

Supplementary information

Regulation of Hfq mRNA and protein levels in *Escherichia coli* and *Pseudomonas aeruginosa* by the *Burkholderia cenocepacia* MtvR sRNA

Christian G. Ramos, André M. Grilo, Sílvia A. Sousa, Joana R. Feliciano, Paulo J. P. da Costa and Jorge H. Leitão*

Department of Bioengineering and Institute for Biotechnology and Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, Torre Sul, Piso 6, 1049-001, Lisboa, Portugal.

*Corresponding author: Fax: +351 218419199; E-mail: jorgeleitao@tecnico.ulisboa.pt

Supplementary Methods

Bioinformatic analysis

BLAST searches were performed using the Integrated Microbial Genomes (IMG) webservice [1], using an E-value $\leq 1e^{-50}$ as cut-off. MtvR putative targets were predicted using the sRNATarget [2], the TargetRNA [3], and the RNAPredator [4] programs, with a cut-off of 1, a minimum seed of 7 nucleotides, an hybridization target region window size of -100 to + 30 around the translation start site.

Supplementary Results

Interaction of MtvR with additional *E. coli* sequences

A Blast search within the genome of *Escherichia coli* strain K-12 sub-strain MG1655, using a low complexity match and a word size of 6 revealed 9 intergenic regions with segments with homology to the *mtvR* sequence. The non-coding region between *lysV* and REPv173 contains the Intergenic Repeat Region (IRR) rtV, and within this region, the sequence 5'-cgcggcgacggcc-3', corresponding to nt 68 to 80 of MtvR. The rlQ, also a IRR found in the non-coding region between *lysQ* and *nadaA*, contains exactly the same sequence of MtvR found on rtV. A H-NS binding sequence of *E. coli* was also found in the non-coding region between *yciG* and *trpA*, sharing homology with nt 53-64 (5'-aatgccgccagc-3') of MtvR. The remaining 6 sequences had no identifiable features worth mentioning (Table S4).

This 13 nucleotide sequence match found within non-coding regions of the *E. coli* genome, together with its conservation along other enteric might represent an unidentified regulatory region in *E. coli*.

Additional sequences in *P. aeruginosa* PA14 genome with possible interaction with MtvR

Blast analysis using the MtvR encoding sequence to query the genome sequence of *P. aeruginosa* UCBPP-PA14, revealed a 100% match in a 17 nt (5'-GCCGCGCGTGCTGG-3') segment within an intergenic region 232 bp at the 5' side of a probable C4-dicarboxylate transporter and 550 bp at the 3' side of a arginine/ornithine antiporter. A thorough search revealed that this sequence is widely distributed along all organisms, including prokaryotes, eukaryotes and virus. Another search, using the

complete intergenic region, revealed a 38 nt region (5'-AAATGAAGAAGCCCGGCGAACGCCGGGCTTCTTCATTT-3') that is present only in prokaryotes, and in general, with a conservation over 80 %, found in intergenic regions. No conserved regulatory elements could be identified in this sequence. This sequence might represent a new uncharacterized regulatory region.

Table S1: Putative MtvR targets in the genome of *E. coli* predicted by TargetRNA

1	<u>hofN</u>	protein required for the utilization of DNA as a carbon source; fimbrial assembly protein homolog	
	Energy: -17.32		p-value: 0.000
sRNA	22	CUUAC-UGGGCGGC	10
		:	
mRNA (<i>hofN</i>)	-2	UGAUGAACCCGCCA	12
2	<u>malS</u>	alpha-amylase	
	Energy: -16.82		p-value: 0.000
sRNA	18	CUGGGCGGCGG-CAGU	4
		: :	
mRNA (<i>malS</i>)	5	AACUCGCCGCCUGUUU	20
3	<u>gidJ</u>	predicted sulfatase/phosphatase	
	Energy: -16.75		p-value: 0.000
sRNA	128	CGCGGGGUUAAUAAGGA	112
mRNA (<i>gidJ</i>)	6	ACGCCCAAUU-UU-CU	20
4	<u>yjbG</u>	conserved protein	
	Energy: -15.58		p-value: 0.000
sRNA	62	ACCGCCGUA	54
mRNA (<i>yjbG</i>)	-13	AGGCGGCAA	-5
5	<u>nagC</u>	DNA-binding transcriptional dual regulator, repressor of N-acetylglucosamine	
	Energy: -14.61		p-value: 0.001
sRNA	40	GCUGU-GUCCGCC	28
		:	
mRNA (<i>nagC</i>)	2	UGACACCAGGCGGA	15
6	<u>yfiQ</u>	CP4-57 prophage; predicted protein	
	Energy: -13.77		p-value: 0.002
sRNA	21	UUACUGGGCGGC	10
		:	
mRNA (<i>yfiQ</i>)	-1	UAUGACCCGUCU	11

