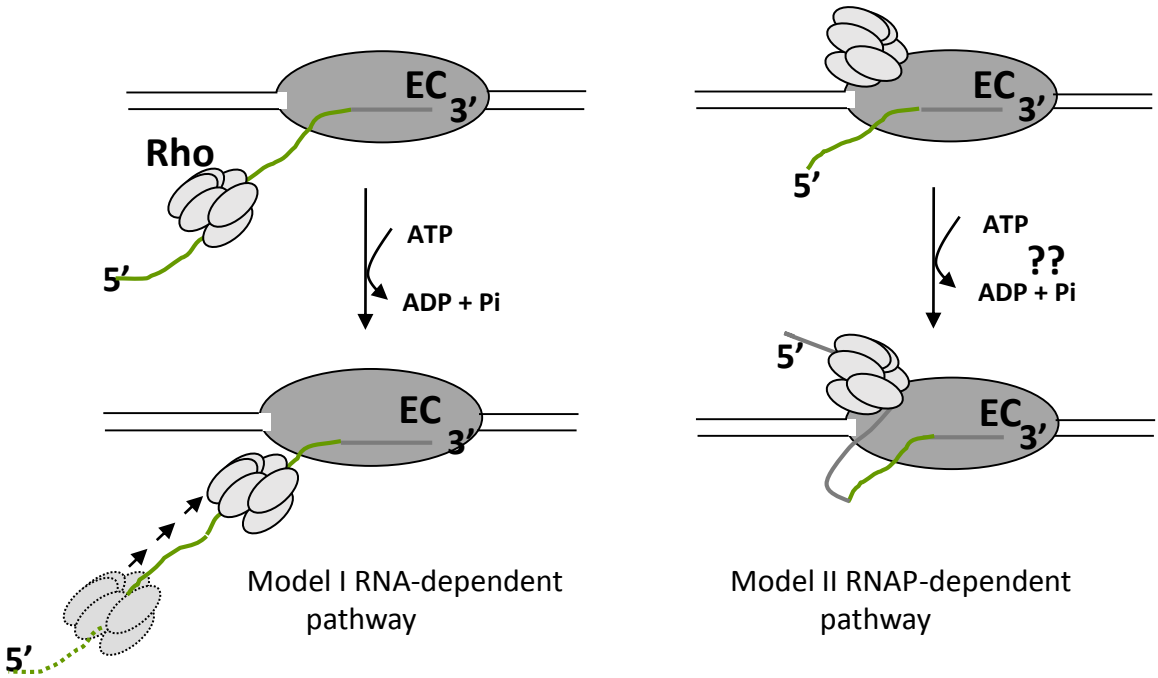
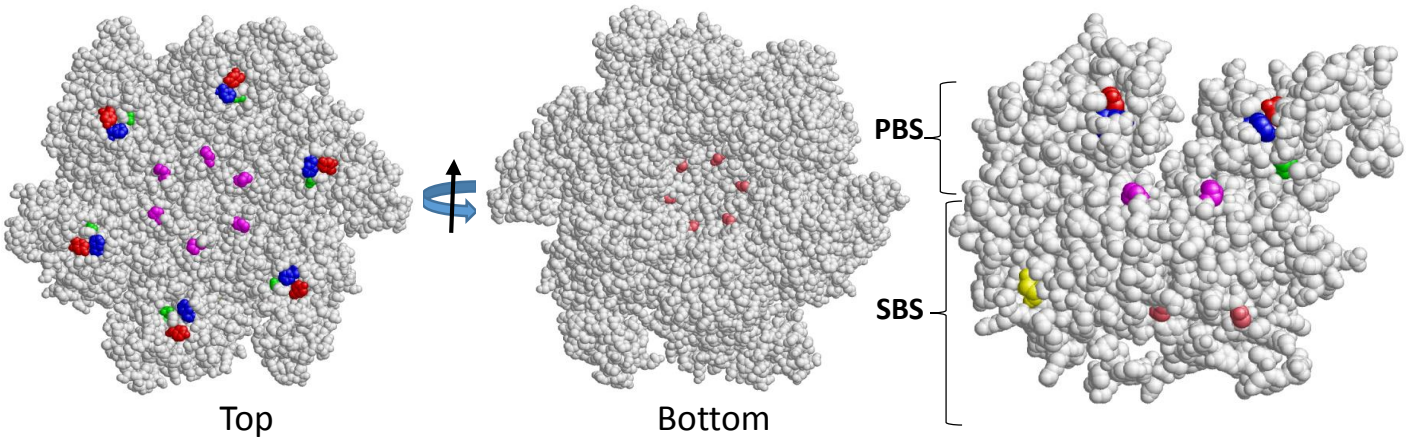


Figure S1:

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 coupled[1].

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].

A)



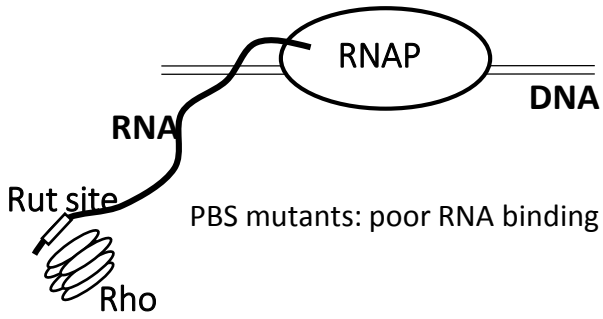
PBS mutants

R66G-Green
F62S-Blue
Y80C- Red

SBS mutants

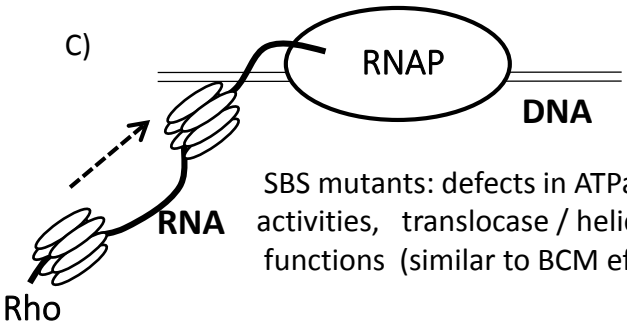
N340S- Yellow
G324D-Pink
P279S- Magenta

B)



PBS mutants: poor RNA binding

C)



SBS mutants: defects in ATPase activities, translocase / helicase functions (similar to BCM effect)

D)

Binding affinity of the PBS Mutants

Rho	K_d
Wild type	10±0.9 $(dC)_{34}$ nM[11]
Y80C	>3000 nM[11] (polyC)
Wild Type	5±1.6 $(rC)_{10}$ μM[16]
F62S	Too low, no binding[16]

