

SUPPLEMENTAL MATERIAL

A dual functioning small RNA/Riboswitch controls the expression of the methionine biosynthesis regulator SahR in *Desulfovibrio vulgaris* Hildenborough

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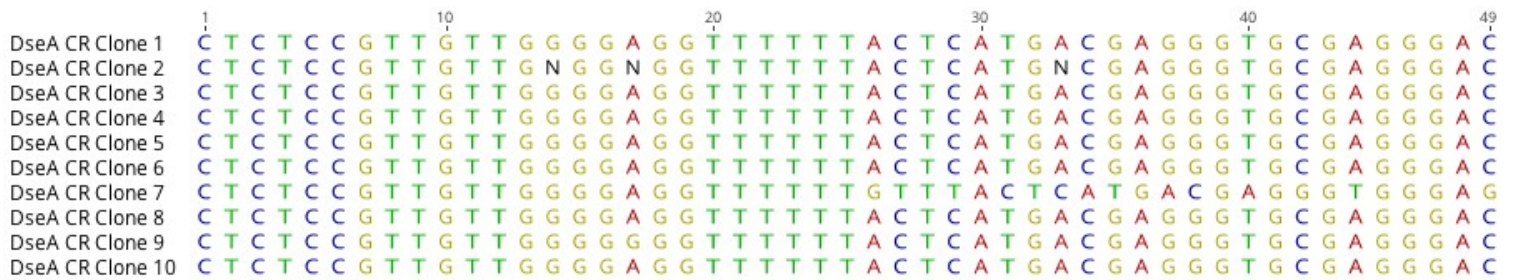


Figure S1. Alignment of RACE clone sequences corresponding to DseA. Positions 1-25 correspond to the 3'-end and position 26 corresponds to the +1 site of the RNA as depicted in Figure 1B.

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DvH      1  ACTCATGACGAG-GGTGCGAGGGA-CTTGGCCCGATGACCACCCGGCAACCTGTGCAGGC
Dv RCH1  1  ACTCATGACGAG-GGTGCGAGGGA-CTTGGCCCGATGACCACCCGGCAACCTGTGCAGGC
Dv DP4   1  ACTCATGACGAG-GGTGCGAGGGA-CTTGGCCCGATGACCACCCGGCAACCTGTGCAGGC
DvM      1  ATTTCATGGAGAGAGGCGCGAGGG-TACGGCCCTGTGACC GCCCGGCAACCTGCCCTGAC
Ds 2638  1  ACTCATGTAGAG-GATGCGAGGGAAATCGGCCCGTCGACCATCCGGCAACCTGCT-----
Da G20   1  ACTCATGAAGAG-GACGAGAGGGG-TCTGGCCCGATGACCCTCCGGCAACCTGCCCG---

DvH      59  GAGACGCCGTCAGCCAGCACAACGGTGCCAA--CCAGCCGCGACGGGAA--CGCGGGGAAC
Dv RCH1  59  GAGACGCCGTCAGCCAGCACAACGGTGCCAA--CCAGCCGCGACGGGAA--CGCGGGGAAC
Dv DP4   59  GAGACGCCGTCAGCCAGCACAACGGTGCCAA--CCAGCCGCGACGGGAA--CGCGGGGAAC
DvM      59  GTGACGCCGTCGGTCCGGGACAA--GGTGCCAAATACCGACCGTGT--AGCG--CACGGGATC
Ds 2638  55  -----TCAG-CAG--CAA--GGTGCCAAATGCCCTCC--CGT-----TACGGGACC
Da G20   56  -----CAAGGCAA--GGTGCCAAAGCCAGCCACGGTGAGATGCGCGGGTGCC