7	<u>uhpA</u>	DNA-binding response regulator in two-component regulatory system with UhpB	
	Energy: -13.01		p-value: 0.004
sRNA	18	CUGGGCGGC	10
mRNA (<i>uhpA</i>)	-19	UACCCGCCA	-11
<hr/>			
8	<u>yceG</u>	predicted aminodeoxychorismate lyase	
	Energy: -12.99		p-value: 0.004
sRNA	130	AUCGCGGGGUAAUA	116
mRNA (<i>yceG</i>)	-17	GAGCGCCCGAAUUAG	-3
<hr/>			
9	<u>dacD</u>	D-alanyl-D-alanine carboxypeptidase (penicillin-binding protein 6b)	
	Energy: -12.91		p-value: 0.004
sRNA	20	UACUGGGCGGCGGC	7
		:	
mRNA (<i>dacD</i>)	1	UUGAAACGCCGUCU	14
<hr/>			
10	<u>csiD</u>	carbon starvation induced gene	
	Energy: -12.72	p-value: 0.005	
sRNA	20	UAC-UGGGC-GGCGGCAG	5
		:	
mRNA (<i>csiD</i>)	4	AUGCA-CUGACCGCCGU-	20
<hr/>			
11	<u>mpaA</u>	murein peptide amidase A	
	Energy: -12.7		p-value: 0.005
sRNA	21	UACUGG-G--CGGCGGC	7
		:	
mRNA (<i>mpaA</i>)	1	AUGACCGUAACCCGCC	17
<hr/>			

Table S2: Putative MtvR targets in the genome of *E. coli* predicted by RNAPredator

Energy [kJ/mol]	z-Score	Interaction	mRNA [Start]	mRNA [End]	sRNA	Gene Annotation
-14.14	-1.88	((((((((((((((((((((((&)))))).....)))))))))))))) mRNA: 5' - AAAUUAUGGCGCCGCC----- -3' sRNA: 3' - UUUAAUAUCGCGG-GGUAAUA -5'	-5	16	109-136	conserved protein; UPF0161 family
-13.63	-1.7	(((((((.((((((&)).)))))).)))))) mRNA: 5' - AGCGCCCGAAUUAGUC -3' sRNA: 3' - UCGCGGGGUAAUAAG -5'	-16	-1	114-129	<u>predicted aminodeoxychorismate lyase</u>
-13.33	-1.59	(((.(((.(((.(((&))))).)).))) mRNA: 5' - GCCCCUCUUACACCUGCU -3' sRNA: 3' - CGGGGUAAUAAGGAC-A -5'	2	20	109-126	predicted anti terminator regulatory protein

Table S3: Putative MtvR targets in the genome of *E. coli* predicted by sRNATarget

1	<i>nfuA_b3414</i>	predicted gluconate transport associated protein
	Probability = 1	
	sRNA (sRNA1)	11 ggCGGGUCAUucuaU 25 . .
	Target (<i>nfuA_b3414</i>)	10 auGCCUAGUAucau -5
2	<i>amiA_b2435</i>	N-acetylmuramoyl-l-alanine amidase I
	Probability = 1	
	sRNA (sRNA1)	74 gACGGCCUGu 83
	Target (<i>amiA_b2435</i>)	1 aUGCCGGACu -9
3	<i>gpmM_b3612</i>	phosphoglycero mutase III, cofactor-independent
	Probability = 1	
	sRNA (sRNA1)	68 cGCGGCGACg 77 . .
	Target (<i>gpmM_b3612</i>)	-1 aCGCUGUUGg -10
	Probability = 1	
	sRNA (sRNA1)	71 gGCGACGGCCUg 82 .
	Target (<i>gpmM_b3612</i>)	-1 aCGCUGUUGGAg -12
4	<i>pmrD_b2259</i>	polymyxin resistance protein B
	Probability = 1	
	sRNA (sRNA1)	90 cGCAUCGCAa 99
	Target (<i>pmrD_b2259</i>)	72 uCGUAGCGUc 63
	Probability = 1	
	sRNA (sRNA1)	10 cggcgGUCAUUCUAUc 26
	Target (<i>pmrD_b2259</i>)	16 acugguCGGUAAGGUAa -1
	Probability = 1	
	sRNA (sRNA1)	55 uGCCGCCAGCA-cgcg 69
	Target (<i>pmrD_b2259</i>)	79 gCGGCGGUCGUagcgu 64

9 *mntR_b0817* DNA-binding transcriptional regulator of mntH
 Probability = 1
 sRNA (sRNA1) 32 cCUGUGUCGc 42
 |||.|||.|
 Target (mntR_b0817) 46 uGACGCAGUGa 36
 Probability = 1
 sRNA (sRNA1) 66 cGCGCGGCGACg 77
 |||||.||||
 Target (mntR_b0817) 15 aCGCGCUGCUGa 4

10 *relE_b1563* Qin prophage; toxin of the RelE-RelB toxin-antitoxin system
 Probability = 1
 sRNA (sRNA1) 77 gGCCUGUUUGc 87
 |||.|.|.|
 Target (relE_b1563) 33 aCGGGCGAGCa 23
 Probability = 1
 sRNA (sRNA1) 19 auucuaucacccgccugugucgcaaaagac-GGGAAUGCcgccag 63
 |||.|. ||| |||| |.||||. | ||||
 Target (relE_b1563) 42 aaggaaauca--cgggcg-agcaguuuagguCUUUUAUG-cggu 1

11 *ymcE_b0991* cold shock gene
 Probability = 1
 sRNA (sRNA1) 85 uCGCGCGCAUc 95
 |||||
 Target (ymcE_b0991) 10 uCGCCGCGUAc -1
 Probability = 1
 sRNA (sRNA1) 6 aCGGCGGCG---ggucau 20
 |.||||| |||||
 Target (ymcE_b0991) 12 gGUCGCCGcuaccaguc -6

12 *hybA_b2996* hydrogenase 2 4Fe-4S ferredoxin-type component
 Probability = 1
 sRNA (sRNA1) 74 gacggcCUGUUUGCGGc 90
 ||| ||||| |||
 Target (hybA_b2996) 13 augca-GACAAGUGCCa -3