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

RS83 →

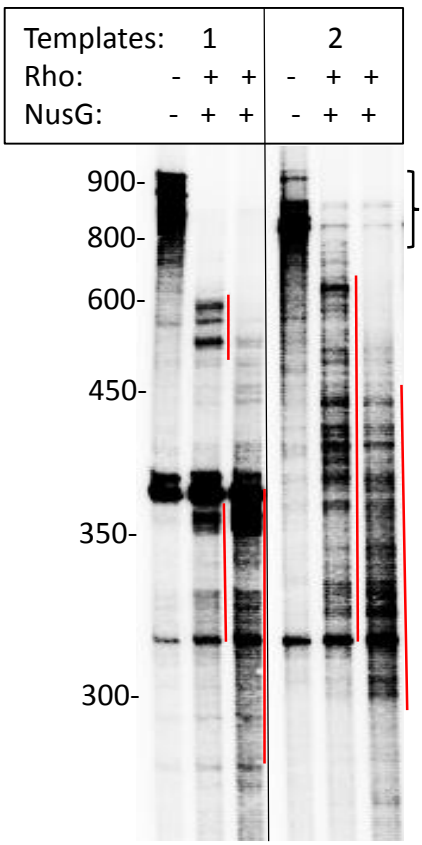
CCATAAACTG CCAGGAATTG GGGATCG***GAA*** ***TTC***GCTTCAC TGACATATTC TGCGAACAAC ATGCCGAACG } P_{RM}
TCGTAAATAT GACCAGTCAA TATCAGGACGAAG TTCTTCGCAC AGAACCTCAC CTCTTGTTGC ACGTTCAATT
GCTGGACATC TCTCGGCAGG CAA***TTGACG***T ACCCCTTTGA TCCATTGATT ***TACGCTT***GGA GGTGATACAC
CTAAAAGCCT AGCCATTGCT GATTGCCCAC CGACAACAGC ACAAGCTTGC TTGAATGAAT AGTTCTCTTT ***Untranslated***
TTTCATCGAA TGAACTCCAA AAACACACAG AAATATTAGG CGACGCCTAA CGCAATTGTC AATAGGCTGT ***region***
GCCTAATGCA GTAAGGGTAG GGATTGCCTA ATGTAATGCG CATAGGAGAA TATTAAGCA ***ATG***CTTAGTG
GTAAAGACTT AGGCCGAGCG ATAGAGCAGG CCATTAACAA AAAATCGCA TCGGGATCCG TCAAATCAAA
GGCGGAGGTC GCACGCCACT TTAAAGTCCA ACCACCATCA ATTTATGACT GGATTAAGAA AGGCTCTATA
AGTAAAGATA AACTTCCAGA ATTATGGCGT TTCTTTTCTG ATGTTGTTGG TCCAGAGCAT TGGGGGCTTA ***racR***
ACGAATACCC CATACCAACC CCCACCAATT CAGATACAAA AAGTGAACTT TTAGATATAA ACAACCTTTA
TCAAGCAGCC TCTGATGAAA TAAGAGCGAT TGTAGCTTTC CTGTTATCTG GAAATGCTAC AGAACCAGAT
TGGGTTGACC ACGATGTTCG CGCCTACATA GCAGCGATGG AAATGAAAGT GGGTAAGTAT CTGAAAGCTC
TTGAATCTGA ACGGAAAAGC CAGAACATCA CAAAAACTGG AACTTAA

RS955 ←
RS956 ←

In vitro termination region

ACTTATATGG TCTGACGGAA AACTCCTGGA TTCCGTTATT TAACCCCCCC ATCACTTTCT GCTGTCGCCA
TCACCTATTA GGTTACGCTC AAAACATTAG GCATAGCCTA TTGACAATCA ATTAGGCATT ACCTATAGTT ***Intragenic***
CCAGCATACC ACCCACCCCG CCCCACAGAA CGCCGGGCAA TACTTCGAGT TACCAGGCAG TGGTAAGGGG ***region***
TTAAGTAGCC AGCCCGAGGC GTATGAACAT GACGGCGGGA TTCAAATTTT GCAGTGCAGC AGTTAGTTCC
GCCACCCGGCGTTAAGGGGA*GAGATAAG****GGATCC***
TAACTAACTAGCGATCCCGACTCACTATAGAGGGACAAACTCAAGGTCATTCGCAAGAGTGGCCTTTATGATTGACC } ***LacZ***
TTCTTCCGGTTAATACGACCGGGATCGAGATCCTAGGTAGGTAGGGGCGCGGCATTTTAACTTTCTTTATCACACAG
GA AACAGCTATG ACCATGATTA CGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAAC

RS845 ←
RS-RK1 ←



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).

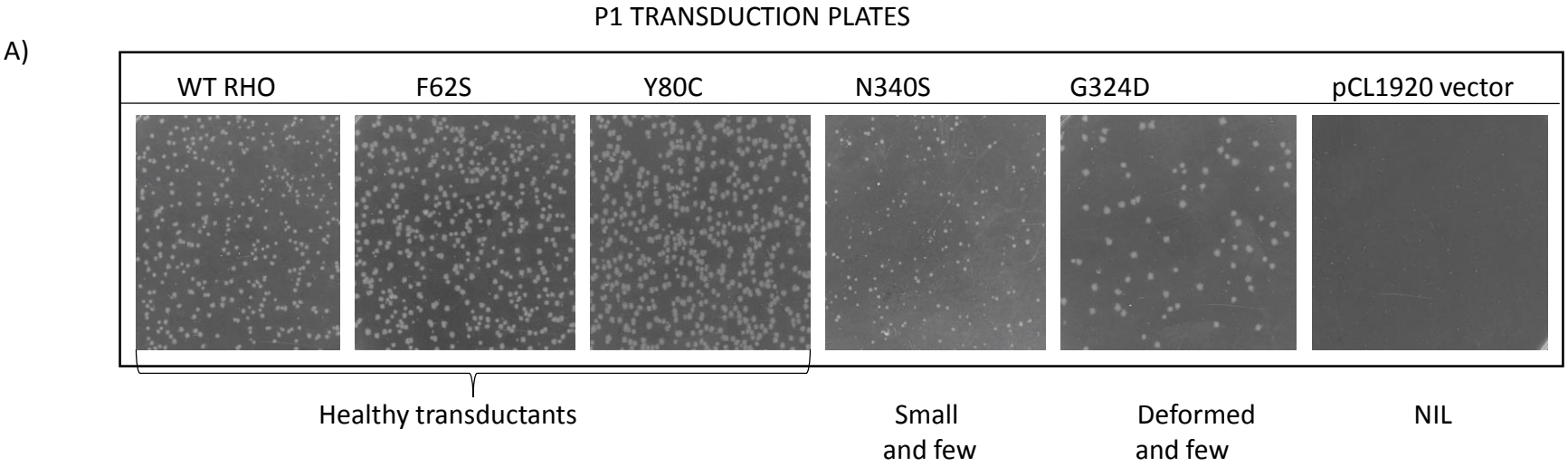


Figure S4A: *MG1655 rac*$^{+}$ strains were at first transformed with pCL1920 plasmids expressing WT and different derivatives of *rho* mutants as indicated on the top of the pictures. Resultant strains were P1 transduced with a *rho:kan*^{R} cassette to delete chromosomal *rho*. The pictures showed 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^{o}C.

