Synthetic Dual-Function RNA Reveals Features Necessary for Target Regulation

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SUPPLEMENTAL MATERIAL

MgtSR
ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCatgCTGGGTAATATGAATGTTTTTATGGCCGTA
CTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaA
TGCCTGTTAGCGTAAAAGCAAAACACAAATCTATCCATGCAAGCATTCACCGCCGGTTTACTGG
CGGTTTTTTTTC

MgtSR$_{\text{STOP}}$
ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCatgTAGGGTAATATGAATGTTTTTATGGCCGTA
CTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaA
TGCCTGTTAGCGTAAAAGCAAAACACAAATCTATCCATGCAAGCATTCACCGCCGGTTTACTGG
CGGTTTTTTTTC

MgtSR$_{\text{BP}}$
ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCatgCTGGGTAATATGAATGTTTTTATGGCCGTA
CTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaA
TGCCTGTTAGCGTAAAAGCAAAACACAAATCTATCCAT**CGTT**GCATTCACCGCCGGTTTACTGG
CGGTTTTTTTTC

MgtSR-no spacer
ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCatgCTGGGTAATATGAATGTTTTTATGGCCGTA
CTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaT
CTATCCATGCAAGCATTCACCGCCGGTTTACTGGCGGTTTTTTTTC

MgtSR-no spacer$_{\text{STOP}}$
ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCatgTAGGGTAATATGAATGTTTTTATGGCCGTA
CTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaT
CTATCCATGCAAGCATTCACCGCCGGTTTACTGGCGGTTTTTTTTC

MgtSR-5′Δ5nt
ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCatgCTGGGTAATATGAATGTTTTTATGGCCGTA
CTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaT
GTTAGCGTAAAAGCAAAACACAAATCTATCCATGCAAGCATTCACCGCCGGTTTACTGGCGGTT
TTTTTTC

MgtSR-5′Δ5nt$_{\text{STOP}}$
ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCatgTAGGGTAATATGAATGTTTTTATGGCCGTA
CTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaT
GTTAGCGTAAAAGCAAAACACAAATCTATCCATGCAAGCATTCACCGCCGGTTTACTGGCGGTT
TTTTTTC

MgtSR-5′Δ10nt
ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCatgCTGGGTAATATGAATGTTTTTATGGCCGTA
CTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaG
CGTAAAAGCAAAACACAAATCTATCCATGCAAGCATTCACCGCCGGTTTACTGGCGGTTTTTTT
TC

MgtSR-5′Δ10nt$_{STOP}$

ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCatgTAGGGTAATATGAATGTTTTTATGGCCGTACTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaGCGTAAAAGCAAAACACAAATCTATCCATGCAAGCATTCACCGCCGGTTTACTGGCGGTTTTTTTTC

MgtSR-3′Δ5nt

ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCatgCTGGGTAATATGAATGTTTTTATGGCCGTACTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaATGCCTGTTAGCGTAAAAGCAAAACTCTATCCATGCAAGCATTCACCGCCGGTTTACTGGCGGTTTTTTTTC

MgtSR-3′Δ5nt$_{STOP}$

ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCatgTAGGGTAATATGAATGTTTTTATGGCCGTACTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaATGCCTGTTAGCGTAAAAGCAAAACTCTATCCATGCAAGCATTCACCGCCGGTTTACTGGCGGTTTTTTTTC

MgtSR-3′Δ10nt

ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCatgCTGGGTAATATGAATGTTTTTATGGCCGTACTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaATGCCTGTTAGCGTAAAAGCTCTATCCATGCAAGCATTCACCGCCGGTTTACTGGCGGTTTTTTTTC

MgtSR-3′Δ10nt$_{STOP}$

ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCatgTAGGGTAATATGAATGTTTTTATGGCCGTACTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaATGCCTGTTAGCGTAAAAGCTCTATCCATGCAAGCATTCACCGCCGGTTTACTGGCGGTTTTTTTTC