13 *apaH_b0049* diadenosine tetraphosphatase
 Probability = 1
 sRNA (sRNA1) 7 cggCGGCGGGUCA 19
 ..|||||.|||
 Target (apaH_b0049) -24 cuuGCCGCUCAGc -36

Probability = 1
 sRNA (sRNA1) 33 cUGUGUCGCCAa 44
 |.|||||||
 Target (apaH_b0049) 12 cAUACAGCGGua 1
 Probability = 1
 sRNA (sRNA1) 36 uGUCGCCAA-aaga 48
 ||||| |||
 Target (apaH_b0049) 25 gCAGCGGUUauucc 12

14 *lamB_b4036* maltose outer membrane porin (maltoporin)
 Probability = 1
 sRNA (sRNA1) 7 cGGCGGCGGGuca 19
 |||||.||.
 Target (*lamB_b4036*) -141 aCCGCCGUCCggc -153
 Probability = 1
 sRNA (sRNA1) 15 ggUCAUUCUAUCacccgccugugucg 40
 .||||||| ||| |||
 Target (*lamB_b4036*) 6 guAGUAAGAUAGa----ggacucagu -16

15 *srmB_b2576* ATP-dependent RNA helicase
 Probability = 1
 sRNA (sRNA1) 120 uUGGGGCGCU 129
 |||||
 Target (*srmB_b2576*) -14 cACCCGCGA -23

16 *dusC_b2140* tRNA-dihydrouridine synthase C
 Probability = 1
 sRNA (sRNA1) 7 cgg-CGGCGGGuC 18
 |||.||||.
 Target (*dusC_b2140*) -42 accaGUCGCCUaa -54
 Probability = 0.997
 sRNA (sRNA1) 22 cuAUCACCCGc 33
 .|||||||
 Target (*dusC_b2140*) -2 agUAGUGGGCGu -13

17 *metC_b3008* cystathionine beta-lyase, PLP-dependent
 Probability = 1
 sRNA (sRNA1) 85 ugc-GGCGCAUCg 96
 || |||||
 Target (*metC_b3008*) -52 gcgcCCGCGUAGg -64
 Probability = 1
 sRNA (sRNA1) 28 cccgcCUGUGUCG----cca 43
 ||| |..||| ||
 Target (*metC_b3008*) 8 aggc-GGUACAGCccuaagga -12

18 *lpxT_b2174* undecaprenyl pyrophosphate phosphatase
 Probability = 1
 sRNA (sRNA1) 8 gGCGGCGGGUcauucuaucac 28
 |||||...| |||
 Target (*lpxT_b2174*) 20 aCGCCGUUUAAAAau-uagua 1
 Probability = 1
 sRNA (sRNA1) 74 gacggc-CUGUUUGCGGCg 91
 .|...| ||.|||||
 Target (*lpxT_b2174*) 32 uuguuguGAUAAACGCCGu 14

19 *hypC_b2728* protein required for maturation of hydrogenases 1 and 3
 Probability = 1
 sRNA (sRNA1) 48 aCGGAAUGCCg 59
 |||||.|||
 Target (*hypC_b2728*) 20 gGCCCUUGCGGa 9
 Probability = 1
 sRNA (sRNA1) 5 gACGGCGGGGUCAuucua 24
 |||||.||||| ||
 Target (*hypC_b2728*) -130 aUGCCGUUGUCCAGUu-gaa -148

20 *idnK_b4268* D-gluconate kinase, thermosensitive
 Probability = 1
 sRNA (sRNA1) 87 cGGCGCAUCGCa 98
 |||.||||.|
 Target (idnK_b4268) -93 uCCGUGUAGUGc -104
 Probability = 1
 sRNA (sRNA1) 24 aUCACCCGCCu 34
 |||||
 Target (idnK_b4268) 12 aAGUGGGCGGu 2
 Probability = 0.995
 sRNA (sRNA1) 66 cgCGCGGCGACg 77
 .|||||.||
 Target (idnK_b4268) 77 uuGCGCCGUUGg 66

21 *zitB_b0752* zinc efflux system
 Probability = 1
 sRNA (sRNA1) 10 cGGCGGGUCAUUCu 23
 |.|||.||||.|.
 Target (zitB_b0752) 1 aUGCUCAGUAGGg -13

22 *yjeS_b4166* predicted Fe-S electron transport protein
 Probability = 1
 sRNA (sRNA1) 3 uugacggcGGCGGGUCAUu 21
 ||||. |.||| |||
 Target (yjeS_b4166) 7 gacugua-CUGCCUGGUAu -11

23 *ydjJ_b1774* predicted oxidoreductase, Zn-dependent and NAD(P)-binding
 Probability = 1
 sRNA (sRNA1) 16 gucauucuaucacCCGCCUGUGUc 39
 ||||| ||.||.||.
 Target (ydjJ_b1774) 5 aaguaaaacuua-GGUGGGCAUaa -18

24 *fliM_b1945* flagellar motor switching and energizing component
 Probability = 1
 sRNA (sRNA1) 29 cCGCCUGUGUCGc 41
 ||||..|||||
 Target (fliM_b1945) 8 aGCGGGUACAGCa -5

25 *recE_b1350* Rac prophage; exonuclease VIII, 5' -> 3' specific dsDNA exonuclease
 Probability = 1
 sRNA (sRNA1) 107 uGACAGGAuaauuggggc---gcua 130
 |.||||||| ||| |||
 Target (recE_b1350) 28 cAUUGCCUucuc-accaaacacgag 3