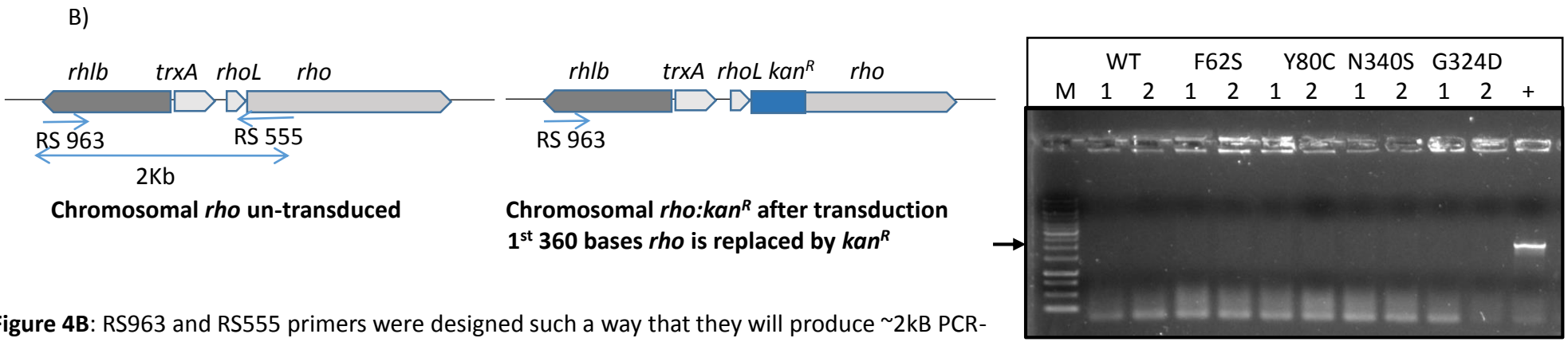


Figure 4B: RS963 and RS555 primers were designed such a way that they will produce ~2kB PCR-product only if an intact *rho* is present in the chromosome, and thereby will detect the occurrence of the duplication of *rho.* This primer pair will not produce any PCR product neither from *kan*^{R} *:rho* nor from the pCL1920 plasmids expressing WT or mutant *rho*s, 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.

