Translational control and Rho-dependent transcription termination are intimately linked in riboswitch regulation

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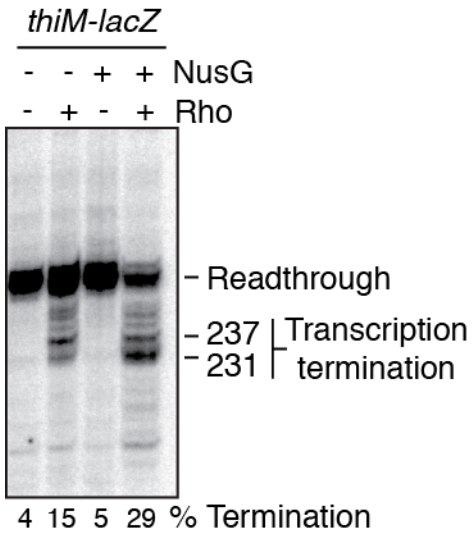
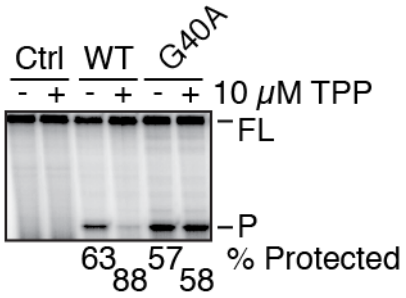
SUPPLEMENTARY INFORMATION



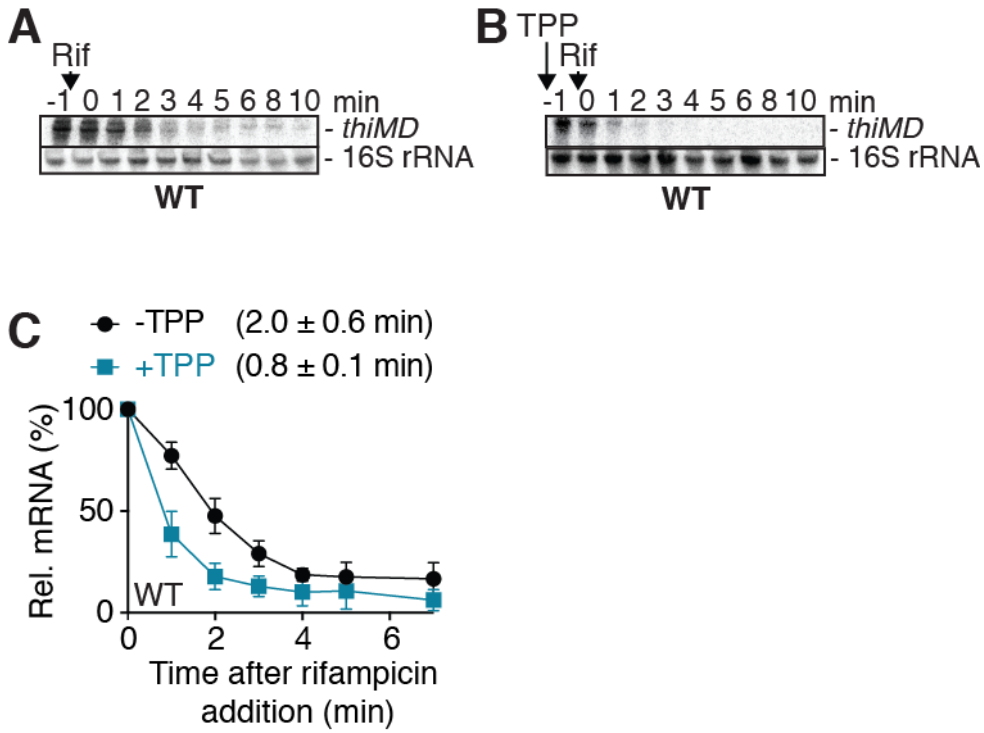
Supplementary Figure S1. Rho-mediated transcription termination using a *thiM-lacZ* fusion.