DvH      115 -ATGAGGCCAG-GCTTGCGGC-----GTCGG-CCTCTC-----CGTT----GTTGGGG
Dv RCH1  115 -ATGAGGCCAG-GCTTGCGGC-----GTCGG-CCTCTC-----CGTT----GTTGGGG
Dv DP4   115 -ATGAGGCCAG-GCTTGCGGC-----GTCGG-CCTCTC-----CGTT----GTTGGGG
DvM      114 -ATGATCGCGG-GCTTGCGGC-----GTCGG-CCTCTC-----C--TT----GC--GAG
Ds 2638  93  CATGAGA--GG-GACTAAAAC-----ATTGAATTCTTAAAGGCATTTCGGATTGGAG
Da G20   101 -ATGAGGTCGACAGCTGCGACC GGTTACC GTTCTGCCTCTCAC--CATT----GT--GAG

DvH      156  AGGTTTTTTT--
Dv RCH1  156  AGGTTTTTTT--
Dv DP4   156  AGGTTTTTTT--
DvM      152  GGGCTTTTTT--
Ds 2638  143  ATGCCTTTTTTT
Da G20   151  AGGTTTTTTT-

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Figure S2. Alignment of conserved DseA sequences. Abbreviations are as follows: DvH, *Desulfovibrio vulgaris* Hildenborough; Dv RCH1, *Desulfovibrio vulgaris* RCH1; Dv DP4, *Desulfovibrio vulgaris* DP4; DvM, *Desulfovibrio vulgaris* Miyazaki F; Ds 2638, *Desulfovibrio salexigens* DSM2638; Da G20, *Desulfovibrio alaskensis* G20. Black shading indicates identically conserved bases while grey shading represents similarly conserved bases.