MgtRS
ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCGATTCGTTATCAGTGCAGGAAAATGCCTGTTAG
CGTAAAAGCAAAACACAAATCTATCCATGCAAGCATTTTGAGATGAAAATTAAGGTAAGCGAGG
AAACACACCACACCATAAACGGAGGAAAATA**atgCTGGGTAATATGAATGTTTTTATGGCCGTA**
CTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaT
GAACGGAGACACCGCCGGTTTACTGGCGGTTTTTTTT

MgtRS-ChiX ARN
ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCGATTCGTTATCAGTGCAGGAAAATGCCTGTTAG
CGTAATAATAAAAAAACACAAATCTATCCATGCAAGCATTTTGAGATGAAAATTAAGGTAAGCG
AGGAAACACACCACACCATAAACGGAGGAAAATA**atgCTGGGTAATATGAATGTTTTTATGGCC**
GTACTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACt
aaTGAACGGAGACACCGCCGGTTTACTGGCGGTTTTTTTT

MgtRS-30nt
ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCGATTCGTTATCAGTGCAGGAAAATGCCTGTTAG
CGTAAAAGCAAAACACAAATCTATCCATGCAAGCATTAACACACCACACCATAAACGGAGGAAA
ATA**atgCTGGGTAATATGAATGTTTTTATGGCCGTACTGGGAATAATTTTATTTTCTGGTTTTC**
TGGCCGCGTATTTCAGCCACAAATGGGATGACtaaTGAACGGAGACACCGCCGGTTTACTGGCG
GTTTTTTTT

MgtRS-15nt
ACCCGTTTTTTTGGGCTAGCAGGAGGAATTCGATTCGTTATCAGTGCAGGAAAATGCCTGTTAG
CGTAAAAGCAAAACACAAATCTATCCATGCAAGCATTAAACGGAGGAAAATA**atgTAGGGTAAT**
ATGAATGTTTTTATGGCCGTACTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCA
GCCACAAATGGGATGACtaaTGAACGGAGACACCGCCGGTTTACTGGCGGTTTTTTTT

FIG. S1 Synthetic dual-function RNA constructs. List of MgtSR (sORF upstream of base pairing region) and MgtRS (base pairing region upstream of sORF) constructs. Bold black font indicates plasmid sequence, blue font indicates *mgtS* mRNA sequence, bold blue font indicates the *mgtS* ORF, black font indicates *mgrR* sequence, yellow indicates *mgrR* ARN motif, green indicates ChiX ARN motif, red indicates the terminator stem, *mgrR* base pairing region is underlined, and mutations are in bold red.

