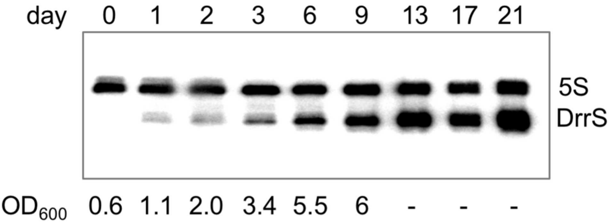
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1
2
3
6
9
13
17
21
5S
DrrS
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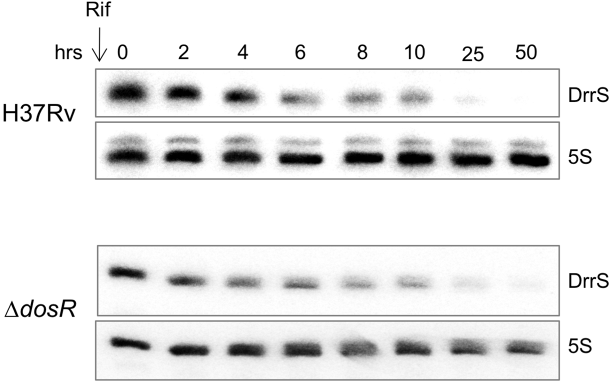
1.1
2.0
3.4
5.5
6
-
-
-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hrs
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2
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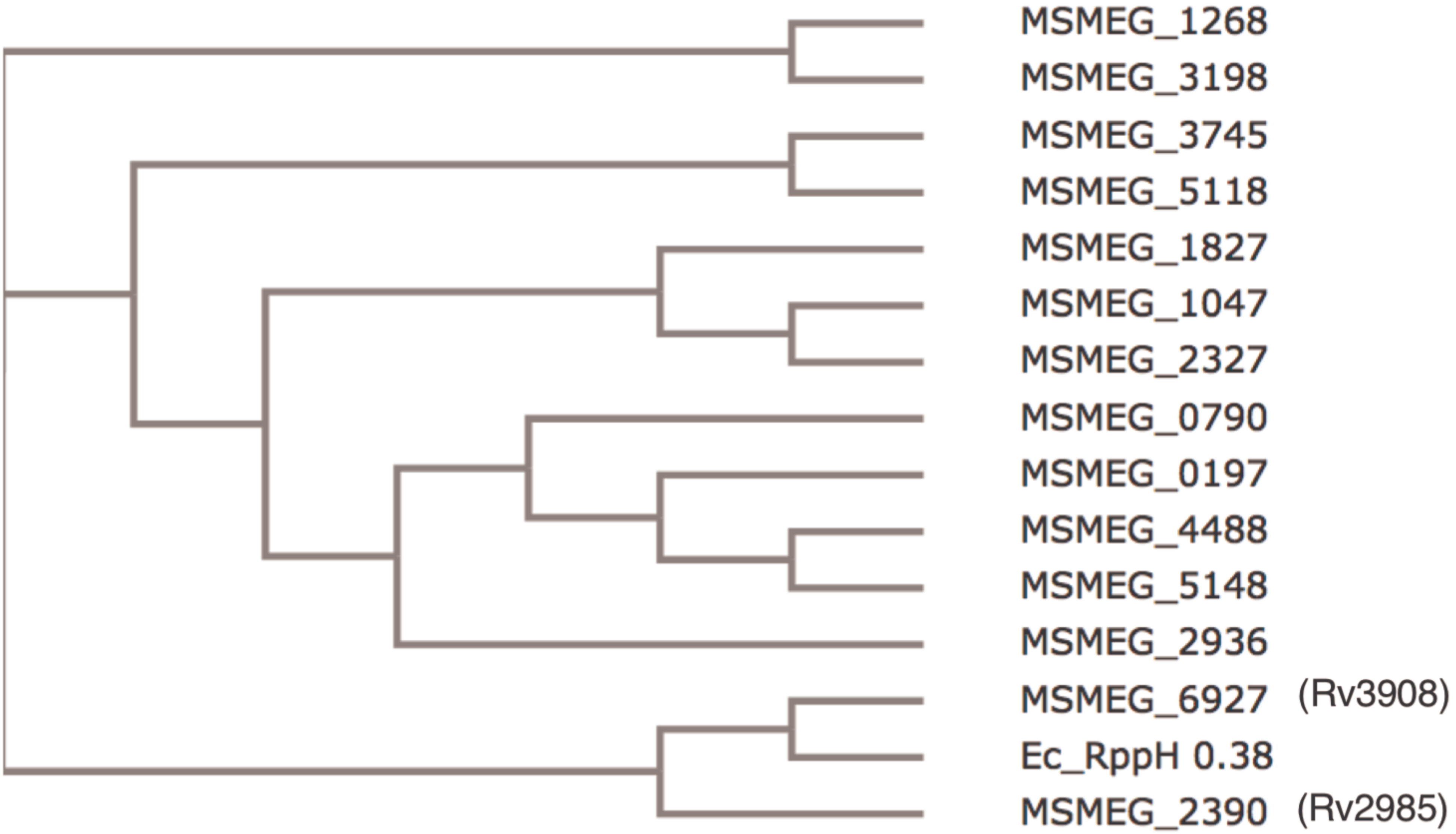
25
50
H37Rv
DrrS
5S
ΔdosR
DrrS
5S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MSMEG_0197
MSMEG_4488
MSMEG_5148
MSMEG_2936
MSMEG_6927 (Rv3908)
Ec_RppH 0.38
MSMEG_2390 (Rv2985)