26 *hrpB_b0148* predicted ATP-dependent helicase
 Probability = 1
 sRNA (sRNA1) 3 ugaCGGCGGCGGGucau 20
 .| |.||..||||.
 Target (hrpB_b0148) 26 ugccGUCGUUGCCCGuu- 10
 Probability = 1
 sRNA (sRNA1) 4 uGACGGCGGCG 14
 |||||.||
 Target (hrpB_b0148) 28 uCUGCCGUCGu 18

Probability = 1
 sRNA (sRNA1) 6 aCGGCGGCGg 15
 |..||.||
 Target (hrpB_b0148) 13 cGUUGCUGCu 4
 Probability = 1
 sRNA (sRNA1) 4 ugACGGCGGC-----gggu---cauucua 24
 .|||.||. | ||| |||||
 Target (hrpB_b0148) 12 guUGCUGCUGuguuuugcgaggucucccaauuguaagaa -27
 Probability = 0.999
 sRNA (sRNA1) 7 cGGCGGCGGg 16
 |.||||.|
 Target (hrpB_b0148) 28 uCUGCCGUCg 19

27 *yhdH_b3253* predicted oxidoreductase, Zn-dependent and NAD(P)-binding
 Probability = 1
 sRNA (sRNA1) 120 uGGGGCGCGuauau 134
 ||..|||| ||||
 Target (yhdH_b3253) -76 uACUUCGCGuuuuu -90
 Probability = 0.996
 sRNA (sRNA1) 19 auucuaucacc--CGCCUGUGUc 39
 ||||| |||||.||
 Target (yhdH_b3253) 22 caagauuuucauuGCGGACGUAu -1

28 *creB_b4398* DNA-binding response regulator in two-component regulatory system with CreC
Probability = 1
sRNA (sRNA1) 71 ggcg-ACGGCCUGUu 84
. ||| |||. |||||
Target (creB_b4398) -126 ucgcgUGCUGGACAU -140
Probability = 1
sRNA (sRNA1) 36 ugucgccaaaagacgggaaUGCCGCCA--gca 65
. ||||| ||||| ||
Target (creB_b4398) -2 uuagcggagacaaua----ACGGCGGUgccgu -29

29 *yahD_b0318* predicted transcriptional regulator with ankyrin domain
Probability = 1
sRNA (sRNA1) 9 gCGGCGGGU-----cauuc-uaucacccg 31
||||. ||| |||||. || |||
Target (yahD_b0318) 26 aGCCGUCCAucuaaaaaauaugaguaaguguaagggga -12
Probability = 1
sRNA (sRNA1) 22 cUAUCACCCgccugugucgcaaa 45
||||||| |||. |||||
Target (yahD_b0318) 56 uAUAGUGGGacaacacgucgguaa 33

30 ***fepB_b0592*** ***iron-enterobactin transporter subunit***
Probability = 1
sRNA (sRNA1) 4 uga-cggcGGCGGGUCA 19
|| ||| ||||. |||
Target (fepB_b0592) 19 ucucgcc-CCGCUCAGa 4
Probability = 1
sRNA (sRNA1) 46 agacgggaaugccGCCAGCACg 67
|| |||||. |||||
Target (fepB_b0592) -98 acucccuugu--CGGUCGUGa -117

31 *polB_b0060* DNA polymerase II
Probability = 1
sRNA (sRNA1) 76 cggCCUGUUGCGgcgca 93
.. ||||.. |||| |.|
Target (polB_b0060) 17 uuuGGACGGACGCggugc -1
Probability = 1
sRNA (sRNA1) 12 gcgggucauucuaucaccGCCUGUGUCGc 42
||||| || ||| |||||. |.|
Target (polB_b0060) 30 agcccauuucuuuuuggaCGGACGGGUGc -1

32 *yfcS_b2336* predicted periplasmic pilus chaperone
Probability = 1
sRNA (sRNA1) 13 cGGGUCAUUCu---aucacc 29
||.|||||. |||||
Target (*yfcS_b2336*) 1 aCCUAGUAAGgaauuaguc -19
Probability = 1
sRNA (sRNA1) 105 uguug--ACAGGAAUAauuggggc 126
|.|| ||||.||. | ||||
Target (*yfcS_b2336*) -29 ucgacuuUGUCUUUGUc--ccccu -50
Probability = 1
sRNA (sRNA1) 66 cGCGCGGCGacggccuguuugc-ggcg 91
|||||.|| |||| ||| |||
Target (*yfcS_b2336*) -114 cGCGCUGCaa-cggaaauacgaccgu -139

33 *fhuE_b1102* ferric-rhodotorulic acid outer membrane transporter
Probability = 1
sRNA (sRNA1) 100 aAAAGUGUgacaggaauaa-uuggg 124
||||..|| |.||| |||||
Target (*fhuE_b1102*) 8 cUUUCGUAAa---cuuagagaacca -15

34 *uhpC_b3667* membrane protein regulates uhpT expression
Probability = 1
sRNA (sRNA1) 91 gcaucgcaaaaaaguguuGACAGGAAu 117
.| || ||| |||. ||||| |||||
Target (*uhpC_b3667*) -41 ugu-gcgcacgg-cacguCUGUCCUUu -65
Probability = 1
sRNA (sRNA1) 4 ugACGGCGGCgggucauucuauca 27
.|||||.|| .||||. |.|
Target (*uhpC_b3667*) 12 uuUGCCGUUG--uaguaguugga -10
Probability = 1
sRNA (sRNA1) 87 cGGCGCAUCG-caaaaaaguguugacaggaau 117
||||||| || |.|. | |||.||.
Target (*uhpC_b3667*) 36 aCCGCGUAGCcguc---cgcgaagucuuug 9
Probability = 1
sRNA (sRNA1) 34 ugugucgcaaaa-AGACGGGAA-----ugccgcc-agca 65
|.|| ||| ||||.|||| | ||||.|| |||
Target (*uhpC_b3667*) -44 gcgca-cggcacgUCUGUCCUUuacacauuacacggugggucgc -86