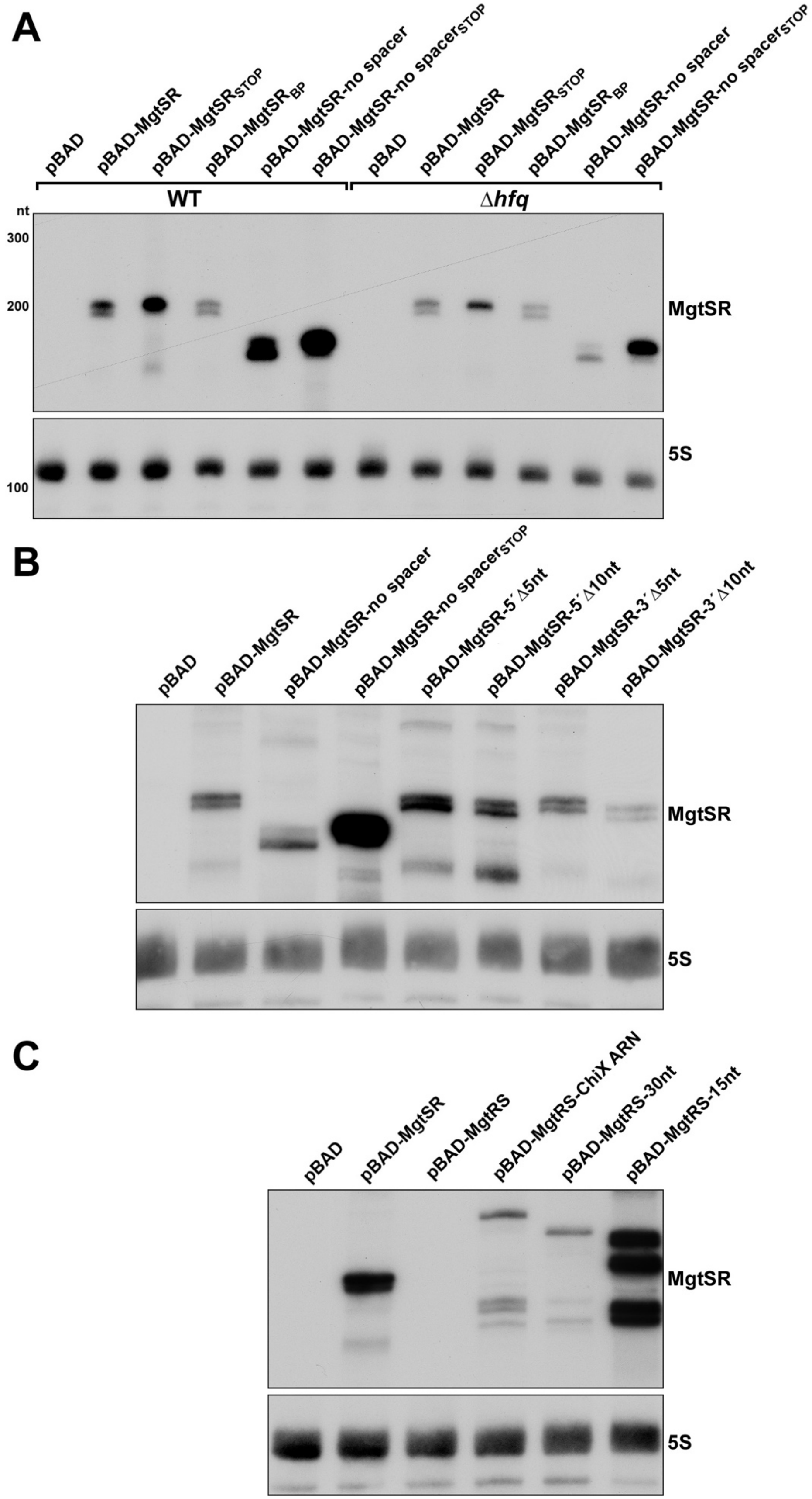
A
pBAD
pBAD-MgtSR
pBAD-MgtSR$_{STOP}$
pBAD-MgtSR$_{BP}$
pBAD-MgtSR-no spacer
pBAD-MgtSR-no spacer$_{STOP}$
pBAD
pBAD-MgtSR
pBAD-MgtSR$_{STOP}$
pBAD-MgtSR$_{BP}$
pBAD-MgtSR-no spacer
pBAD-MgtSR-no spacer$_{STOP}$
WT
Δ*hfq*
nt
300
200
100
MgtSR
5S
B
pBAD
pBAD-MgtSR
pBAD-MgtSR-no spacer
pBAD-MgtSR-no spacer$_{STOP}$
pBAD-MgtSR-5′Δ5nt
pBAD-MgtSR-5′Δ10nt
pBAD-MgtSR-3′Δ5nt
pBAD-MgtSR-3′Δ10nt
MgtSR
5S
C
pBAD
pBAD-MgtSR
pBAD-MgtRS
pBAD-MgtRS-ChiX ARN
pBAD-MgtRS-30nt
pBAD-MgtRS-15nt
MgtSR
5S