Table S1

DNA sequence	**DrrS position**				**108**	**109**	**110**	**111**	**112**	**113**	**114**	**115**	**Total**
Willdtype 3’ end (2 repeats) **aTCCTCaTCCTC**	Sequence	U	C	C	**U**	C	A	U	C	C	U	C	
	%				**85**	9	-	3	3				
	n of clones				29	3		1	1				34
Extended 3’ end (3 repeats) **aTCCTCaTCCTCaTCCT**	Sequence	U	C	C	U	**C**	A	U	C	C	U	C	
	%			5	21	**42**	-	11	5	5	11		
	n of clones			**1**	4	8		2	1	1	2		19
Cropped 3’ end (1 repeat) **aTCCT**	Sequence	U	C	C	**U**	C	U	C	G	A	G	A	
	%				**33**	6		28	28	-	6	-	
	n of clones				6	1		5	5		1		18

3’ RACE results of DrrS variants expressed in *M. smegmatis*. A-residues were not assigned as the RACE was done by adding a poly-A tail.

Table S2: extended 3’ RACE of DrrS

Clone No.	Position from TSS	No. of clones/total	Two nt*	Four nt*
Extension 1 (CTTCGGGCAGATCCTCATCCTGTTAC)				
1	122	1/15	**C|G**	**GC|GG**
2	123	1/15	g|g	cg|gc
3	125	3/15	**C|G**	**GC|GC**
4				
5				
6	135	2/15	c|a/a|g	uc|ag / ca|gc
7				
8	162	1/15	**C|G**	**GC|GC**
9	164	2/15	**C|G**	**GC|GC**
10				
11	198	1/15	g|g	cg|gg
12	204	1/15	**C|G**	**GC|GU**
13	215	1/15	**C|G**	**AC|GG**
14	227	1/15	**C|G**	**AC|GU**
15	228	1/15	g|u	cg|ug
Extension 2 (GTTACTGCGGCGCACCGCGTC)				
16	138	1/21	c|u	gc|uc
17	140	3/21	**C|G**	**UC|GU**
18				
19				
20	142	1/21	u|u	gu|ug
21	146	1/21	u|c	au|cg
22	148	1/21	g|a	cg|ac
23	162	2/21	**C|G**	**GC|GC**
24				
25	164	4/21	**C|G**	**GC|GC**
26				
27				
28				
29	212	1/21	g|c	ag|ca
30	228	1/21	g|u	cg|ug
31	258	1/21	u|u	gu|ug
32	259	1/21	u|g	uu|gu
33	261	1/21	u|g	gu|gc
34	271	1/21	c|c	gc|cg
35	273	1/21	g|a/a|a	cg|aa / ga|aa
36	297	1/21	u|g	gu|gg