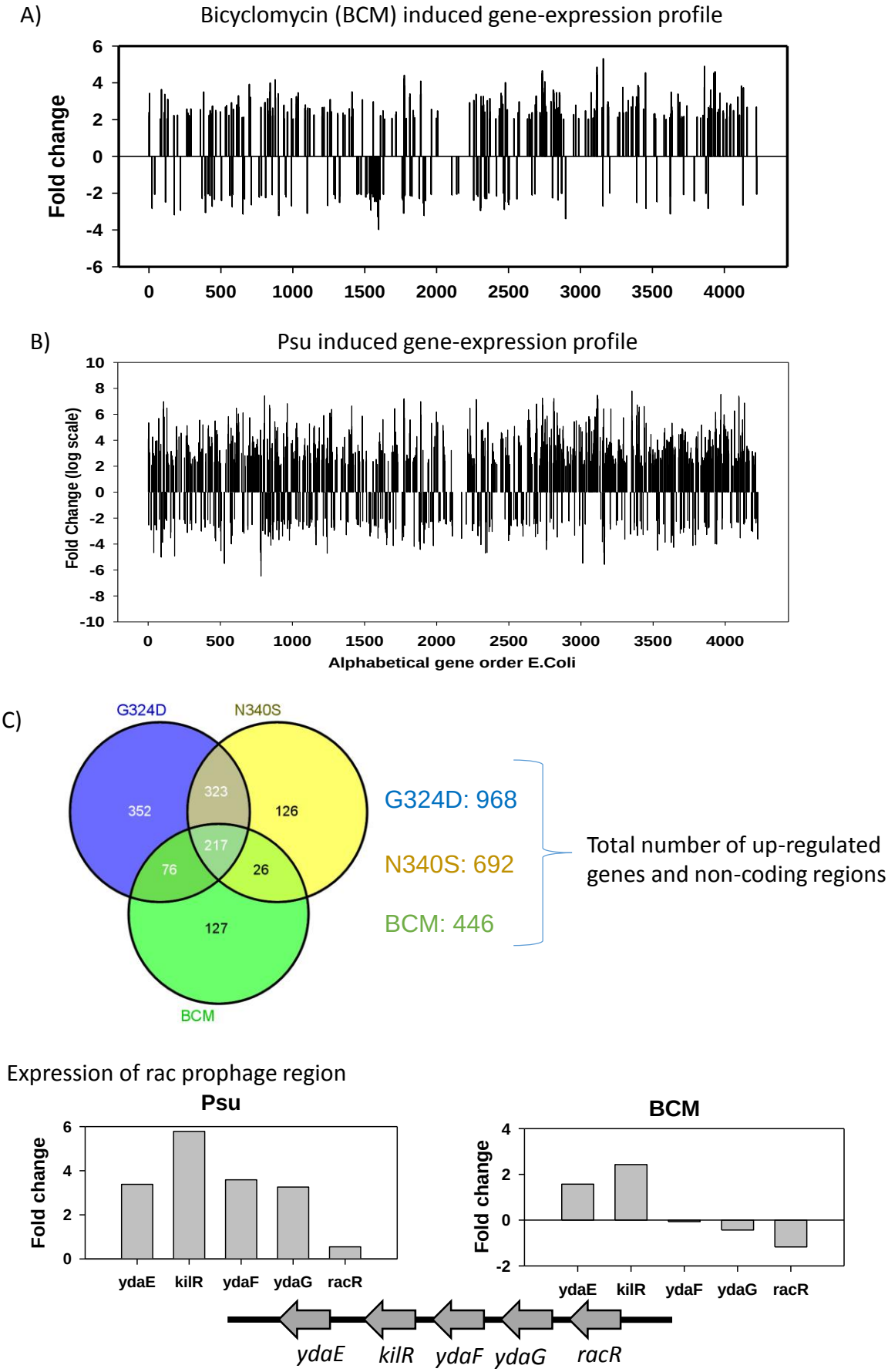


Figure S5. Microarray profiles of genome wide expression patterns of MG1655 treated with A) BCM and B) due to expression of Psu. Psu is expressed from a plasmid and has a drastic effect compared to SBS mutants or BCM. C) Venn diagram showing the overlaps between up-regulated genes of Rho mutants and 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)	
	PBS mutants	SBS mutants
aaeA	0.8560	3.7106
aaeB	1.9583	3.7579
aaeX	0.6806	4.5353
allB	0.2170	3.0600
aqpZ	-0.1951	3.8572
aslB	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** CP4-6
yagM	**1.4124**	**3.2965**
ybcK	**0.1373**	**4.4540**
ybcL	**-0.0450**	**3.7694** DLP12
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** Qin
ydhS	2.6417	7.0625
ydiO	0.1592	3.1277
yeeR	**2.7713**	**3.8542**
yeeS	**2.7728**	**3.2037** CP-44
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

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
yjgZ	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)	
	PBS mutants	SBS mutants
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			*Non-coding regions*	
Name	*Fold change in gene expression level (log_2)*		*Region (nt)*	*Fold change in gene expression level (log_2)*
	PBS	SBS		
cysA	3.402	3.89	1285750-1285931	4.462
cysC	4.516	2.126	1922994-1923131	3.060
cysD	4.457	3.162	2008514-2008623	3.392
cysH	4.624	3.837	261981-262373	3.170
cysI	4.878	3.961	262374-262436	5.773
cysJ	4.361	3.409	262437-262551	4.130
cysN	3.613	2.83	264768-264843	3.393
cysP	4.102	2.44	266000-266191	3.405
cysU	4.123	2.87	2815526-2816982	3.366
cysW	3.300	2.77	4529675-4530072	3.305
fhuF	3.555	4.421	4530073-4530333	3.355
gfcB	3.333	4.536	4603687-4603826	6.537
gfcD	3.012	5.182	497044-497278	3.635
pgaB	3.287	3.417	688237-688565	3.485
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		
ykfI	5.350	3.17		
ypjJ	4.238	1.506		
yqaA	3.314	4.14		
yqaB	4.576	3.65		

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

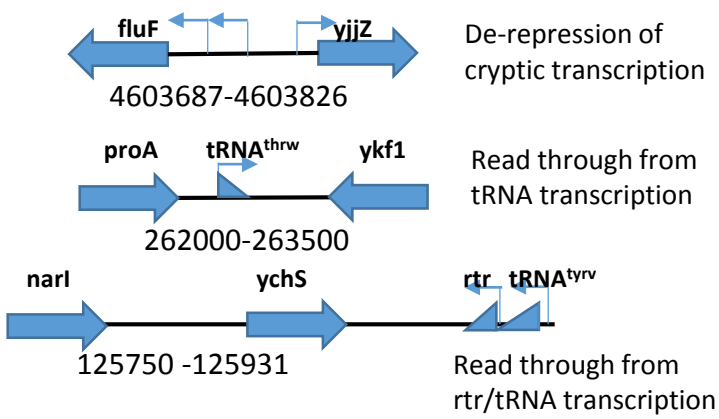


Figure S7. Genes and non-coding regions those are severely affected by PBS mutants.

A)

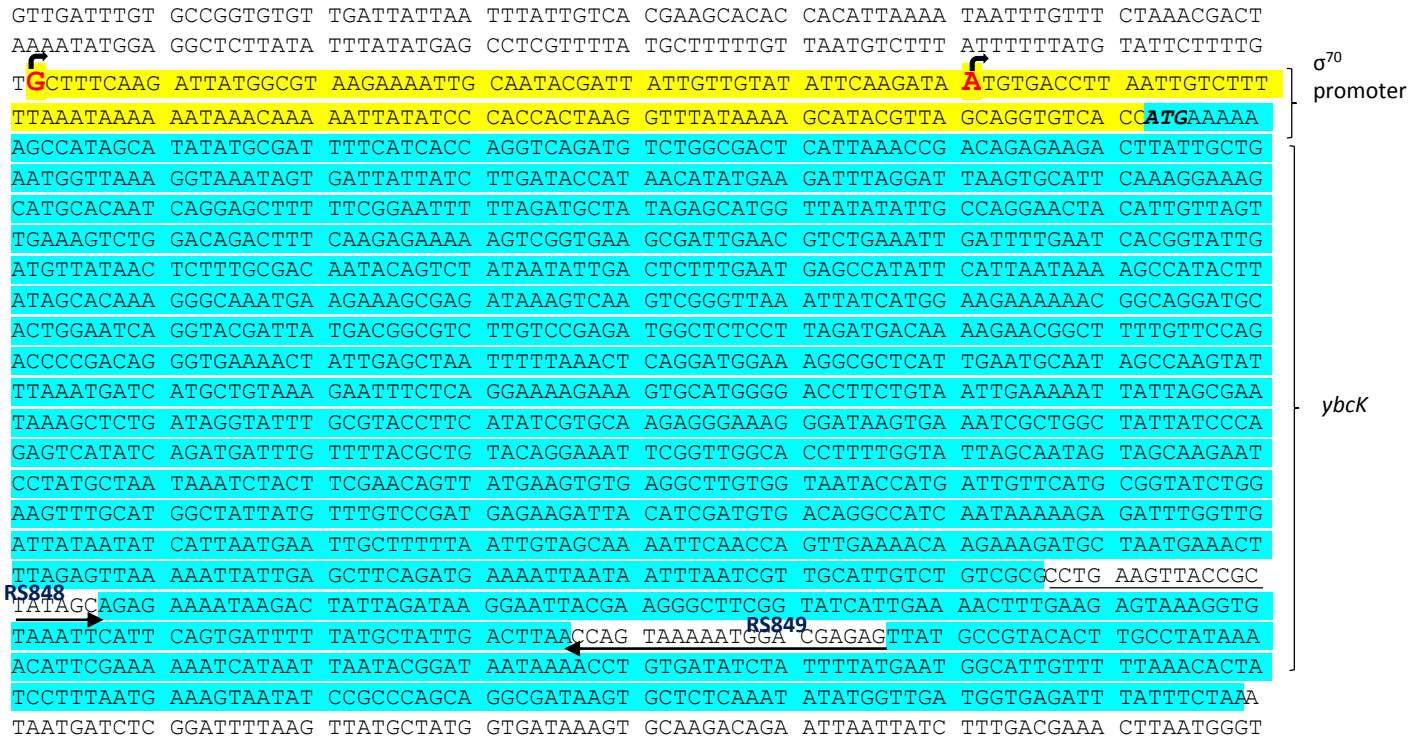


Figure 8A. Different elements of *ybcK* are indicated. Untranslated region is shown in yellow. Transcription start sites are shown by bent arrows. Primer pair RS848/849 was used for RT-PCR reactions to measure *in vivo* transcriptions described in figure 3E. It is likely that the untranslated region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

