site regions of different terminators determined by the M-fold program. The terminators *trp t'* and *tR1* are shown in A) and B). Rut sites are indicated by dotted ovals. Compositions of the bases in each of these regions are indicated together with the free energy (ΔG) of the secondary structure formation. C) The 210 nt untranslated region preceding the *racR* region is folded as a whole and part wise. The part, 60-119nt has the lowest ΔG and also is G-poor. The region 1-59 nt has also G-poor sequence. And hence, these regions of the untranslated part of *t_{rac}* would have the Rho-loading sites.

M-Fold plots of 151nt *ybcK* untranslated region

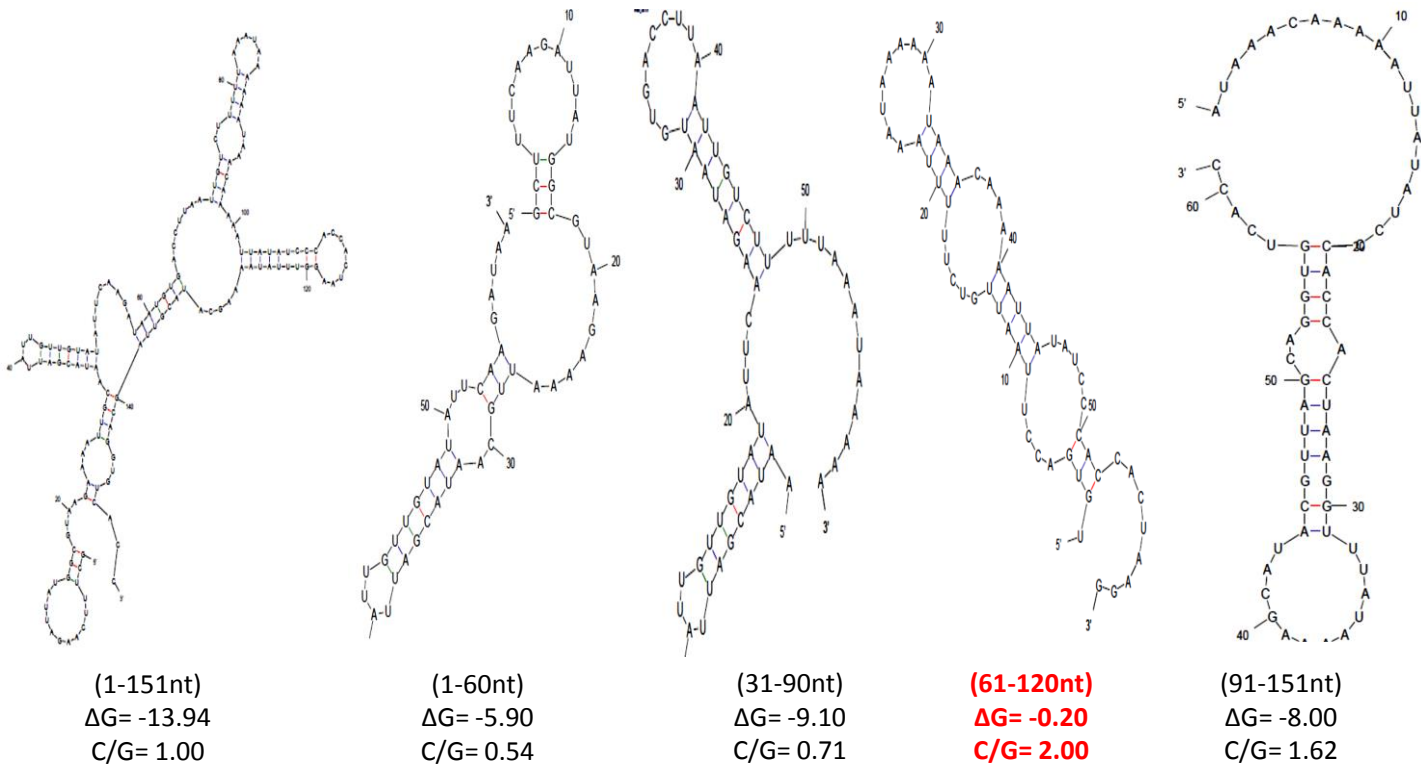


Figure 10A. The region (in red), 61-120nt, has the lowest free energy of secondary structure formation and high C/G ratio, and hence is likely to have Rho-loading site(s).