50 *mscS_b2924* mechanosensitive channel
 Probability = 0.995
 sRNA (sRNA1) 1 -UAUUGACGGCgg-cgggucauucuauc 26
 |||.|||.|. |...|| |||.||
 Target (*mscS_b2924*) 26 gAUAGCUGUUGuaaguuuag-aagguaa -1

51 *mak_b0394* manno(fructo)kinase
 Probability = 0.99
 sRNA (sRNA1) 34 uGUGUCGCCaaaagacgggaaugccgccagcacgcgcgacgg-ccug 82
 |||.|||| ||| || ||.|| | |||.||| || |||
 Target (*mak_b0394*) 30 cCACGGCGGauua-gc---uauggaua---ugcgugcca-ugagaggaa -12

52 *dapA_b2478* dihydrodipicolinate synthase
 Probability = 0.99
 sRNA (sRNA1) 67 gCGCGGCGAcgg--ccuguuugc---ggcgcauc-----gcaaaaaaguguugaca-ggaauaaauugg---ggcgc 128
 |||||.|.| ||.|| .| ||| |||| | |||| | |||||. |||. |||| ||||.
 Target (*dapA_b2478*) 25 aGCGCUGUUaugaagggcacuuguacccg-guaggagacacguuuguucacagaguuaccaugcaaaccuaccgua -51

6- (1500400..1500411) non-coding region between *tehB* and *ydcL*

Score = 24.3 bits (12), Expect = 28

Identities = 12/12 (100%)

Strand = Plus / Minus

Query: 98 aaaaaagtgttg 109
 |||
 Sbjct: 1500411 aaaaaagtgttg 1500400

7- (1405859..1405870) non-coding region between *ydaM* and *ydaN*

Score = 24.3 bits (12), Expect = 28

Identities = 12/12 (100%)

Strand = Plus / Plus

Query: 93 atcgcaaaaaag 104
 |||
 Sbjct: 1405859 atcgcaaaaaag 1405870

8- (1314409..1314420) non-coding region between *yciG* and *trpA*

Score = 24.3 bits (12), Expect = 28

Identities = 12/12 (100%)

Strand = Plus / Plus

Query: 53 aatgccgccagc 64
 |||
 Sbjct: 1314409 aatgccgccagc 1314420

9- (741938..741949) non-coding region between *dtpD* and *ybgI*

Score = 24.3 bits (12), Expect = 28

Identities = 12/12 (100%)

Strand = Plus / Minus

Query: 39 cgccaaaagacg 50
 |||
 Sbjct: 741949 cgccaaaagacg 741938

Table S5: Putative MtvR targets in the genome of *P. aeruginosa* UCBPP-PA14 predicted by TargetRNA

1 PA14_15920 major facilitator transporter

Energy: -21.91 p-value: 0.000

sRNA 21 UUACUGGGCGGCGGC 7
 |||| |

mRNA (PA14_15920) -1 CAUGA-CCGCCGCCA 13

2 PA14_39850 MFS transporter

Energy: -21.71 p-value: 0.000

sRNA 21 UAC---U-GGGCGGCGGCAGU 4
 ||| | ||||| ||

mRNA (PA14_39850) 1 AUGACCACCCGCCGCCUC- 20

3 katA catalase

Energy: -21.03 p-value: 0.000

sRNA 24 AUCUUACUGGGCGGC 10
 |||| |

mRNA (*katA*) 7 GAGAA-GACCCGCCU 20

4 fepB iron-enterobactin transporter periplasmic binding protein

Energy: -20.33

p-value: 0.000

sRNA 19 ACUGGGCGGCGGCA 6
 |||||

mRNA (*fepB*) 5 CGACCCGCCGCCGC 18

5 PA14_69340 ABC transporter ATP-binding protein

Energy: -20.29 p-value: 0.000

sRNA 17 UGGGCGGCGGC 7
 :|||

mRNA (PA14_69340) -10 CUCCGCCGCCA 1

6 PA14_18050 hypothetical protein

Energy: -20.18 p-value: 0.000

sRNA 17 UGGGCGGCGGCA 6
 |||||

mRNA -20 CCCC GCCCGCA -9

(PA14_18050)

7 PA14_69880 LysR family transcriptional regulator

Energy: -19.74 p-value: 0.000

sRNA 22 CUUACUGGGCGGCGGC 7

|||||||:||||

mRNA (PA14_69880) -2 CAAUGACCCGUCGCCU 14

8 modA molybdate-binding periplasmic protein precursor modA

Energy: -18.98 p-value: 0.000

sRNA 21 UUACUGG-GCGGCGGC 7

||||| | ||||

mRNA (*modA*) -1 CAUGACCAC-CCGCCU 14**9 ureD** urease accessory protein

Energy: -18.81 p-value: 0.000

sRNA 21 UUACUGGGCGGCGGCAGU 4

:|||| ||||| ||

mRNA (*ureD*) -2 GAUGA-CCGCCGCCUCC 16**10 PA14_56960** hypothetical protein

Energy: -18.31 p-value: 0.000

sRNA 20 UACUGGG--CGGCGGCAGU 4

||||| |||||

mRNA (PA14_56960) 1 AUGACCCAGGCCCGUCC 19

11 gacA response regulator GacA

Energy: -17.85 p-value: 0.000

sRNA 71 GC--G--CGCACGACCGCCGU 55

:| : | |||||:|:

mRNA (*gacA*) 1 UGAUUAAG-GUGCUGGUGGU- 20**12 PA14_53090** transcriptional regulator