FIG. S2 Levels of transcripts expressed from dual-function RNA constructs. (A) Northern analysis of Δ*mgtS* (GSO786) and Δ*mgtS* Δ*hfq* (GSO1112) cells transformed with pBAD, pBAD-MgtSR, pBAD-MgtSR$_{STOP}$, pBAD-MgtSR$_{BP}$, pBAD-MgtSR-no spacer, or pBAD-MgtSR-no spacer$_{STOP}$. (B) Northern analysis of Δ*mgtS* (GSO786) cells transformed with pBAD, pBAD-MgtSR, pBAD-MgtSR-no spacer, pBAD-MgtSR-no spacer$_{STOP}$, pBAD-MgtSR-5´Δ5nt, pBAD-MgtSR-5´Δ10nt, pBAD-MgtSR-3´Δ5nt, or pBAD-MgtSR-3´Δ10nt. (C) Northern analysis of Δ*mgtS* (GSO786) cells transformed with pBAD, pBAD-MgtSR, pBAD-MgtRS, pBAD-MgtRS-ChiX ARN, pBAD-MgtRS-30nt, or pBAD-MgtRS-15nt. For all panels, cells were grown in LB to OD~1.0. Total RNA was isolated, separated on an acrylamide gel, transferred to a membrane and sequentially probed for the 3´ region of *mgtS* and 5S (as a loading control).

Table S1. Strains used in study

Name	**Genotype**	**Source**
MG1655	*E. coli* WT strain (*crl*$^{+}$) (AZ282, GSO982)	lab stock
TOP10	F- *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80*lacZ*ΔM15 Δ*lacX74 nupG recA1 araD139* Δ(*ara-leu*)7697 *galE15 galK16 rpsL*(Str^{R}) *endA1* λ^{-}	Invitrogen
KM100	*mal::lac*Iq, Δ*araBAD*, *leu* +, *araC*+, *araE*	(1)
KM132	Δ*mgrR::kan*	(1)
KM238	*eptB-lacZ* Δ*mgrR::kan* positions -150 to +94 of *eptB* promoter	(2)
GSO229	MG1655 Δ*mgtS::kan*	(3)
GSO786	MG1655 *mgtA-HA* Δ*mgtS::kan*	(3)
GSO954	MG1655 Δ*hfq-cat::sacB*	(4)
GSO1112	MG1655 *mgtA-HA* Δ*mgtS::kan* Δ*hfq::cat-sacB*	This study

Table S2. Plasmids used in study

Name	Relevant features	Source
pBRplac	derivative of the pBR322 vector	(5)
pBR-MgrR	pBR with *mgrR* cloned into EcoRI and HindIII, Amp^R	(2)
pBAD24		(6)
pBAD-MgtSR	Initial construct with MgtS ORF 30-nt upstream of *mgrR*	This study
pBAD-MgtSR$_{STOP}$	pBAD-MgtSR with TAG stop mutant as second amino acid	This study
pBAD-MgtSR$_{BP}$	pBAD-MgtSR with GCAA>GCTT mutation to disrupt base-pairing	This study
pBAD-MgtSR-no spacer	pBAD-MgtSR with 30-nt intervening sequence removed	This study
pBAD-MgtSR-no spacer$_{STOP}$	pBAD-MgtSR$_{NO\ SPACE\ MUT}$ with TAG stop mutant as second amino acid	This study
pBAD-MgtSR-5´Δ5nt	pBAD-MgtSR with 5-nt removed from 5´ end of intervening sequence	This study
pBAD-MgtSR-5´Δ10nt	pBAD-MgtSR with 10-nt removed from 5´ end of intervening sequence	This study
pBAD-MgtSR-3´Δ5nt	pBAD-MgtSR with 5-nt removed from 3´ end of intervening sequence	This study
pBAD-MgtSR-3´Δ10nt	pBAD-MgtSR with 10-nt removed from 3´ end of intervening sequence	This study
pBAD-MgtSR-5´Δ5nt$_{STOP}$	pBAD-MgtSR with 5-nt removed from 5´ end of intervening sequence and stop mutant	This study
pBAD-MgtSR-5´Δ10nt$_{STOP}$	pBAD-MgtSR with 10-nt removed from 5´ end of intervening sequence and stop mutant	This study
pBAD-MgtSR-3´Δ5nt$_{STOP}$	pBAD-MgtSR with 5-nt removed from 3´ end of intervening sequence and stop mutant	This study
pBAD-MgtSR-3´Δ10nt$_{STOP}$	pBAD-MgtSR with 10-nt removed from 3´ end of intervening sequence and stop mutant	This study
pBAD-MgtRS	Reverse construct	This study
pBAD-MgtRS-ChiX ARN	Reverse construct with MgrR ARN motif replaced with ChiX ARN motif	This study
pBAD-MgtRS-30nt	Reverse construct with 30 nt intervening sequence	This study
pBAD-MgtRS-15nt	Reverse construct with 15 nt intervening sequence	This study