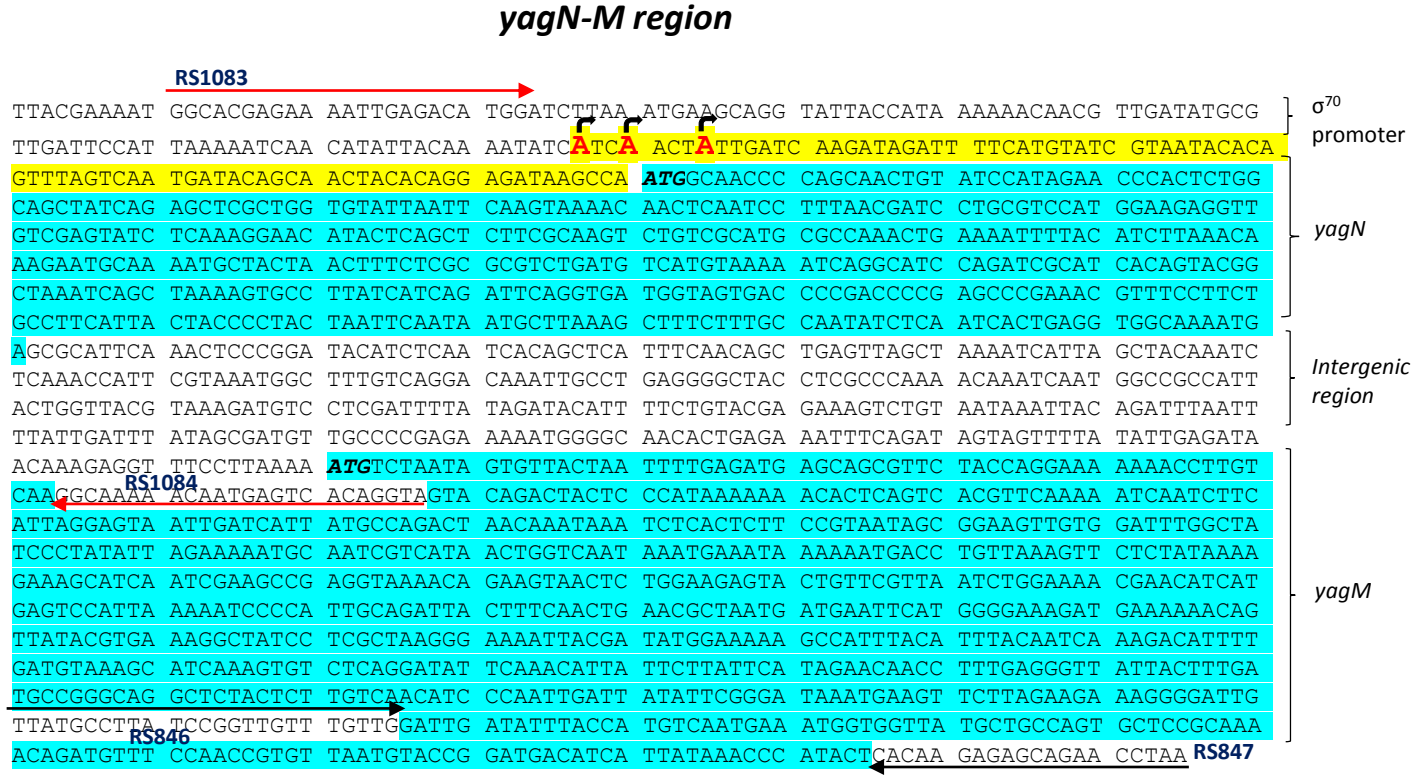


Figure 8B. Different elements of yagN-M operons are indicated. Untranslated region is shown in yellow. transcription start sites are shown by bent arrows. Primer pairs RS1083/1084 and RS846/847 were used to make DNA templates for *in vitro* transcription by PCR and for qPCR reactions, respectively.