Energy: -17.85 p-value: 0.000

sRNA 19 ACUGGGCGGCGGC 7

||||||| ||

mRNA (PA14_53090) 7 CGACCCGCC-CCA 18

13 PA14_12610 transporter

Energy: -17.78 p-value: 0.000

sRNA	130	AUCGCGGGGUU	120

mRNA (PA14_12610)	-9	AAGCGCCCAU	2
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14 PA14_28980 Fe²⁺-dicitrate sensor

Energy: -17.7 p-value: 0.000

sRNA	21	UACUGGGCGGCGGC	7
		:	

mRNA (PA14_28980)	-20	AUGACCCGUGCCU	-7
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15 fadH1 2,4-dienoyl-CoA reductase

Energy: -17.64 p-value: 0.000

sRNA	20	UACUGGGCGGCGGCAGU	4
		:	

mRNA (<i>fadH1</i>)	-1	AUGA-CCGCCCGUUC	16
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16 PA14_53920 transcriptional regulator

Energy: -17.63 p-value: 0.000

sRNA	21	UACUGGG-C---GGCGGC	7

mRNA (PA14_53920)	1	AUGACCCAGAACCCGCC	18
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17 PA14_18060 hypothetical protein

Energy: -17.58 p-value: 0.000

sRNA	18	CUGGGCGGCGGC	7
		: :	

mRNA (PA14_18060)	6	CAUCCGCCGCUA	17
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18 aceK bifunctional isocitrate dehydrogenase kinase/phosphatase

Energy: -17.53 p-value: 0.000

sRNA	130	AUCGCGGGUUA	119
		:	

mRNA (<i>aceK</i>)	9	GAGCGCCCAGC	20
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19 PA14_15280 hypothetical protein

Energy: -16.95 p-value: 0.000

sRNA	21	UUACUGGGCGGCGG	8

mRNA (PA14_15280)	-1	CAUGACCCACCGCA	13
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20 PA14_20491 acetyltransferase

Energy: -16.87 p-value: 0.000

sRNA	83	UGUCCGGCAGCGGC	70

mRNA (PA14_20491)	6	CCAGACCGUCGCCA	19
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21 PA14_50850 hypothetical protein

Energy: -16.5 p-value: 0.000

sRNA	23	UCUUACUGGGCGGC	10

mRNA (PA14_50850)	-3	CGAAUGACCC-CCU	10
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22 PA14_22280 pirin-related protein

Energy: -16.39 p-value: 0.000

sRNA	21	UUACUGGGCGGCGCA	6
		:	

mRNA (PA14_22280)	-1	CAUGACCCGCUACCGU	15
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23 PA14_44770 guanine deaminase

Energy: -16.34 p-value: 0.000

sRNA	21	UUACUGGGCG--GCGGC	7

mRNA (PA14_44770)	-1	CAUGACCCGCAACGCC-	15
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24 PA14_29090 periplasmic spermidine/putrescine-binding protein

Energy: -15.88 p-value: 0.000

sRNA	23	UCUUACUGGGCG--GCGGC	7
		:	

mRNA (PA14_29090)	2	UGAA-GACCCGCAUCGCUU	19
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25 PA14_40280 hypothetical protein

Energy: -15.57 p-value: 0.000

sRNA	23	UCUU--ACUGGGCGGCGGCA	6
		:	
mRNA (PA14_40280)	2	UGAGCCUUUCCC-CCGCCGA	20

26 PA14_31070 hypothetical protein

Energy: -15.57 p-value: 0.000

sRNA	77	GCAGCGGCGCGCACGACCGCC	57
		: : :	
mRNA (PA14_31070)	2	UGUCGUCGCGGUGC-AGU-G	20

27 fepC ferric enterobactin transport protein FepC

Energy: -15.5 p-value: 0.000

sRNA	21	UUACUGGGCGGCGGC	7
		:	
mRNA (<u>fepC</u>)	-1	CAUGACCCAUCGCCU	14

28 PA14_38950 hypothetical protein

Energy: -15.49 p-value: 0.000

sRNA	18	CUGGGC-GGCGGCAG	5
		:	
mRNA (PA14_38950)	2	UGCCCACC GCCGUA	16

29 pykA pyruvate kinase

Energy: -15.45 p-value: 0.000

sRNA	81	UCCGGC-AGCGGCGC	68
mRNA (pykA)	2	UGUCCGUUCGCCGCA	16

30 phaD poly(3-hydroxyalkanoic acid) depolymerase

Energy: -15.26 p-value: 0.001

sRNA	15	GCGGCGGCA	6
mRNA (phaD)	-20	CGCCGCCGA	-12

31 PA14_29760 chemotaxis transducer

Energy: -15.25 p-value: 0.001

sRNA	17	UGGGCGGGCGG-CAGU	4
		:	
mRNA (PA14_29760)	7	CUUCGCCGCCUGUC-	20

32 PA14_26350 hypothetical protein

Energy: -15.08 p-value: 0.001

sRNA	75	AGCGGCGCGC	66
mRNA (PA14_26350)	-20	ACGCCGCGCA	-11

33 algF alginate o-acetyltransferase AlgF

Energy: -15.01 p-value: 0.001

sRNA	22	CUUACUGGGCGGCG	9
		:	
mRNA (<i>algF</i>)	8	CGAUGACCCGCCG-	20

34 PA14_15190 hypothetical protein

Energy: -15 p-value: 0.001

sRNA	17	UGGGCGGGCGGCAGUU	3
		:	
mRNA (PA14_15190)	-4	UCCC-AUGCCGUCAU	10

35 PA14_43650 hypothetical protein

Energy: -14.84 p-value: 0.001

sRNA	76	CAGCGGCGCGCACGA	62
		:	
mRNA (PA14_43650)	7	AUCGCUUCGCGUGC-	20

36 PA14_17990 hypothetical protein

Energy: -14.83 p-value: 0.001

sRNA	21	UUACUGGGCGGCGGC	7
		:	