M-Fold plots of 87nt untranslated region of *yagN-M* operon

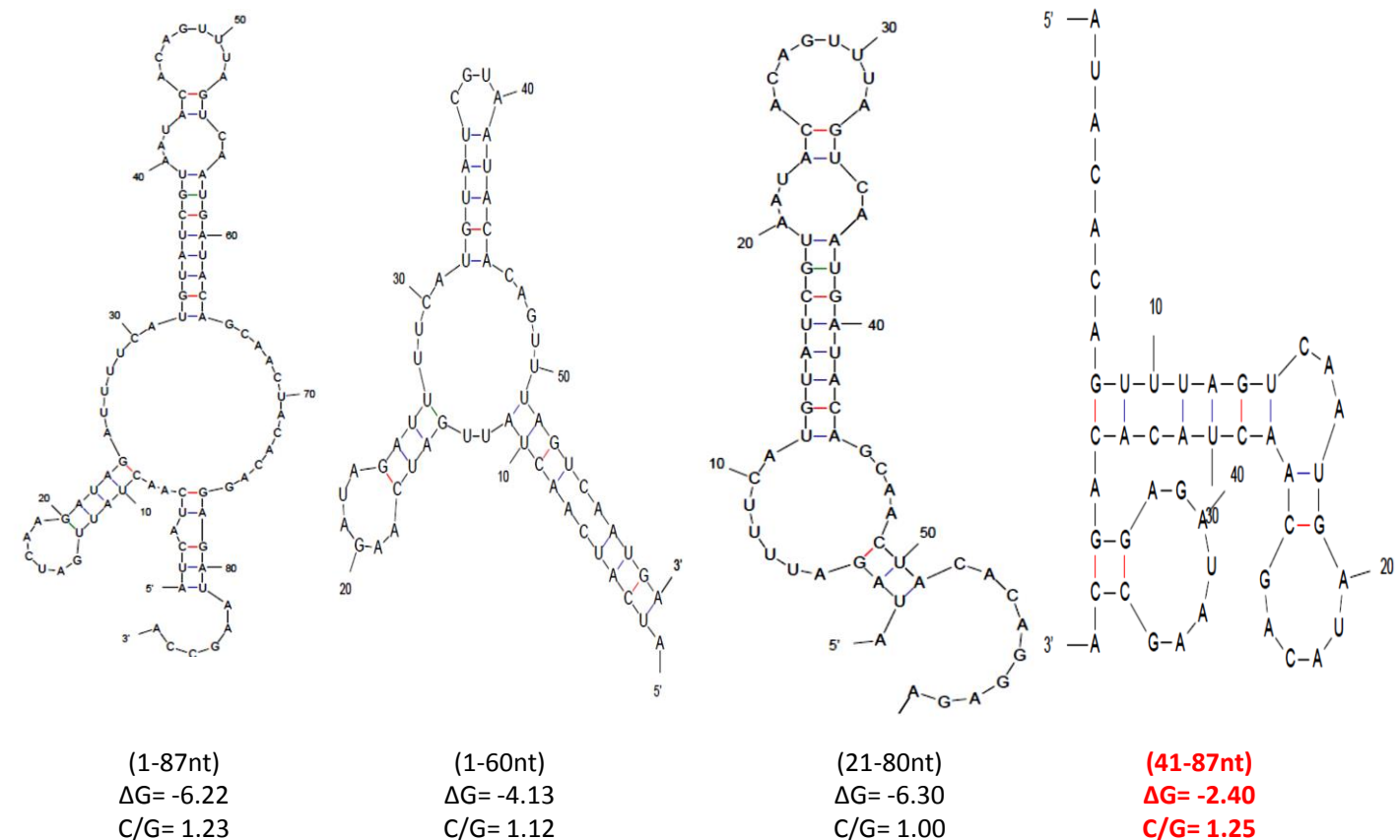


Figure 10B. The region (in red), 41-87nt, has the lowest free energy of secondary structure formation and high C/G ratio, and hence is likely to have Rho-loading site(s).