* Two nt and four nt refer to the cleavage sites seen in a two-nucleotide and four-nuclotide context, respectively

Table S3

Oligonucleotide name	Sequence
Riboprobes	
5S_RP	GTCCCATTCCGAACCCGGAAGCTAAGCCTGCCAGCGCCTGTCTC
DrrS_RP	GATCTGCCCGAAGTGCTGGGCGATTGAGCGGGTATGTACACCTGTCTC
DrrS_RPExt	GACCTACCGTCCCAAGACGGGGCTACCGCCTTCGGGCAGACCTGTCTC
MTS2823RP	GCACCCACGCGGAGTCATAGCCACGATAACGGCAGAAGCCTGTCTC
DrrS promoter fusions in pEJ414	
DrrS.coreF	CTAGATCCCGCCTGCTGTGTTTGGTGGCAGTATTGGTGATA
DrrS.coreR	AGCTTATCACCAATACTGCCACCAAACACAGCAGGCGGGAT
DrrS.coreStemloopf	CTAGATCCCGCCTGCTGTGTTTGGTGGCAGTATTGGTGATACCGGGGAAACCCGGTA
DrrS.coreStemloopr	AGCTTACCGGGTTTCCCCGGTATCACCAATACTGCCACCAAACACAGCAGGCGGGAT
DrrS.DosR2f	CTAGACAGGCGAAGTTTTATTGTCGGATAAGGGACTTTCGCCCCTTCCCGCCTGCTGTGTTTGGTGGCAGTATTGGTGATA
DrrS.DosR2r	AGCTTATCACCAATACTGCCACCAAACACAGCAGGCGGGAAGGGGCGAAAGTCCCTTATCCGACAATAAAACTTCGCCTGT
DrrS.DosR2Stemloopf	CTAGACAGGCGAAGTTTTATTGTCGGATAAGGGACTTTCGCCCCTTCCCGCCTGCTGTGTTTGGTGGCAGTATTGGTGATACCGGGGAAACCCGGTA
DrrS.DosR2Stemloopr	AGCTTACCGGGTTTCCCCGGTATCACCAATACTGCCACCAAACACAGCAGGCGGGAAGGGGCGAAAGTCCCTTATCCGACAATAAAACTTCGCCTGT
DrrS wt, 3’ variants and 5’extended in pIRaTE	
DrrS.wtf	GCGCTCTCGAGAGGATGAGGAT
DrrS.wtr	TAACTTACTAGTACCGGGGAAACCC
DrrS.extended3’f	TATACTCGAGAGGATGAGGATGAGGATCT
DrrS.cropped3’f	TATACTCGAGAGGATCTGCCCGAAG
DrrS.3’r	TAATCCATGGTAGCTTCCTTAGCTCCTG
DrrS.5’A_1r	TAACTTACTAGTAACCGGGGAAACCC
DrrS.5’A_2r	TAACTTACTAGTAAACCGGGGAAACCC
DrrS.5’A_3r	TAACTTACTAGTAAAACCGGGGAAACCC
DrrS.5’A_4r	TAACTTACTAGTAAAAACCGGGGAAACCC
3’ and 5’ RACE oligonucleotides	
DrrS.3’RACEf	GATCTGCCCGAAGTGCTGGGCGAT
DrrS.Ext1RACEf	CTTCGGGCAGATCCTCATCCTGTTAC
DrrS.Ext2RACEf	GTTACTGCGGCGCACCGCGTC