mRNA (PA14_17990)	-1	CAUGACCC-CUGCCA	13
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37 PA14_53210 hypothetical protein

Energy: -14.56 p-value: 0.001

sRNA	19	ACUGGGCGGCGGCA	6

mRNA (PA14_53210)	7	GGAACCGCCCGCA	20
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38 PA14_03490 hypothetical protein

Energy: -14.47 p-value: 0.001

sRNA	21	UUACUGG-GCGGCGGCAG-U	4

mRNA (PA14_03490)	-1	CAUGACCAC-CCGCC-UCGA	17
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39 PA14_46360 two-component response regulator

Energy: -14.41 p-value: 0.001

sRNA	24	AUCUUACUGGGCGGCG	9
		:	

mRNA (PA14_46360)	-4	AAGAAUGACUGGCCG-	11
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40 PA14_15720 transglycosylase

Energy: -14.25 p-value: 0.001

sRNA	71	GCGCGCACGACCGCCG	56

mRNA (PA14_15720)	-5	AGCGGUGCAGAAGG-	10
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41 pilF type 4 fimbrial biogenesis protein PilF

Energy: -14.24 p-value: 0.001

sRNA	78	GGCAGCGGCGCGC	66

mRNA (<i>pilF</i>)	9	ACG-CGCCGCGCU	20
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42 recO DNA repair protein RecO

Energy: -14.18 p-value: 0.001

sRNA	19	ACUGGGCGGCGGCAGU	4
		:	
mRNA (<i>recO</i>)	-9	CGACCCGCC-AUGUCC	6

43 PA14_20880 hypothetical protein

Energy: -14.12 p-value: 0.002

sRNA	17	UGGG---CGGCGGCAGUU	3
mRNA (PA14_20880)	-16	CCCCUGAGCCGCCGACAU	2

44 PA14_26260 hydrolase

Energy: -14.03 p-value: 0.002

sRNA	27	ACUAUCUUACUGG-GCGGC	10
mRNA (PA14_26260)	-6	AGA-AGAAUGACCGAGCCU	12

45 nosF NosF protein

Energy: -13.92 p-value: 0.002

sRNA	17	GGGCGGCGGCA	6
mRNA (<i>nosF</i>)	-20	CCCGCCGCGGA	-10

46 PA14_11650 hypothetical protein

Energy: -13.81 p-value: 0.002

sRNA	17	GGGCGGCGGCAGUUA-	2
		:	
mRNA (PA14_11650)	-20	CCCGCCGCC--CAGUG	-7

47 PA14_12670 hypothetical protein

Energy: -13.74 p-value: 0.002

sRNA	66	CACG-ACCGCCGUAAGGGC	49
		:	
mRNA (PA14_12670)	1	AUGCGGCGGCAAGCUCU	19

48 PA14_49720 hypothetical protein

Energy: -13.64 p-value: 0.002

sRNA	20	UACUG-GGCGGCGGCAG	5
		:	

mRNA (PA14_49720)	1	AUGUCACUGCCGCGUA	17
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49 PA14_68920 LysR family transcriptional regulator

Energy: -13.57 p-value: 0.002

sRNA	19	ACUGG--GCGGCGGC	7

mRNA (PA14_68920)	6	CGACCUACGCCGCCU	20
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50 PA14_40650 hypothetical protein

Energy: -13.31 p-value: 0.003

sRNA	23	UCUUAC-UGGGCGGCGGC	7
		:	

mRNA (PA14_40650)	-15	CGGA-GAACCCGCC-CCA	1
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51 PA14_46310 hypothetical protein

Energy: -13.25 p-value: 0.003

sRNA	23	UCUUACUGGGCGGC-GGCA	6
		:	

mRNA (PA14_46310)	-3	CGCAUGACCCG-UGACCG-	14
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52 PA14_42920 hypothetical protein

Energy: -13.18 p-value: 0.003

sRNA	39	CUGUGUCCGCCACUA	24
		:	

mRNA (PA14_42920)	-20	GGCA-AGGCGGGUGAC	-6
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53 PA14_29210 MFS transporter

Energy: -13.17 p-value: 0.003

sRNA	19	AC-UGGGCGGCGGC	7

mRNA (PA14_29210)	6	CGCACCCGCCGCCU	19
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54 PA14_37910 LysR family transcriptional regulator

Energy: -13.14 p-value: 0.003

sRNA	19	ACUGGGC-GGCGGCAGUUA	2
		: :	

mRNA (PA14_37910)	3	GGACCUGAAUGCCGUCAA-	20
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55 PA14_58440 ABC transporter permease

Energy: -13.12 p-value: 0.003

sRNA	24	AUCUACUGGGCGGC	10
		: :	

mRNA (PA14_58440)	-17	AGGAGUUACCCGCCA	-3
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56 PA14_16740 hypothetical protein