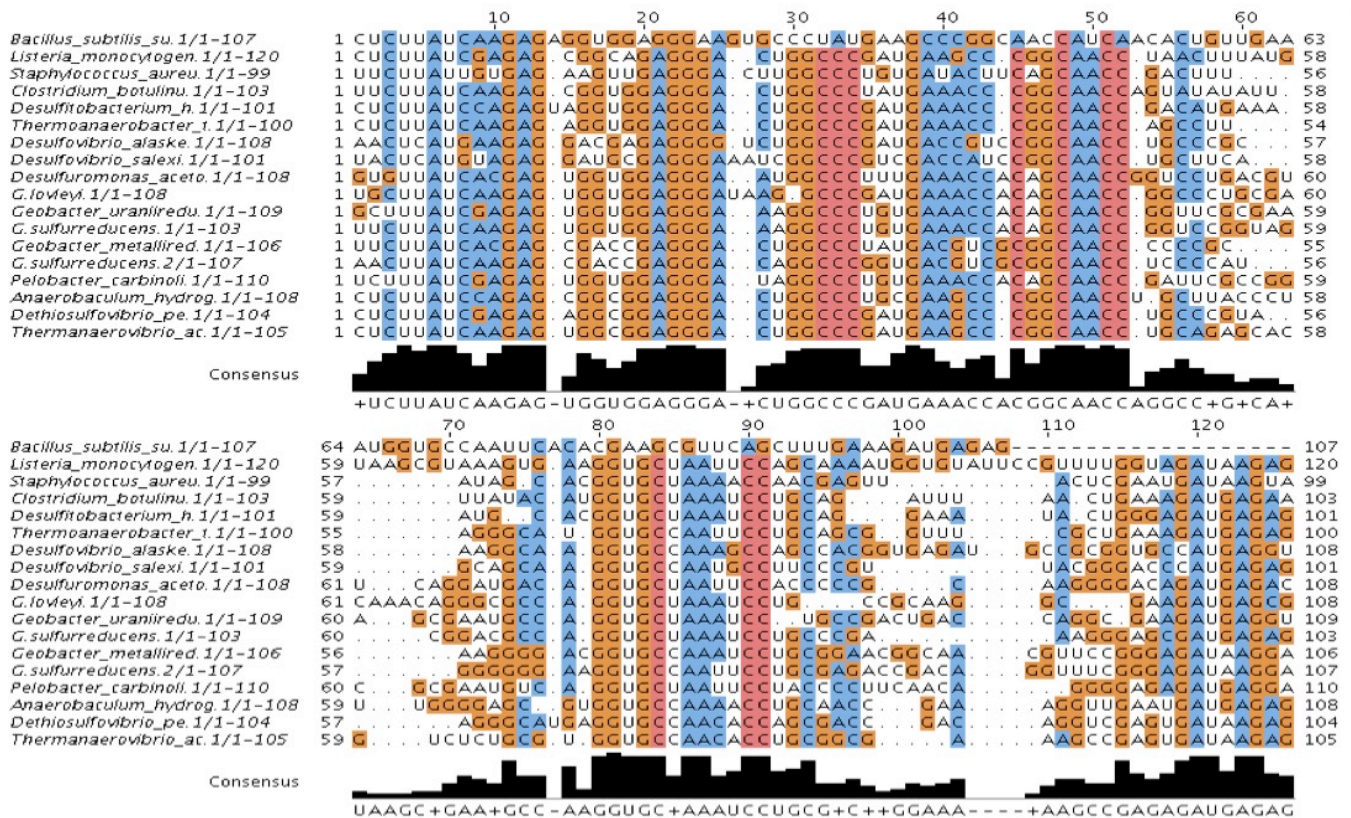


Figure S3. Alignment of the predicted riboswitch region of two *Desulfovibrio* species and other known SAM riboswitches. The colors represent the consensus base for that location.