Oligo(d)T	GCTGTCAACGATACGCTACGTAACGGCATGACAGTGT_{24}
GeneRacer3’f	GCTGTCAACGATACGCTACGTAACG
GR5’	CGACTGGAGCACGAGGACACTGA
RNA linker	CGACUGGAGCACGAGGACACUGACAUGGACUGAAGGAGUAGAAA
DrrS.5’RACEr	AGGATCTGCCCGAAGGCGGTAGC
Mint	ACGAGGGGCATTCACACCAGATTG
518R	TTTCCCAGTCACGACGTTGTAAAA

Table S4

Plasmid ID	**Plasmid name**	**Construction**	**Origin, Marker**	**Reference**
pEJ414		Integrating promoterless *lacZ* reporter vector	Kan	[73]
pAM100	DrrS.core	Transcriptional fusion of DrrS core promoter region (-35 to +1) to *lacZ*; insert amplified with DrrS.coref/DrrS.corer	pEJ414, KanR	This study
pAM101	DrrS.DBS2	Transcriptional fusion of DrrS core promoter region with 1DBS and 2nd putative DosR binding site (-75 to +1) to *lacZ*; insert amplified with DrrS.DosR2f/ DrrS.corer	pEJ414, KanR	This study
pAM102	DrrS.coreStemloop	Transcriptional fusion of DrrS core promoter region with the addition of DrrS 5’ stem loop (ACCGGGGAAACCCGGT) to *lacZ*; insert amplified with DrrS.coreStemloopf/DrrS.coreStemloopr	pAM100, Kan	This study
pAM103	DrrS.DBS2Stemloop	Transcriptional fusion of DrrS core promoter region with 1DBS and 2nd putative DosR binding site with the addition of DrrS 5’ stem loop (ACCGGGGAAACCCGGT) to *lacZ*; insert amplified with DrrS.DosR2Stemloopf/ DrrS.DosR2Stemloopr	pAM101, Kan	This study
pEJ425		Integrating promoterless *lacZ* reporter vector	pEJ414, HygR	[72]
pKA425		Integrating promoterless *lacZ* reporter vector with NcoI site at lacZ ATG	pEJ425, HygR	This Study
pIRaTE		Integrating Regulator and Target Expression control plasmid, regulator overexpressed from *rrnB* (-80 to -8) promoter and terminated by SynB synthetic terminator	pKA425, HygR	This study
pIRaTE.001	DrrSwt	Wild type DrrS (+1 to +114) overexpressed from *rrnB* promoter; insert amplified with DrrS.wtf/DrrS.wtr	pIRaTE, HygR	This study
pIRaTE.002	DrrS.extended3’	DrrS with an extended 3’end (+1 to +114 plus CATCCT), overexpressed from *rrnB* promoter; insert amplified with DrrS.extended3’f/DrrS.wtr	pIRaTE.001, HygR	This study
pIRaTE.003	DrrS.cropped3’	DrrS with a cropped 3’end (+1 to 109), overexpressed from *rrnB* promoter; insert amplified with DrrS.cropped3’f/DrrS.wtr	pIRaTE.001, HygR	This study
pIRaTE.004	DrrS.5’A_1	DrrS (+1 to +114) extended at the 5’end with one adenine (A), overexpressed from *rrnB* promoter; insert amplified with DrrS.wtf/DrrS.5’A_1r	pIRaTE, HygR	This study
pIRaTE.005	DrrS.5’A_2	DrrS (+1 to +114) extended at the 5’end with two adenines (AA), overexpressed from *rrnB* promoter; insert amplified with DrrS.wtf/DrrS.5’A_2r	pIRaTE, HygR	This study
pIRaTE.006	DrrS.5’A_3	DrrS (+1 to +114) extended at the 5’end with three adenines (AAA), overexpressed from *rrnB* promoter; insert amplified with DrrS.wtf/DrrS.5’A_3r	pIRaTE, HygR	This study
pIRaTE.007	DrrS.5’A_4	DrrS (+1 to +114) extended at the 5’end with four adenines (AAAA), overexpressed from *rrnB* promoter; insert amplified with DrrS.wtf/DrrS.5’A_4r	pIRaTE, HygR	This study