In vitro Rho-dependent transcriptions performed using the *thiM-lacZ* transcriptional fusion. Transcriptions were done in the absence (-) or presence (+) of 50 nM NusG or 50 nM Rho. Readthrough and termination transcripts are indicated at the right. Termination efficiencies are indicated below.



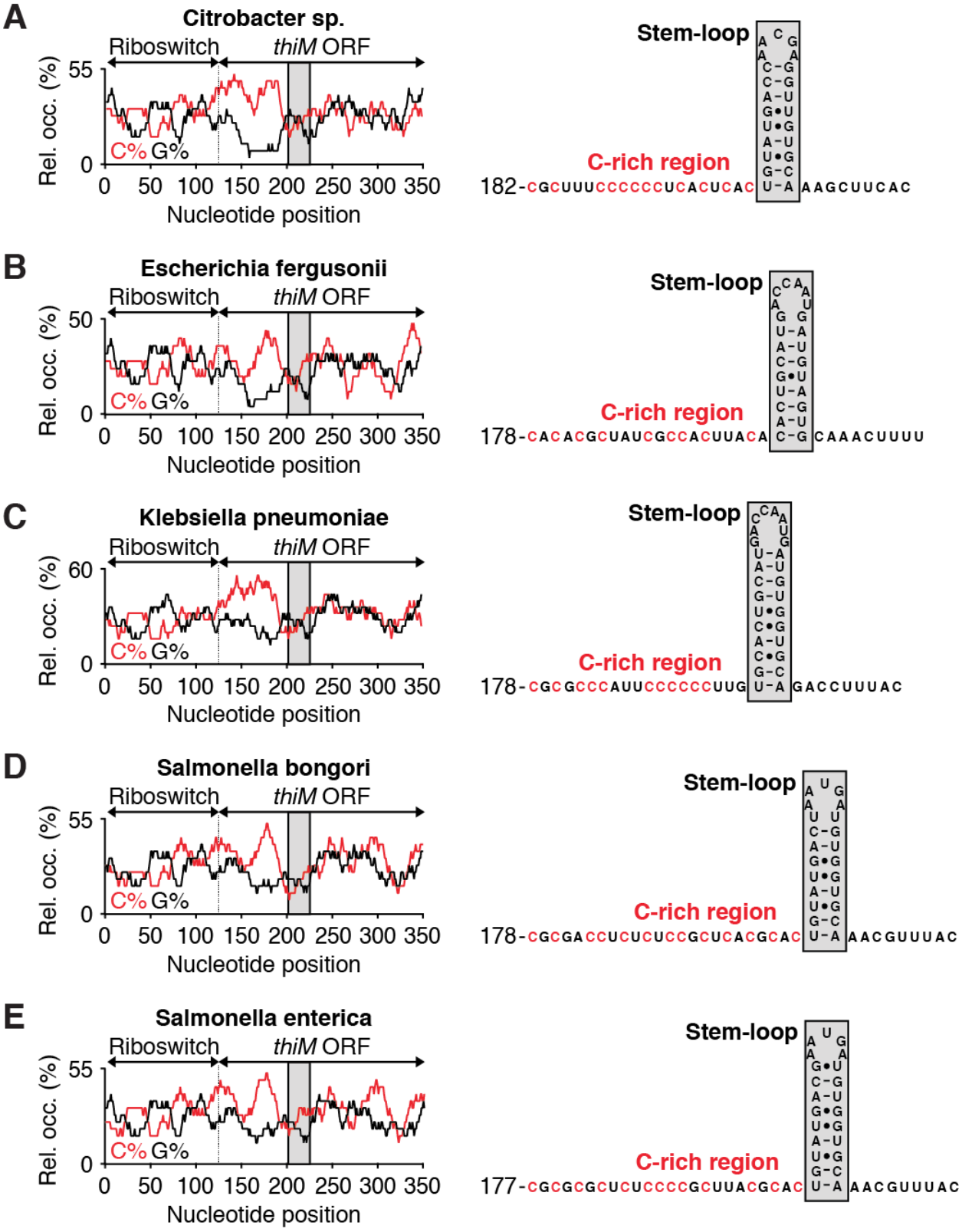
Supplementary Figure S2. RNase H cleavage assays monitoring TPP-induced structural modulation of the *thiM*- riboswitch.