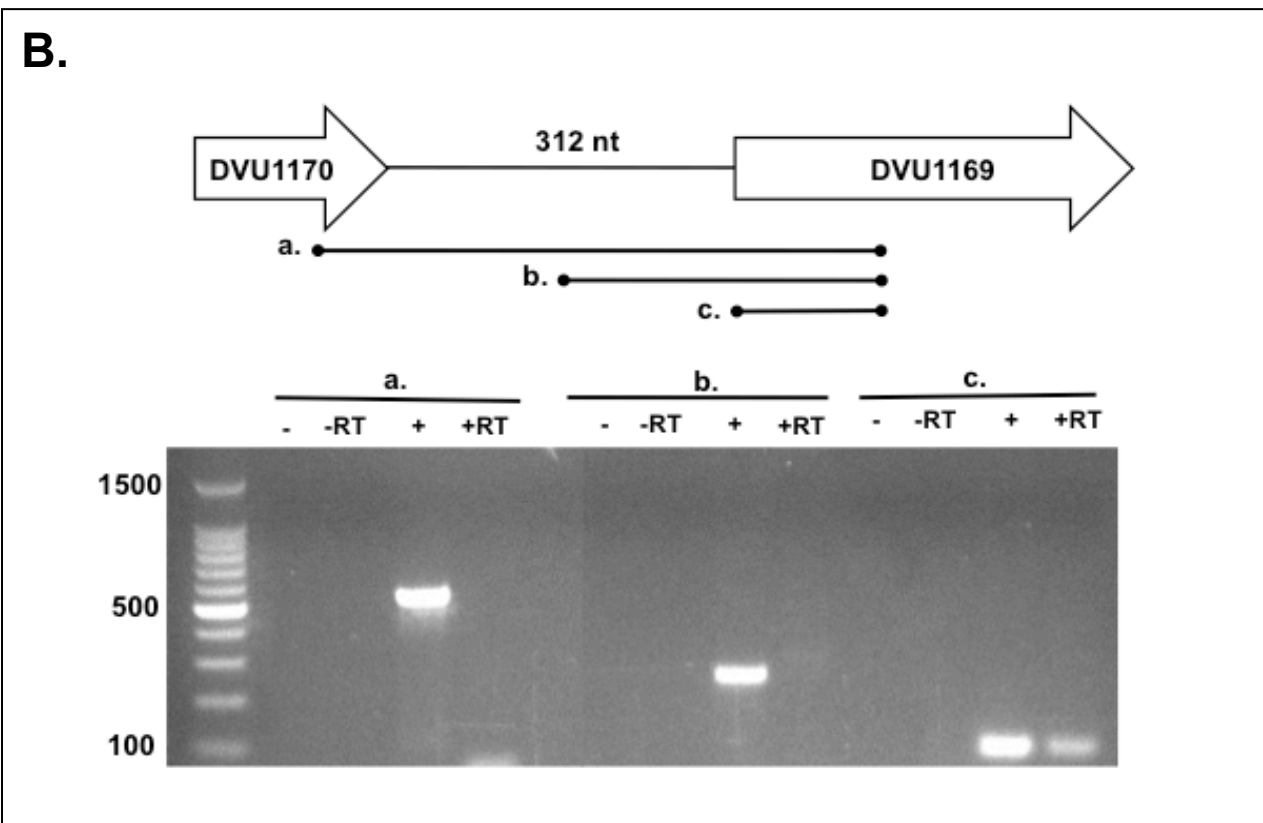
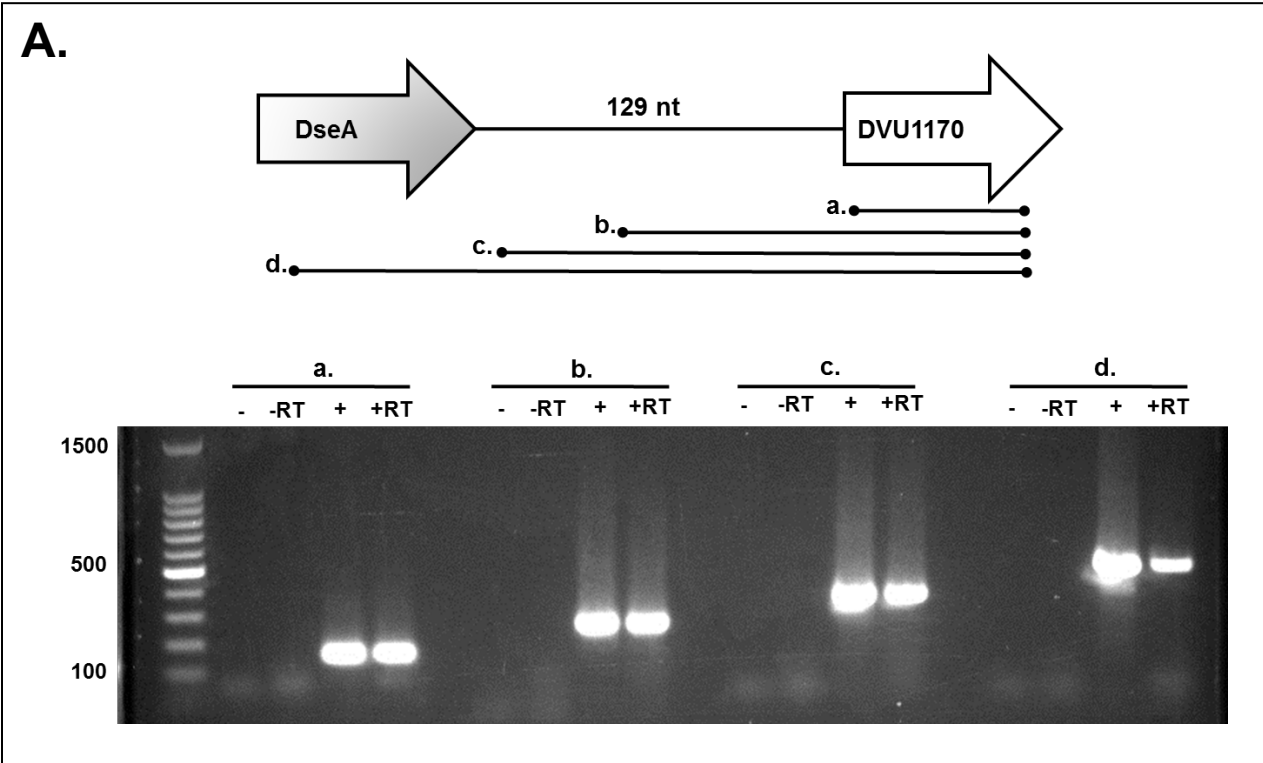