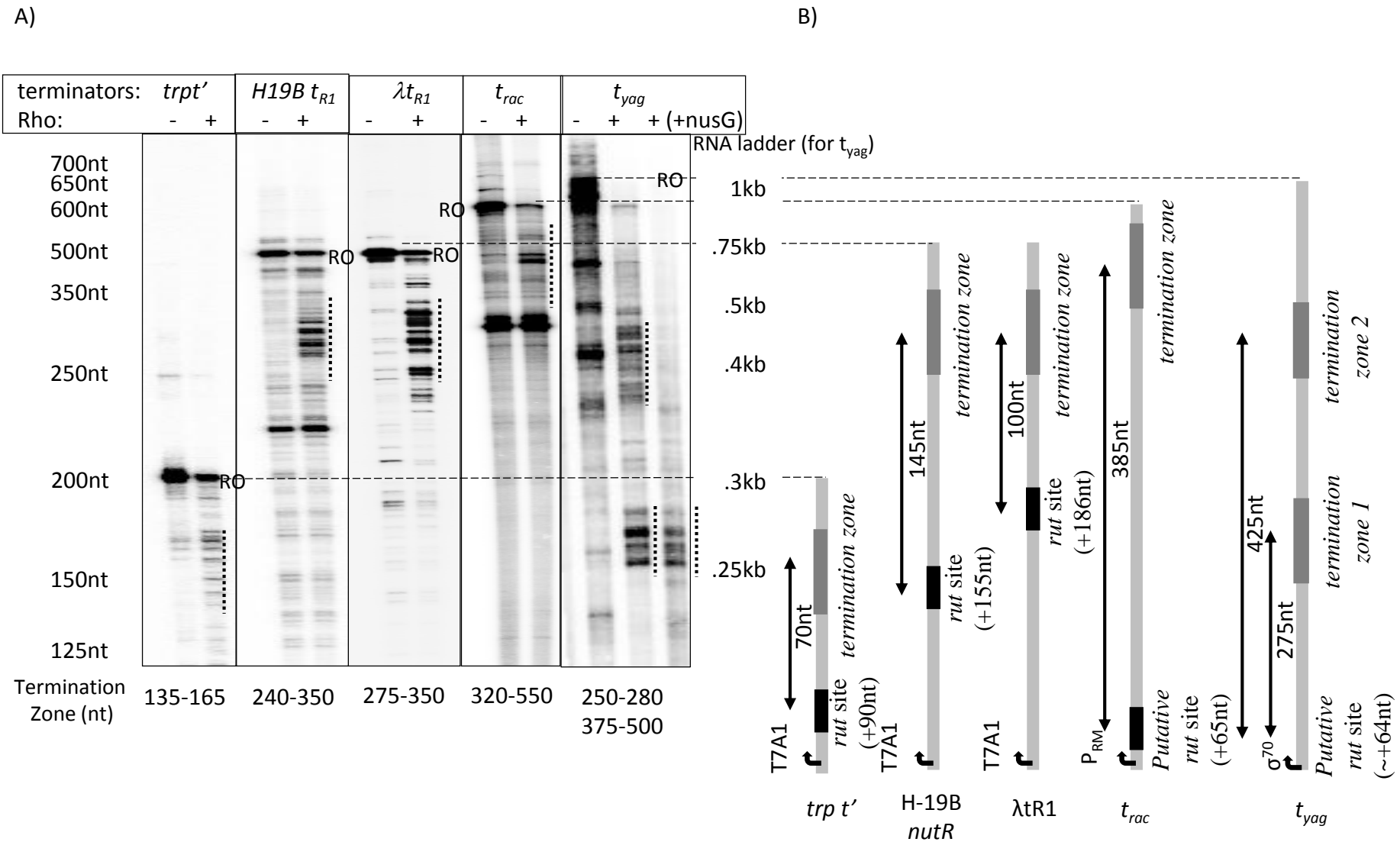


Figure S11: A) *In vitro* termination assays on different terminators. Termination zones for each are indicated by dashed vertical lines, and their mean distances from the start site are indicated below the autoradiograms. *t_{yag}* has two termination zones. Last lane of this panel is in the presence of NusG. Size of the transcripts of the *t_{yag}* panel aligns to the RNA ladder. It should be noted that like *t_{rac}*, *t_{yag}* is also highly NusG-dependent, as indicated from the early termination bands. **B)** Cartoons showing the mean distances between the *rut* site and the termination zones for different terminators. The positions of *rut* sites and the termination zones are w.r.t. the transcription start site. DNA template ends are roughly aligned with the position of the run-off (RO).