In vitro RNase H assays performed using the *thiM*-riboswitch in the absence (-) or presence (+) of 100 uM TPP. Full-length (FL) and cleavage product (P) are indicated at the right. Protection efficiencies are indicated below. Absence of products is observed in the absence of RNase H (Ctrl).



Supplementary Figure S3. Study of *thiMD* mRNA stability as a function of TPP.

(A and B) Northern blot analysis of *thiMD* mRNA levels in the context of the wild-type strain. The strain was grown to mid-log phase in M63 minimal medium with 0.2% glucose at 37°C and total RNA was isolated at different times before (-1) and after (0) the addition of rifampicin (250 µg/mL). The experiment was performed in the absence (A) or presence of 500 µg/mL TPP (*B*). The loading control was 16S rRNA. (C) Quantification analysis of Northern blots shown in panels A and B. The quantification represents the stability of the full-length *thiMD* mRNA in the context of the wild-type strain. Half-lives obtained in the absence and presence of TPP are indicated. The average values of three independent experiments with SDs are shown.


A
Citrobacter sp.
Riboswitch
thiM ORF
Rel. occ. (%)
55
0
C% G%
0 50 100 150 200 250 300 350
Nucleotide position
Stem-loop
C-rich region
182-CGCUUUCCCCCCUCACUCAC U-A AAGCUUCAC
B
Escherichia fergusonii
Riboswitch
thiM ORF
Rel. occ. (%)
50
0
C% G%
0 50 100 150 200 250 300 350
Nucleotide position
Stem-loop
C-rich region
178-CACACGCUAUCGCCACUUACA C-G CAAACUUUU
C
Klebsiella pneumoniae
Riboswitch
thiM ORF
Rel. occ. (%)
60
0
C% G%
0 50 100 150 200 250 300 350
Nucleotide position
Stem-loop
C-rich region
178-CGCGCCCAUUCCCCCCUUG U-A GACCUUUAC
D
Salmonella bongori
Riboswitch
thiM ORF
Rel. occ. (%)
55
0
C% G%
0 50 100 150 200 250 300 350
Nucleotide position
Stem-loop
C-rich region
178-CGCGACCUCUCUCCGCUCACGCAC U-A AACGUUUAC
E
Salmonella enterica
Riboswitch
thiM ORF
Rel. occ. (%)
55
0
C% G%
0 50 100 150 200 250 300 350
Nucleotide position
Stem-loop
C-rich region
177-CGCGCGCUCUCCCCGCUUACGCAC U-A AACGUUUAC

Supplementary Figure S4. The region important for Rho transcription termination is conserved across various bacterial species.

(A to E) Sequence analysis of *thiM* in Citrobacter sp. (A), *Escherichia fergusonii* (B), *Klebsiella pneumoniae* (C), *Salmonella bongori* (D) and *Salmonella enterica* (E). In each case, the left panel shows the relative occurrence (Rel. occ.) of cytosine (C%) and guanine (G%) in the upstream region of the *thiM* sequence. A scanning window of 25 nt was used to determine C and G occurrences and the shaded region represents the position of the stem-loop. Right panels show a portion of the C-rich region and the secondary structure of the stem-loop, which is based on mfold analysis (1).

Supplementary Table S1. Summary of strains/plasmids used in this study.