Figure S4. RT-PCR of DVU1169 transcript. **(A)** Genomic view of the DVU1170 and DVU1169 region. Lines a, b, and c indicate regions of the locus amplified by PCR as shown in panel B. **(B)** RT-PCR results. Lanes 1–4 corresponds to line a, lanes 5–8 to line b, lanes 9–12 to line c. Within each set of 4 wells, PCR to amplify lines a–c was performed with RNA after RT (+RT), RNA without RT as a control (-RT), PCR without DNA template as a control (-), and PCR with genomic DNA from *DvH* as a positive control (+).

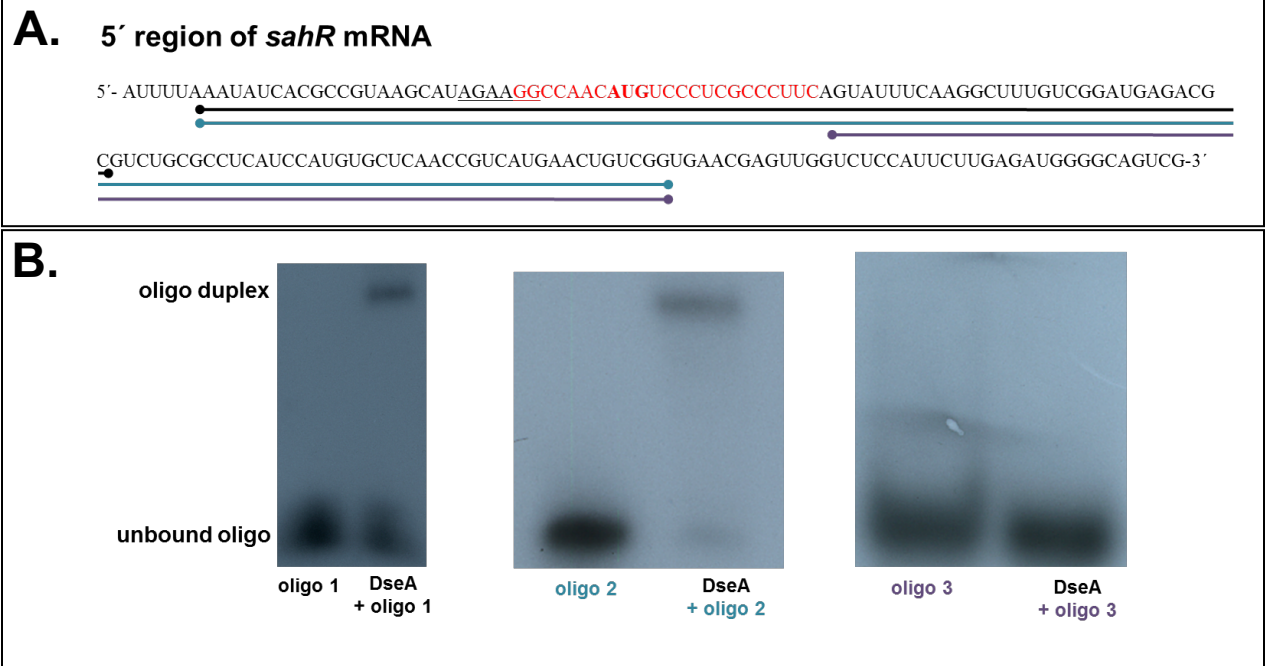


Figure S5. (A) The sequence encompassing the -36 to +126 (in reference to the start codon) region of the *sahR* mRNA. The RBS is underlined and the start codon is bolded. The predicted region of interaction between DseA and *sahR* is shown in red. **(B)** EMSA showing interaction between DseA and RNA oligos of portions of the *sahR* mRNA. The sequence of oligo 1, oligo 2, and oligo 3 is underlined with a black, blue, and purple line, respectively.