Table S3. Oligonucleotides used in study

Name	5´-3´ sequence
Northern probes	
MgtSR	GTCATCCCATTTGTGGCTGAAATACGCGGCCAG
gBlocks	
MgtSR gblock	CTAAGGAATTCatgCTGGGTAATATGAATGTTTTTATGGCCGTACTGGGAATAATTT TATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaATGCCTGT TAGCGTAAAAGCAAAACACAAATCTATCCATGCAAGCATTCACCGCCGGTTTACT GGCGGTTTTTTTTCAAGCTTTGTGG
MgtRS gblock	CTCCTGGAATTCGATTCGTTATCAGTGCAGGAAAATGCCTGTTAGCGTAAAAGCA AAACACAAATCTATCCATGCAAGCATTTTGAGATGAAAATTAAGGTAAGCGAGGA AACACACCACACCATAAACGGAGGCAAATAatgCTGGGTAATATGAATGTTTTTAT GGCCGTACTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCACA AATGGGATGACtaaTGAACGGAGACACCGCCGGTTTACTGGCGGTTTTTTTTCAAGC TTCAGCCA
MgtRS-ChiX gblock	CAGGAGGAATTCGATTCGTTATCAGTGCAGGAAAATGCCTGTTAGCGTAATAATA AAAAAACACAAATCTATCCATGCAAGCATTTTGAGATGAAAATTAAGGTAAGCGA GGAAACACACCACACCATAAACGGAGGAAAATAatgCTGGGTAATATGAATGTTTT TATGGCCGTACTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTATTTCAGCCA CAAATGGGATGACtaaTGAACGGAGACACCGCCGGTTTACTGGCGGTTTTTTTTCAA GCTTGGCTG
MgtRS-30nt gblock	CAGGAGGAATTCGATTCGTTATCAGTGCAGGAAAATGCCTGTTAGCGTAAAAGCA AAACACAAATCTATCCATGCAAGCATTAACACACCACACCATAAACGGAGGAAA ATAatgCTGGGTAATATGAATGTTTTTATGGCCGTACTGGGAATAATTTTATTTTCTG GTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaTGAACGGAGACACCGC CGGTTTACTGGCGGTTTTTTTTCAAGCTTGGCTG
MgtRS-15nt gblock	CAGGAGGAATTCGATTCGTTATCAGTGCAGGAAAATGCCTGTTAGCGTAAAAGCA AAACACAAATCTATCCATGCAAGCATTAAACGGAGGAAAATAatgTAGGGTAATAT GAATGTTTTTATGGCCGTACTGGGAATAATTTTATTTTCTGGTTTTCTGGCCGCGTA TTTCAGCCACAAATGGGATGACtaaTGAACGGAGACACCGCCGGTTTACTGGCGGT TTTTTTTCAAGCTTGGCTG
Sequencing primers	
lacZ F	GCAATGACCATTGAACAGGCAGC
lacZ R	GCGATCGGTGCGGGCCTCTTCGCTA
pBAD f	AAACCAGAAAATAAAATTAT
pBAD r	GTTTTTATGGCCGTACTGGG
MgtSR seq f	GTCACACTTTGCTATGCCAT
MgtSR seq r	ACCCCACACTACCATCGGCG
Plasmid construction	
MgtSR stop f	GGGCTAGCAGGAGGAATTCatgtaGGGTAATATGAATGTTTTTATG
MgtSR stop r	CATAAAAACATTCATATTACCCtacatGAATTCCTCCTGCTAGCCC
MgtSR_bp_f	CAAAACACAAATCTATCCATcgttGCATTCACCGCCGGTTTACTG
MgtSR_bp_r	CAGTAAACCGGCGGTGAATGCaacgATGGATAGATTTGTGTTTTG
MgtSR_nospace_f	AATTCatgCTGGGTAATATGAATGTTTTTATGGCCGTACTGGGAATAATTTTATTTTC TGGTTTTCTGGCCGCGTATTTCAGCCACAAATGGGATGACtaaTCTATCCATGCAAG CATTCACCGCCGGTTTACTGGCGGTTTTTTTTCA