Strains	Relevant marker	References
AM147	JW5741-1(11550), rnr-729(del)::kan, BL21(DE3) pIA247 (nusG) BL21(DE3) pET28b-rho	CGSC, Yale
BTU1	PM1205 lacI′::PBAD-BtuB$_{6cd}$	This study
BTU2	PM1205 lacI′::PBAD-*btuB*$_{6cd}$	This study
CRB016	MG1655 *rho*-R66S *ilvD*500::Tn10	Laboratory collection
DAL1	BL21(DE3) F–ompT hsdS(rB– mB–) gal dcm λ(DE3)	Laboratory collection
DAL2	JW1279-1 (99150) *Δrnb-723::kan*,	CGSC, Yale
EM1055	MG1655 Δ*lacZ* X174	(2)
EM1047	DH5-alpha + pACYC184	Laboratory collection
EM1237	DY330 [W3110 delta-lacU169 gal490 lambda-cI857 delta-(*cro-bioA*)]	(3)
EM1321	EM1055 rnc14 [P1 from EM1320]	Laboratory collection
JF185	EM1055 *rppH::Tet*	Laboratory collection
KP1487	*thiM-lacZ*$_{34cd}$ (250) trx	This study
KP1500	*rho*R66S Tn10	This study
KP1502	*thiM-lacZ*$_{34cd}$ (250) trx *rho*R66S Tn10	KP1487 + P1(KP1500)
PM1205	*lacI*::P$_{BAD}$-*cat-sacB-lacZ*, mini|tet^{R}	(4)
TPP3	PM1205 lacI′::PBAD-t*hiM*$_{6cd}$	(5)
TPP4	PM1205 lacI′::PBAD-ThiM$_{6cd}$	(5)
TPP5	PM1205 lacI′::PBAD-*thiM*$_{50cd}$	This study
TPP9	PM1205 lacI′::PBAD-*thiM*$_{10cd}$	This study
TPP10	PM1205 lacI′::PBAD-*thiM*$_{20cd}$	This study
TPP12	PM1205 lacI′::PBAD-*thiM*$_{100cd}$	This study
TPP13	PM1205 lacI′::PBAD-*thiM*$_{167cd}$	This study
TPP14	PM1205 lacI′::PBAD-*thiM*$_{34cd}$mutRBS	This study
TPP15	PM1205 lacI′::PBAD-*thiM*$_{34cd}$mutAUG	This study
TPP16	PM1205 lacI′::PBAD-*thiM*$_{6cd}$mutRBS	This study
TPP17	PM1205 lacI′::PBAD-*thiM*$_{6cd}$mutAUG	This study
TPP18	PM1205 lacI′::PBAD-ThiM$_{6cd}$mutRBS	This study
TPP19	PM1205 lacI′::PBAD-ThiM$_{6cd}$mutAUG	This study
TPP20	PM1205 lacI′::PBAD-*btuBthiM*	This study
TPP21	PM1205 lacI′::PBAD-*thiM*$_{34cd}$M3	This study
TPP22	PM1205 lacI′::PBAD-*thiM*$_{34cd}$M2	This study
TPP23	PM1205 lacI′::PBAD-*thiM*$_{34cd}$M1	This study
TPP24	PM1205 lacI′::PBAD-*thiM*$_{50cd}$M4	This study
TPP25	PM1205 lacI′::PBAD-*thiM*$_{50cd}$M5	This study
TPP29	PM1205 lacI′::PBAD-*thiM*$_{50cd}$M2	This study
TPP30	PM1205 lacI′::PBAD-*thiM*$_{100cd}$M2	This study
TPP31	PM1205 lacI′::PBAD-*thiM*$_{167cd}$M2	This study
TPP32	PM1205 lacI′::PBAD-*thiM*$_{34cd}$M6	This study
TPP33	PM1205 lacI′::PBAD-*thiM*$_{34cd}$M7	This study

Supplementary Table S2. Summary of *lacZ* fusions used in this study.