TABLE S1. Bacterial strains and plasmids used in this study.

Strains	Genotype/Description	Source/Reference
<i>Desulfovibrio vulgaris</i> Hildenborough	Wild-type strain Hildenborough	ATCC 29579 ^T
$\Delta dseA$	<i>DvH</i> , $\Delta dseA$, Km ^R , G418 ^R	Judy Wall Lab (University of Missouri)
<i>E. coli</i> TOP10	F- <i>mcrA</i> Δ (<i>mrr-hsdRMS-mcrBC</i>) $\Phi 80lacZ\Delta M15$ $\Delta lacX74 recA1 araD139$ Δ (<i>araleu</i>)7697 <i>galU galK rpsL</i> (StrR) <i>endA1 nupG</i>	Invitrogen
Plasmids		
pRS415	<i>E. coli</i> expression vector, promoterless <i>lacZ</i> , Amp ^R	Simons et al., 1983
pRS415-DseA	pRS415 with the promoter region of DseA, Amp ^R	This study
<i>pdseA</i>	pSIL300 with DseA, Spec ^R	This study

TABLE S2. Primers and probes used in this study.

Name	Sequence (5' – 3')
DseA 30mer Probe	GCAAGCCTGGCCTCATGTTCCCGCGTTCGTCGC
DvH 5S 40mer Probe	TTTCCCACGAGCTACCCCGCAGTATCATCGGCGATGGAGGGC
DseA CR F	AACATGAGGCCAGGCTTGCGG
DseA CR R	GTTCCCGTCGCGGCTGGTTG
1170 RT F1	ATGACCACCCGGCAACCTGTGCAG
1170 RT F2	CACATGTCGTGGCTGCAGGTGGGT
1170 RT F3	GCCACGCGCATAGGGGAACACGATG
1170 RT F4	ATGCCCGTCCAATGGACCGATCCC
1170 RT R	TGTTCTTGCGGAAGGCTCGGCCATT
DVU1169 RT F1	AAGGAGCGGAGACGGTCTGGAATGG
DVU1169 RT F2	TCTGCACCGCATCAACGCGACTCC
DVU1169 RT F3	TTCTCCGTCATCTGCGTCCTGTCTCTCG
DVU1169 RT R	GCCTCGCCGAACGATTCGTTGTAGTCC
DseA prom/EcoRI F	GAATTCGTTGACGTTTTTTCTGCGACC
DseA prom/ BamHI R	GGATCCACGACATGTGTCTGTGCTG
16S qRT-PCR F2	GTGCGAAAGCGTGGGGAGCA
16S qRT-PCR R2	ACGGCACCGAAGCTCAAGGC
DVU1170 qRT-PCR F	AGATGCGACGCAGATGGACGAATTGC
DVU1170 qRT-PCR R	ATGATGTTCTTGCGGAAGGCTCGGC
sahR qRT-PCR F	GCCGATTTGCGCCAGCATCAACATGG
sahR qRT-PCR R	CGAAATCCGTGACCATGAGTTGGCCT
DseA T7 prom F	TAATACGACTCACTATAGGGACTCATGACGAGGGTGCAGGGAC
DseA T7 prom R	CAAAAAACCTCCCAACAACGGAGAGGCC
DseA R	GGCATGGGCGCTTCCTCGTTGTGGTA
DseA T7A1 prom F	TTGACTTAAAGTCTAACCTATAGGATACTATGTAGTAAGGAGGTTGTAT GGAAGAACTCATGACGAGGGTGCGA
DseA T7A1 R	CGTGCAACGGCCTGAAATCCACCCACCT
SahR T7 prom F	TAATACGACTCACTATAGGGTAAATATCACGCCGTAAGCATAGAA
SahR T7 prom R	GCAAACCGGCACCCGTGAGAATCTT
SahR Middle F	TAATACGACTCACTATAGGGAGTATTTCAAGGCTTTGTCCGAT
SahR Rev 2	GCGTCTCATCCGACAAAGCCTTGAAATACT
SahR Rev 3	CCGACAGTTCATGACGGTTGAGCACATG
Dv sRNA-2 T7 prom F	TAATACGACTCACTATAGGGAGGAGCCGGTCTTGATGGTCAT
Dv sRNA-2 T7 prom R	CCCGGAGGCTTCGAGGAAGGAACA
DVU0277 qRT-PCR F	GACACCCTGCATCGTTATGA
DVU0277 qRT-PCR R	CCCATCACAGTGGAGAAGATAC
DVU1165 qRT-PCR F	GAAGGATGTGGAGGTGATGTT
DVU1165 qRT-PCR R	CTATCTGTTCCGGCTCAAGTT
DVU1362 qRT-PCR F	GCTTCTCTCCCGTGTTC