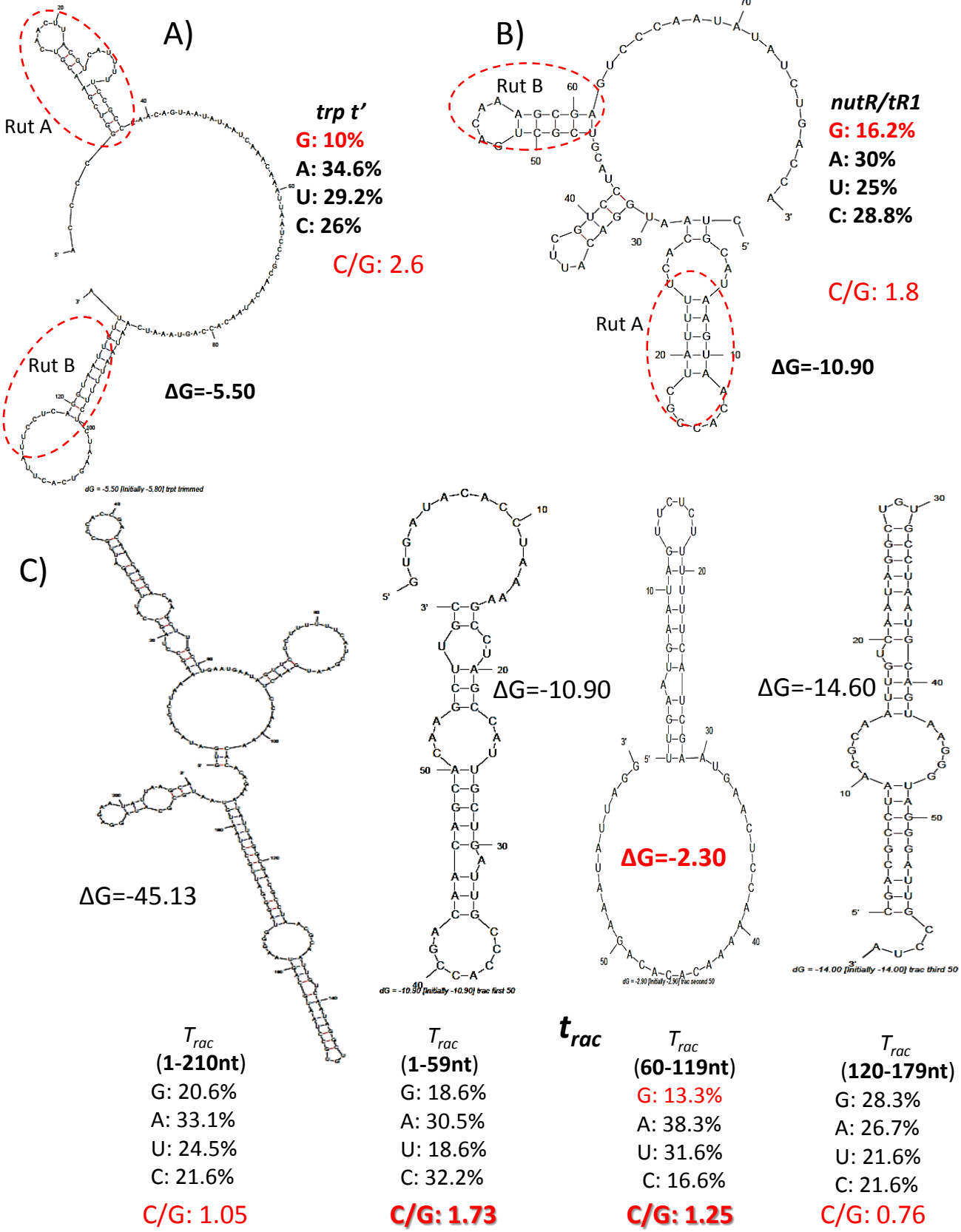
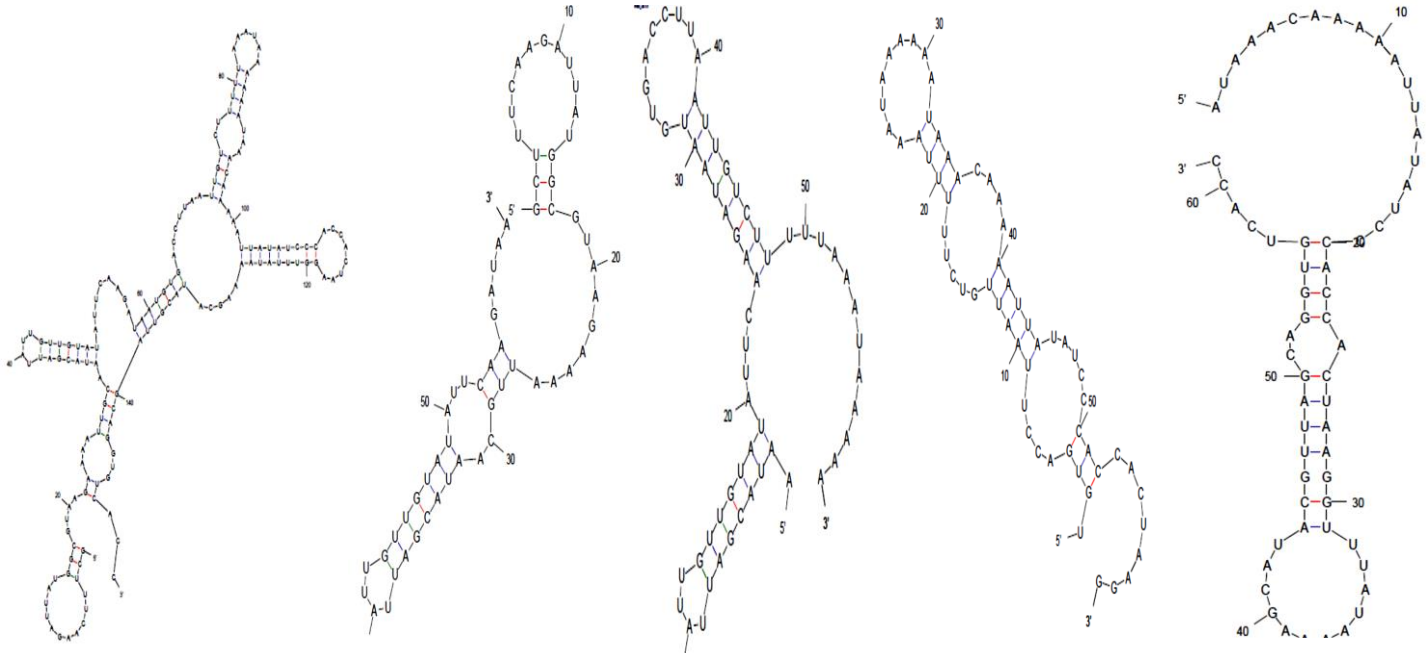


Figure S9: The predicted secondary RNA structures of rut site regions of different terminators determined by the M-fold program. The terminators *trpt'* and *tR1* are shown in A) and B). Rut sites are indicated by dotted ovals. Compositions of the bases in each of these regions are indicated together 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.