MgtSR_nospace_r	AGCTTGAAAAAAAACCGCCAGTAAACCGGCGGTGAATGCTTGCATGGATAGAttaG TCATCCCATTTGTGGCTGAAATACGCGGCCAGAAAACCAGAAAATAAAATTATTC CCAGTACGGCCATAAAAACATTCATATTACCCAGcatG
MgtSR_5pdel_-5_f	TGTTAGCGTAAAAGCAAAACA
MgtSR_5pdel_-10_f	GCGTAAAAGCAAAACACAAAT
MgtSR_5pdel_inv_r	ttaGTCATCCCATTTGTGGCT
MgtSR_3pdel_-5_r	GTTTTGCTTTTACGCTAACAG
MgtSR_3pdel_-10_r	GCTTTTACGCTAACAGGCA
MgtSR_3pdel_inv_f	TCTATCCATGCAAGCATTCA
MgtRS_bp_f	GTAAAAGCAAAACACAAATCTATCGTACGTTGCATTTTGAGATG
MgtRS_bp_r	CTTAATTTTCATCTCAAAATGCAACGTACGATAGATTTGTGTTTTGC
MgtRS_stop_f	CATAAACGGAGGCAAATAatgTAGGGTAATATGAATGTTTTTATG
MgtRS_stop_r	GTACGGCCATAAAAACATTCATATTACCCTAcatTATTTGCCTC
RS-stop-f	ATAAACGGAGGAAAATAatgTAGGGTAATATGAATGTTTTTA
RS-stop-r	TAAAAACATTCATATTACCCTAcatTATTTTCCTCCGTTTAT

Supplemental References

1. Moon K, Gottesman S. 2009. A PhoQ/P-regulated small RNA regulates sensitivity of Escherichia coli to antimicrobial peptides. Mol Microbiol 74:1314-1330.
2. Moon K, Six DA, Lee HJ, Raetz CR, Gottesman S. 2013. Complex transcriptional and post-transcriptional regulation of an enzyme for lipopolysaccharide modification. Mol Microbiol 89:52-64.
3. Wang H, Yin X, Wu Orr M, Dambach M, Curtis R, Storz G. 2017. Increasing intracellular magnesium levels with the 31-amino acid MgtS protein. Proc Natl Acad Sci USA 114:5689-5694.
4. Melamed S, Adams PP, Zhang A, Zhang H, Storz G. 2020. RNA-RNA interactomes of ProQ and Hfq reveal overlapping and competing roles. Mol Cell 77:411-425.
5. Guillier M, Gottesman S. 2006. Remodelling of the *Escherichia coli* outer membrane by two small regulatory RNAs. Mol Microbiol 59:231-247.
6. Guzman LM, Belin D, Carson MJ, Beckwith J. 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. J Bacteriol 177:4121-4130.