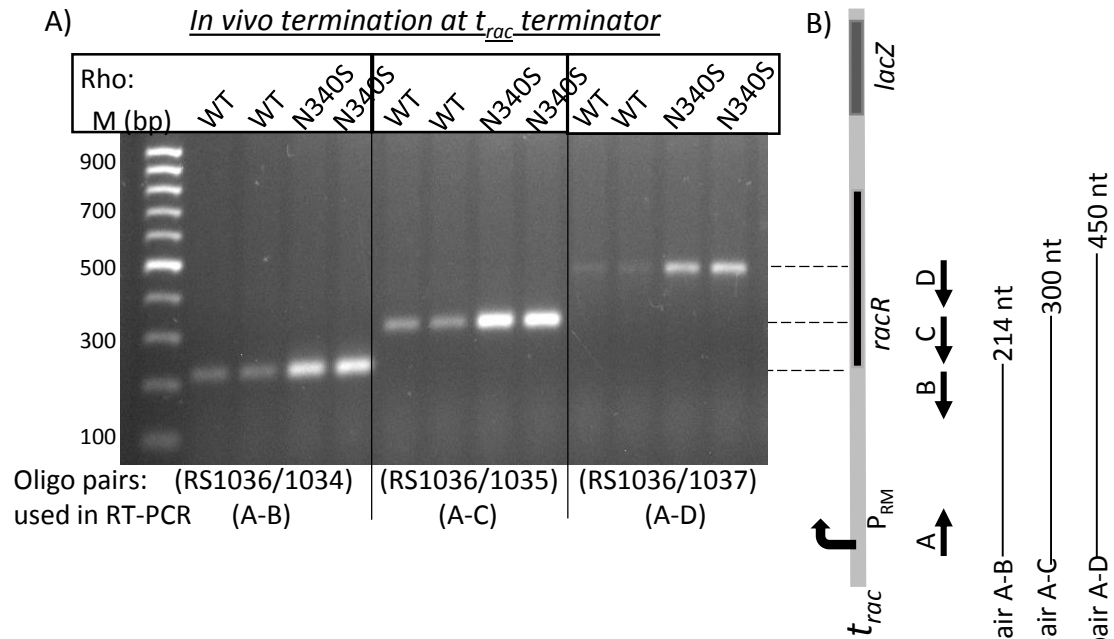


Figure S12. A) RT-PCR products of the total RNA obtained from the strains expressing either WT or N340S Rho mutant (RS1428). Different oligo-pairs, as indicated, were used to probe the terminated RNA products initiated from P_{RM} promoter. Two reactions were loaded for each case. These cDNAs were from terminated RNA because the amount of the product is much higher in the presence of N340S mutant. **B)** Cartoon showing the positions of various oligos used and the lengths of cDNA products aligned to different regions of *racR* region. This indicates that the major *in vivo* termination events occurred inside the *racR* region.

Table S1: List of oligos used.