Strains	Construct	Oligonucleotides
Transcriptional and translational fusions in PM1205		
BTU1	$BtuB_{6cd}$	LB18-LB19 (genomic DNA)
BTU2	$btuB_{6cd}$	LB18-LB20 (genomic DNA)
TPP3	$thiM_{6cd}$	LB1-LB3 (genomic DNA)
TPP4	$ThiM_{6cd}$	LB1-LB2 (genomic DNA)
TPP5	$thiM_{50cd}$	LB1-LB7 (genomic DNA)
TPP9	$thiM_{10cd}$	LB1-LB4 (genomic DNA)
TPP10	$thiM_{21cd}$	LB1-LB5 (genomic DNA)
TPP11	$thiM_{34cd}$	LB1-LB6 (genomic DNA)
TPP12	$thiM_{100cd}$	LB1-LB8 (genomic DNA)
TPP13	$thiM_{167cd}$	LB1-LB9 (genomic DNA)
TPP14	$thiM_{34cd}$mutRBS	PCR1: LB1-LB11 (genomic DNA) PCR2: LB10-LB6 (genomic DNA) PCR3: LB1-LB6 (PCR1-2)
TPP15	$thiM_{34cd}$mutAUG	PCR1: LB1-LB12 (genomic DNA) PCR2: LB13-LB6 (genomic DNA) PCR3: LB1-LB6 (PCR1-2)
TPP16	$thiM_{6cd}$mutRBS	LB1-LB3 ($thiM_{34cd}$mutRBS)
TPP17	$thiM_{6cd}$mutAUG	LB1-LB15 ($thiM_{34cd}$mutAUG)
TPP18	$ThiM_{6cd}$mutRBS	LB1-LB2 ($thiM_{34cd}$mutRBS)
TPP19	$ThiM_{6cd}$mutAUG	LB1-LB14 ($thiM_{34cd}$mutAUG)
TPP20	*btuBthiM*	PCR1: LB18-LB17 (genomic DNA) PCR2: LB16-LB6 (genomic DNA) PCR3: LB18-LB6 (PCR1-2)
TPP21	$thiM_{34cd}$M3	PCR1: LB1-LB21 (genomic DNA) PCR2: LB22-LB6 (genomic DNA) PCR3: LB1-LB6 (PCR1-2)
TPP22	$thiM_{34cd}$M2	PCR1: LB1-LB24 (genomic DNA) PCR2: LB23-LB6 (genomic DNA) PCR3: LB1-LB6 (PCR1-2)
TPP23	$thiM_{34cd}$M1	PCR1: LB1-LB26 (genomic DNA) PCR2: LB25-LB6 (genomic DNA) PCR3: LB1-LB6 (PCR1-2)
TPP24	$thiM_{50cd}$M4	PCR1: LB1-LB28 (genomic DNA) PCR2: LB27-LB7 (genomic DNA) PCR3: LB1-LB7 (PCR1-2)
TPP25	$thiM_{50cd}$M5	PCR1: LB1-LB30 (genomic DNA) PCR2: LB29-LB7 (genomic DNA) PCR3: LB1-LB7 (PCR1-2)
TPP29	$thiM_{50cd}$M3	LB1-LB7 ($thiM_{167cd}$M3)
TPP30	$thiM_{100cd}$M3	LB1-LB8 ($thiM_{167cd}$M3)
TPP31	$thiM_{167cd}$M3	PCR1: LB1-LB24 (genomic DNA) PCR2: LB23-LB9 (genomic DNA) PCR3: LB1-LB9 (PCR1-2)
TPP32	$thiM_{34cd}$M6	PCR1: LB1-LB48 (genomic DNA) PCR2: LB49-LB9 (genomic DNA) PCR3: LB1-LB6 (PCR1-2)
TPP33	$thiM_{34cd}$M7	PCR1: LB1-LB50 (genomic DNA) PCR2: LB51-LB9 (genomic DNA) PCR3: LB1-LB6 (PCR1-2)

Supplementary Table S3. PCR constructs used for *in vitro* RNA synthesis.