Energy: -13.11 p-value: 0.003

sRNA	19	ACU-GGGCGGCGGC	7

mRNA (PA14_16740)	-13	GGAGCCCGCCGCCA	1
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57 PA14_05040 coproporphyrinogen III oxidase

Energy: -13.1 p-value: 0.003

sRNA	17	UGGGC--GGCGGCAGUU	3

mRNA (PA14_05040)	-12	CCCCGCCCGCCGUGAC	5
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58 PA14_71100 hypothetical protein

Energy: -13.09 p-value: 0.003

sRNA	18	CUGGGCGGCGGCAGUUA	2

mRNA (PA14_71100)	6	CACCCUCC-CCGUCAA-	20
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59 napF ferredoxin protein NapF

Energy: -13.02 p-value: 0.004

sRNA	80	CCGGCAGCGG--CGCGC	66

mRNA (<i>napF</i>)	4	AGCAGUCGCCGAGAGCU	20
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60 PA14_06460 hypothetical protein

Energy: -12.99 p-value: 0.004

sRNA 75 GCGGCGCGC 66

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mRNA (PA14_06460) -20 CGCCGCGCA -12

61 adhA alcohol dehydrogenase

Energy: -12.97 p-value: 0.004

sRNA 27 CUAUCU--UACUGGG-CGGCGG 8

||| || ||||| ||| |

mRNA (*adhA*) -7 GAU-GACCAUGACCCUGCCACA 14**62 PA14_61990** hypothetical protein

Energy: -12.84 p-value: 0.004

sRNA 19 ACUGGGCGGCGCA 6

|||||: ||

mRNA (PA14_61990) 8 AGACCCGCU-ACGG 20

63 PA14_60550 ATP-dependent protease

Energy: -12.77 p-value: 0.004

sRNA 79 CGGCAGCGGCGC 68

|||||: ||

mRNA (PA14_60550) 10 UCCGUCGUCG- 20

64 PA14_58110 Maf-like protein

Energy: -12.65 p-value: 0.005

sRNA 83 UGUCCGGCAGCGGCGC 68

|| |||||: |:

mRNA (PA14_58110) -2 CCAUGCCGUCGUGUA 14

Table S6: Putative MtvR targets in the genome of *P. aeruginosa* UCBPP-PA14 predicted by RNAPredator

Energy [kJ/mol]	z-Score	Interaction	mRNA [Start]	mRNA [End]	sRNA	Gene Annotation
-16.06	-2.49	((((.(((((((..(((((((&)))))))))))))).))) mRNA: 5'- GAUCGCAUGACCCAGGCCGCCG -3' sRNA: 3'- CUAUCUUACUGGG--CGGCGGC -5'	-6	17	5-26	hypothetical protein 56960
-16.04	-2.48	(((((...(((((((&)))))))))).)))) mRNA: 5'- GAUGGACUGGACCCGCCGC -3' sRNA: 3'- CUAUCUUA-CUGGGCGGCG -5'	-1	18	8-26	transcriptional regulator
-15.91	-2.43	(((((...((((((((&)))))))))).))..)))) mRNA: 5'- GCGGGUGCCGGCAUGACCCAGCCGC -3' sRNA: 3'- CGCCACUAUCUUACUGGG-CGGCG -5'	-12	13	8-32	hypothetical protein 34940
-15.49	-2.28	(((((....(((((((..(((((((&)))))))))).)).....)))))) mRNA: 5'- GUAGCCCAUGC-----CCCA-UCCCAU -3' sRNA: 3'- UAUCG----CGGGGUUAA-UAAGGACA -5'	-6	20	104-131	putative hydrolase
-15.39	-2.24	(((((.((((((((&)))))))))).))) mRNA: 5'- CGUG-GCAUGAGCGGCAUUC -3' sRNA: 3'- GCGCAC-GA-CCGCCGUAAGG -5'	-5	16	49-69	phenazine biosynthesis protein PhzD

Energy [kJ/mol]	z-Score	Interaction	mRNA [Start]	mRNA [End]	sRNA	Gene Annotation
-14.54	-1.94	(((((((((....((&)).....))))))))))	-7	8	113-129	putative transporter
		mRNA: 5' - AGCGCCCCAUGAG-UC -3' sRNA: 3' - UCGCGGGGUAAUAAG -5'				
-14.39	-1.88	((((((((((((&))))))....))))))	-13	-2	109-125	hypothetical protein 70860
		mRNA: 5' - CCCCA-----UC -3' sRNA: 3' - GGGGUAAUAAG -5'				
-14.27	-1.84	(((((((((.(((((((&))))))..))))))..))))))	-3	19	8-30	endonuclease III
		mRNA: 5' - GGAGUGA-UUGAAU-GAAUGCCG -3' sRNA: 3' - CC-CACUA-UCUUACUGGGCGGC -5'				
-14.22	-1.82	(((((((((((((&))))))))))))))	6	20	5-18	iron enterobactin transporter periplasmic binding protein
		mRNA: 5' - GACCCGCCGCCGCU -3' sRNA: 3' - CUGGGCGGGCGGC-A -5'				

Supplementary References

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3. Tjaden B, Goodwin SS, Opdyke JA, Guillier M, Fu DX, et al. (2006) Target prediction for small, noncoding RNAs in bacteria. *Nucleic Acids Res* 34: 2791-2802.
4. Eggenhofer F, Tafer H, Stadler PF, Hofacker IL (2011) RNApredator: fast accessibility-based prediction of sRNA targets. *Nucleic Acids Res* 39: W149-W154.