Oligos	Description
RS83	ATAAACTGCCAGGAATTGGGGATC; upstream oligo of pTL61T vector sequence, 5' biotinylated
RS139	TTAATACGACTCACTATAGGGAGATCGAGAGGGACACGGGCG; T7 promoter fused to the start site of T7A1 promoter
RS147	GCGCGCGGATCCCCCATTCAAGAACAGCAAGCAGC; lambda <i>TR1</i> reverse primer with BamHI site.
RS401	GCGCGCGTGGTGCAACGGGCGCTGGG; <i>lacZ</i> specific FP for qPCR
Rs402	GCGCGCCAACCTCGCCGCACATCTG; <i>lacZ</i> specific RP for qPCR
RS404	GAATTGTGAGCGCTCACAATTTCGGATGCCAGACCGCGCTGGGTAAGC G; RP with 5'-lacO fusion, used to generate roadblock downstream of rut sites of for <i>T7A1-H19B TR1</i> template
RS555	CTG CTT CAG GAT GGC AAA AAT AAT GTC C ; internal RP (123-96bp) of <i>rho</i> gene used for checking rho deletion by PCR
RS845	GAATTGTGAGCGCTCACAATTCTTAGCGCCTCGGGCTGGCTA; RP with 5'-lacO fusion used to generate roadblock on $P_{RM-racR/t_{rac}}$ template
RS846	CCTTATCCGTTGTTTGTG; <i>yagM</i> specific FP for qPCR
RS847	TTAGGTTCTGCTCTCTTGTG; <i>yagM</i> specific RP for qPCR
RS848	CCTGAAGTTACCGCTATAGC; <i>ybcK</i> specific FP for qPCR
RS849	CTCTCGTCCATTTTACTGG; <i>ybcK</i> specific RP for qPCR
RS852	AATCGCAAAGCAATCTTGGT; <i>gshA</i> specific FP for qPCR
RS853	GCAACACGTTGCTGTTGATT; <i>gshA</i> specific RP for qPCR
RS854	CTGGTAACAAACCAGAGTGG; <i>rpoC</i> specific FP for qPCR
RS855	TCAGACGGTTGTTACGGTTA; <i>rpoC</i> specific RP for qPCR
RS955	GAATTGTGAGCGCTCACAATTCTTAGGTATTCGTTAAGCCCC; RP with 5'-lacO fusion, used to generate roadblock on $P_{RM-racR/t_{rac}}$ template
RS956	GAATTGTGAGCGCTCACAATTCTTAGGGCGCGAACATCGTGG; RP with 5'-lacO fusion, used to generate roadblock on $P_{RM-racR/t_{rac}}$ template.
RS963	AACAATGTCGACTTAACCTGAACGACGACGATTAC; <i>rhlB</i> specific primer for checking <i>rho</i> deletion by PCR
RS995	TTAATACGACTCACTATAGGGAGAGTGATACACCTAAAAGCCTAGCC; T7 promoter fused to the start site of P_{RM} promoter.
RS1034	CATTGCTTAATATTCTCCTATGCGC; RP for RNA preparation on RS1352
RS1035	TGCGACCTCCGCCTTTGATTTGACGG; RP for RNA preparation on RS1352
RS1036	GAGGTGATACACCTAAAAGCCTAGCC; FP at transcription start site of T7A1 of pRS1352
RS1037	TTGGTATGGGGTATTCGTTAAGCCCC; RP at <i>racR</i> gene of pRS1352.
RSRK-1	GTTTTCCAGTCACGAC; reverse primer in <i>lacZ</i> gene.
RK23B	TGGAGTTCAGACGATACG; reverse oligo to generate <i>T7A1-H19B/TR1</i> terminator template
RS1083	GGCACGAGAAAATTGAGACATGG; FP in <i>yagN</i> region, to be used to prepare t_{yag} terminator template
RS1084	CTACCTGTGACTCATTGTTTTTGCC; RP in <i>yagM</i> region, to be used to prepare t_{yag} terminator template

Table S2: β -galactosidase activities of different lacZ fusions under different conditions.

<i>NusG</i> <i>alleles</i>	<i>T_{RI}-LacZ</i> <i>β-galactosidase</i> <i>activity</i>	<i>T_{RI-trpt'}-LacZ</i> <i>β-galactosidase</i> <i>activity</i>	<i>T_{rac}-LacZ</i> <i>β-galactosidase</i> <i>activity</i>
WT	259.4 \pm 5	20.2 \pm 0.08	61 \pm 0.5
V160N	634.3 \pm 60	34 \pm 0.8	2421 \pm 46
G146D	461.3 \pm 9	49 \pm 0.9	1865 \pm 64
L158Q	460 \pm 10	48 \pm 2	2766 \pm 75