Constructions	Oligonucleotides
***In vitro* transcription assays**	
PlacUV5thiM$_{34cd}$*lacZ*	PCR1: LB45-LB46 (Strain TPP11) PCR2: AC1-LB65 (PCR1)
PlacUV5thiM$_{300}$	AC1-AC4 (genomic DNA)
PlacUV5thiM$_{300}$-G40A	PCR1: AC1-AC5 (genomic DNA) PCR2: AC6-AC4 (genomic DNA) PCR3: AC1-AC4 (PCR1-2)
PlacUV5thiM$_{400}$	AC1-AC2 (genomic DNA)
Northern Blot RNA probes	
thiM probe	LB39-LB40 (genomic DNA)
thiD probe	LB41-LB42 (genomic DNA)
16S RNA probe	EM293-EM294 (genomic DNA)

Supplementary Table S4. Summary of oligonucleotides used in this study.

Oligonucleotides	Sequence 5'-3'
130	GCCATAACGT
201	TTGGTGAAAA
AC1	GGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGCTGCG ATTTATCATCGCAACC
AC2	CACGCATCGCCTGAGCGCGT
AC3	CCGAGTCGTT
AC4	CATCGCTGGCGATGCACCGAG
AC5	ACGGGTATTTCTTAGCCTTCACGCA
AC6	TGCGTGAAGGCTAAGAAATACCCGT
EM293	TAATACGACTCACTATAGGGAGACGCTTTACGCCCAGTAATTCC
EM294	CTCCTACGGGAGGCAGCAGT
LB1	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATGATTTATCATCGCAAC CAAAC
LB2	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACCAGCAGGTCGACTT GCATAG
LB3	GTGTGATAAAGAAAGTTAAAATGCCGGATCCAGCAGGTCGACTTGCATAG
LB4	GTGTGATAAAGAAAGTTAAAATGCCGGATCCGCAGATTGCGCTGAACC
LB5	GTGTGATAAAGAAAGTTAAAATGCCGGATCCGCAGATTGCGCTGAACC
LB6	GTGTGATAAAGAAAGTTAAAATGCCGGATCTTGCACCACATCATTGGTCAT
LB7	GTGTGATAAAGAAAGTTAAAATGCCGGATCCATCGCTGGCGATGCACCGAG
LB8	GTGTGATAAAGAAAGTTAAAATGCCGGATCTGGATCAAGCGTCCAGGGTGT
LB9	GTGTGATAAAGAAAGTTAAAATGCCGGATCGATTGCGCCAGTTTCCCGTGC
LB10	TATGGCAGCAGCAAACTATGCAAGTCG
LB11	CGACTTGCATAGTTTGCTGCTGCCATA
LB12	GGAGCAAACTATACAAGTCGAC
LB13	GTCGACTTGTATAGTTTGCTCC
LB14	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACCAGCAGGTCGACTT GCTTAG
LB15	GTGTGATAAAGAAAGTTAAAATGCCGGATCCAGCAGGTCGACTTGCTTAG
LB16	AAAAGCCGGTCAATCTGCGCACGCGTTACACCT
LB17	GTGCGCAGATTGACCGGCTTTTTTAATCATTGTAAAG
LB18	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATGCCGGTCCTGTGAGT TAATAG
LB19	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACCGAAGCTTTTTTAAT CATTGTAAAGC
LB20	GTGTGATAAAGAAAGTTAAAATGCCGGATCCGAAGCTTTTTTAATCATTGTAAAGC
LB21	GTCATGCAGTGCACAGGGGAATGTTGGTGAAAAAGGTG
LB22	CATTCCCCTGTGCACTGCATGACCAATGATGTGGTGC
LB23	CCAACATTCCCCGGTTGTGCACTGCATGACCAATGATG
LB24	CAGTGCACAACCGGGGAATGTTGGTGAAAAAGGTG
LB25	CCCCTCGGGTGCACTGCATGACCAATG
LB26	CAGTGCACCCGAGGGGAATGTTGGTGAAAAAGG
LB27	GATGTGGTGCAAACCGCCAATACCTTGCTGGCGCTC
LB28	GTATTGGCGGTTTGCACCACATCATTGGTCATGCAG
LB29	GCAATGGTTTACCGCCAATACCTTGCTGGCGC
LB30	GGCGGTAAACCATTGCACCACATCATTGGTCATGCAGTG