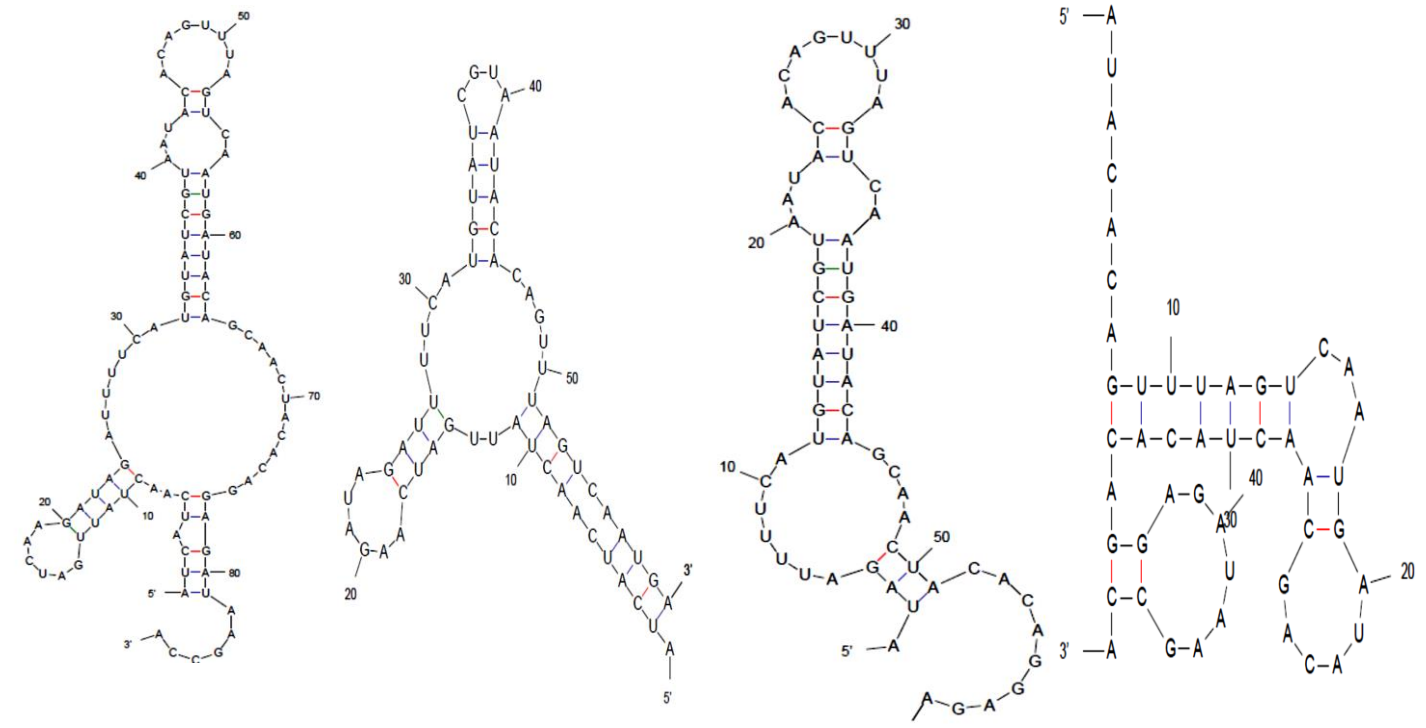
M-Fold plots of 151nt ybcK untranslated region



(1-151nt)	(1-60nt)	(31-90nt)	**(61-120nt)**	(91-151nt)
ΔG= -13.94	ΔG= -5.90	ΔG= -9.10	**ΔG= -0.20**	ΔG= -8.00
C/G= 1.00	C/G= 0.54	C/G= 0.71	**C/G= 2.00**	C/G= 1.62

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



(1-87nt)	(1-60nt)	(21-80nt)	**(41-87nt)**
ΔG= -6.22	ΔG= -4.13	ΔG= -6.30	**ΔG= -2.40**
C/G= 1.23	C/G= 1.12	C/G= 1.00	**C/G= 1.25**

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).

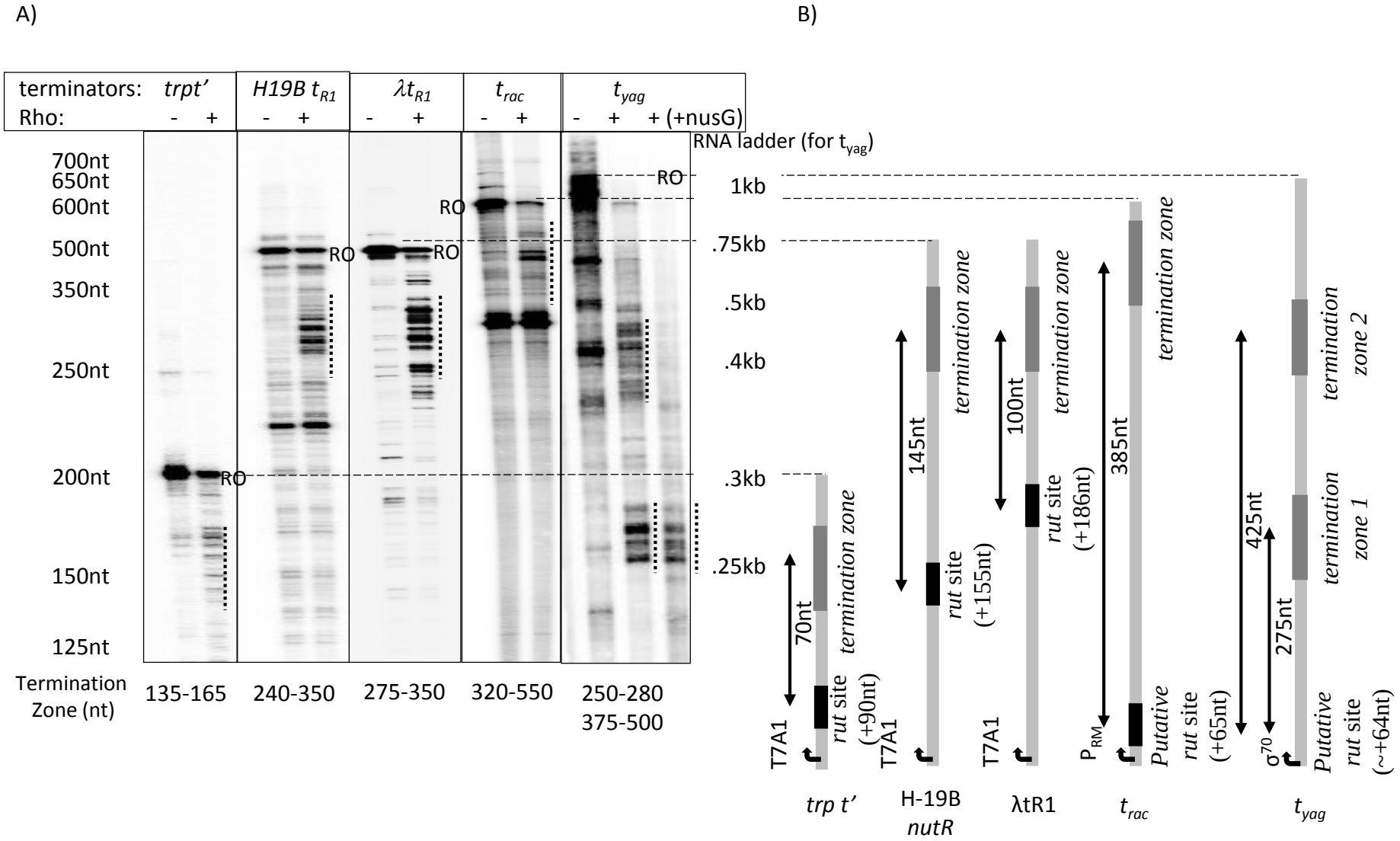


Figure S11: **A)** *In vitro* termination assays on different terminators. Termination zones for each are indicated by dashed vertical lines, and their mean distances from the start site are indicated below the autoradiograms. t_{yag} has two termination zones. Last lane of this panel is in the presence 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).