LB39	AATAATACGACTCACTATAGGGTGGATCAAGCGTCCAGGGTGTTTG
LB40	CCTTTACCGCCAATACCTTGCTG
LB41	AATAATACGACTCACTATAGGGCGCCGCTTTTTGCCAGCATAACGG
LB42	GGCGTACAGTCGGTGTATCGCA
LB45	GAATCTGGTGTATATGGCGAGC
LB46	GGGGGATGTGCTGCAAGGC
LB47	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACCATAGCTGTTTCCTG TGTGATAAAGAAAGTTAAAATGCCGGATC
LB48	CAAGAGGGGAAAAAAGGTGTAACGCGTGCGCAGATTGCG
LB49	CGCGTTACACCTTTTTTCCCCTCTTGTGCACTGCATGACC
LB50	CAAGACCCCAATGTTGGTGAAAAAGGTGTAACGCGTG
LB51	CAACATTGGGGTCTTGTGCACTGCATGACCAATGATG
LB65	AAGAAAGTTAAAATGCCGGATCTTGCACCACATCATTGGTCAT
F-rho-UTR	CAACGCTTCCCGTTTTATCT
R-rho-UTR	TTGTGAATGGTATGGCAGGA
F-rho-ORF	TGTGTGCTGATGGTTCTG
R-rho-ORF	CTTCTCGATCACCATTTCC
F-thiM-UTR	TGAGAAATACCCGTATCACC
R-thiM-UTR	GTAAAGGTTTGCACCACATC
F-thiM-ORF	ATGGACATCGTATCATTGGT
R-thiM-ORF	GGAAATGTGGAACAAAACTG
F-thiC-UTR	TTAATCTGCTATCGCATCG
R-thiC-UTR	ATAAATGCGTTTTGAGTTGG
F-thiC-ORF	ACATCTGCCGGAAAATATCA
R-thiC-ORF	GCTTTTCCACTTCTTCTTCG
F-lysC-UTR	GGGTGAAAATAGTAGCGAAG
R-lysC-UTR	TCAGAAAGCACAATATCAGC
F-lysC-ORF	ATGTACGTAAAGTGATGCGT
R-lysC-ORF	GGTCCAGATATCAACACGAG
F-ribB-UTR	GGATGGGAGAGAGTAACGAT
R-ribB-UTR	GTGCATTTTCAACACGTTC
F-ribB-ORF	CTTGATGATGAAGACCGTGA
R-ribB-ORF	TTGGCAGATCGAGTTGTTTA
F-mgtA-UTR	GTCCCTGCTCAGCTTTATTA
R-mgtA-UTR	GACCACCGTATTCACTGTCT
F-mgtA-ORF	CGCTGATTGTGCATATGAT
R-mgtA-ORF	TCATATACCCTGCCAGAATC
F-btuB-UTR	CATCTGGTTCTCATCATCG
R-btuB-UTR	AAAACGGTTAGCAGTAACGA
F-btuB-ORF	GTATGACTTCGACTGGGGTA
R-btuB-ORF	GCAGTTTGGTAGCCATAGAC
F-thiB-UTR	GAGAAAATACCCGTCGAAC
R-thiB-UTR	GCGAAGGAATCGTAGGTAT
F-thiB-ORF	CCTGAAAGAACTGGTTGAGA
R-thiB-ORF	TGGTGTAACTCAGTACCAGA

Supplementary References

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