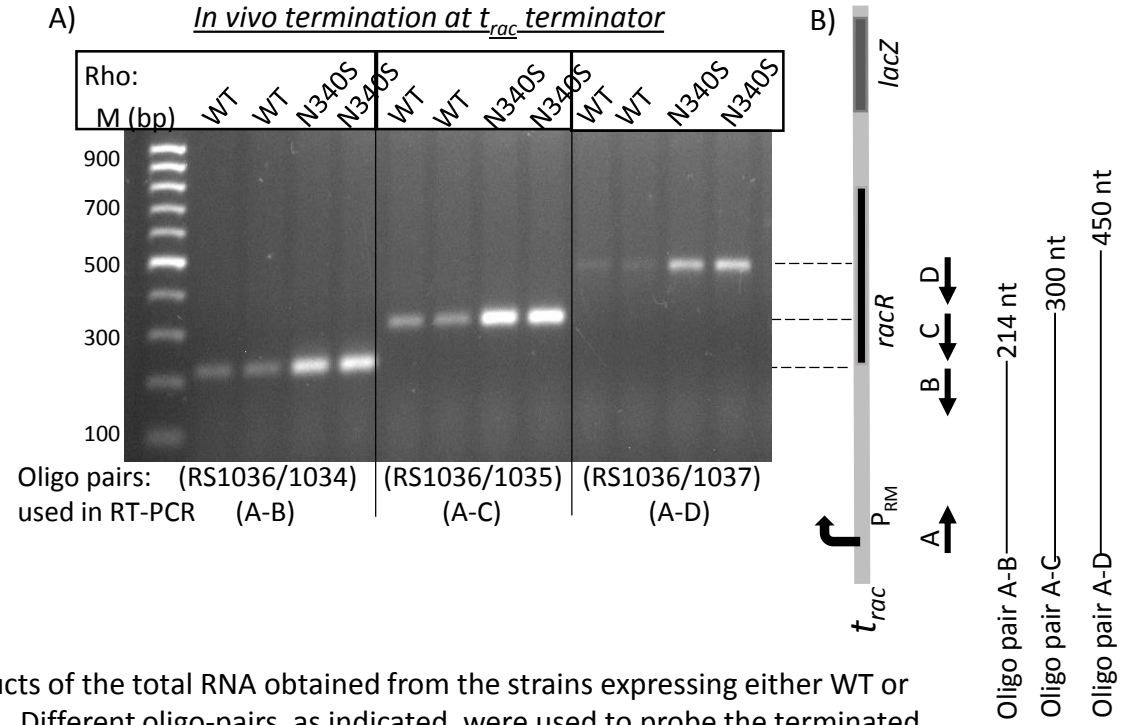


Figure S12. **A)** RT-PCR products of the total RNA obtained from the strains expressing either WT or N340S Rho mutant (RS1428). Different oligo-pairs, as indicated, were used to probe the terminated RNA products initiated from P_{RM} promoter. Two reactions were loaded for each case. These cDNAs were 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	GCGCGCGGATCCCCCCATTCAAGAACAGCAAGCAGC; lambda *TR1* reverse primer with BamHI site.
RS401	GCGCGCGTGGTGCAACGGGCGCTGGG; *lacZ* specific FP for qPCR
Rs402	GCGCGCCAACTCGCCGCACATCTG; *lacZ* specific RP for qPCR
RS404	*GAATTGTGAGCGCTCACAATTC*GGATGCCAGACCGCGCTGGGTAAGC G; RP with 5'-lacO fusion, used to generate roadblock downstream of rut sites of for *T7A1-H19B TR1* template
RS555	CTG CTT CAG GAT GGC AAA AAT AAT GTC C ; internal RP (123-96bp) of *rho* gene used for checking rho deletion by PCR
RS845	*GAATTGTGAGCGCTCACAATTC*TTAGCGCCTCGGGCTGGCTA; RP with 5'-lacO fusion used to generate roadblock on P_{RM}-*racR*/t_{rac}template
RS846	CCTTATCCGGTTGTTTGTTG; *yagM* specific FP for qPCR
RS847	TTAGGTTCTGCTCTCTTGTG; *yagM* specific RP for qPCR
RS848	CCTGAAGTTACCGCTATAGC; *ybcK* specific FP for qPCR
RS849	CTCTCGTCCATTTTTACTGG; *ybcK* specific RP for qPCR
RS852	AATCGCAAAGCAATCTTGGT; *gshA* specific FP for qPCR
RS853	GCAACACGTTGCTGTTGATT; *gshA* specific RP for qPCR
RS854	CTGGTAACAAACCAGAGTGG; *rpoC* specific FP for qPCR
RS855	TCAGACGGTTGTTACGGTTA; *rpoC* specific RP for qPCR
RS955	*GAATTGTGAGCGCTCACAATTC*TTAGGTATTCGTTAAGCCCC; RP with 5'-lacO fusion, used to generate roadblock on P_{RM}-*racR*/t_{rac}template
RS956	*GAATTGTGAGCGCTCACAATTC*TTAGGGCGCGAACATCGTGG; RP with 5'-lacO fusion, used to generate roadblock on P_{RM}-*racR*/t_{rac}template.
RS963	AACAATGTCGACTTAACCTGAACGACGACGATTAC; *rhlB* specific primer for checking *rho* deletion by PCR
RS995	*TTAATACGACTCACTATAGGGAGA*GTGATACACCTAAAAGCCTAGCC; 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 *racR* gene of pRS1352.
RSRK-1	GTTTTCCCAGTCACGAC; reverse primer in *lacZ* gene.
RK23B	TGGAGTTCCAGACGATACG; reverse oligo to generate *T7A1-H19B/TR1* terminator template
RS1083	GGCACGAGAAAATTGAGACATGG; FP in *yagN* region, to be used to prepare t_{yag} terminator template
RS1084	CTACCTGTGACTCATTGTTTTTGCC; RP in *yagM* region, to be used to prepare t_{yag} terminator template

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

NusG alleles	*T_{R1}-LacZ β–galactosidase activity*	*T_{R1} –trpt'-LacZ β–galactosidase activity*	*T_{rac}-LacZ β–galactosidase activity*
WT	259.4 ± 5	20.2 ± 0.08	61 ± 0.5
V160N	634.3 ± 60	34 ± 0.8	2421 ± 46
G146D	461.3 ± 9	49 ± 0.9	1865 ± 64
L158Q	460 ± 10	48± 2	2766 ± 75