DVU1362 qRT-PCR R	GCCAGTATCTTGACCGTGAT
DVU1411 qRT-PCR F	TCCGTCCCACCCTTCTT
DVU1411 qRT-PCR R	CACCTTCTGCATTTTCGCATTC
DVU1977 qRT-PCR F	GGCGACATGGTGCTGTT
DVU1977 qRT-PCR R	TACTCGATGATGGCGAGGAT
DVU 2373 qRT-PCR F	CATCTGGGATCTCGGCTATTTTC
DVU2373 qRT-PCR R	GAGCCTTCGATGACGATGTT
DVU2514 qRT-PCR F	CATCTTCCGCCTCAACTTCT
DVU2514 qRT-PCR R	CGATTTTCGCCTATCCGTATCTT
DVU2938 qRT-PCR F	CCCACCATCAAGACGTCGAA
DVU2938 qRT-PCR R	TCCGGGGTAGACCTTGTTCA
DVU3156 qRT-PCR F	AAATCCAACGACGGTTCTCTC
DVU3156 qRT-PCR R	TCTCGGAGTGGAAGACGTAG
rplS qRT-PCR F from ref. (59)	TGTCTTCCCCCTGCACTCG
rplS qRT-PCR R from ref. (59)	CTTGATGCGGGCAGCCTTAC
Xba-c3pro-F	GAGCTCTAGAGTCCCAAACCGCCATGAATCTAG
c3proBamSca-R	TATTAGTACTATATGGATCCCCCAAGCGGGATGGTATTGTGTC
DseA-pSIL300-BamHI-F	AATAGGATCCACTCATGACGAGGGTGCGAG
DseA-pSIL300-R	AAAAAACCTCCCCAACCAACG

TABLE S3. IntraRNA predicted targets of DseA.

Target	Energy kcal/mol	Gene	Annotation
DVU2373	-25.6163		OMP85 family outer membrane protein
DVU0277	-24.8905		AraC family transcriptional regulator
DVU1165	-21.5161		pyridine nucleotide-disulfide oxidoreductase
DVU2594	-21.4385		
DVU1977	-19.828	<i>groES</i>	co-chaperonin GroES
DVU1362	-19.4161		
DVU3156	-19.2118	<i>glmS</i>	glucosamine--fructose-6-phosphate aminotransferase (isomerizing)
DVU0606	-19.1926		ArsR family transcriptional regulator
DVU2514	-19.1684	<i>pyk</i>	pyruvate kinase
DVU2605	-18.978		
DVU1411	-18.8335	<i>thiC</i>	thiamine biosynthesis protein ThiC
DVU1902	-18.7894		
DVU2623	-18.7117		
DVU2620	-18.6032		
DVU0297	-17.9608		
DVU1923	-17.919	<i>hupD</i>	hydrogenase expression/formation protein HupD
DVU2282	-17.8726		
DVU1824	-17.7374		
DVU0917	-17.5822	<i>atpE</i>	ATP synthase F0 subunit C
DVU2331	-17.4978		Smr family protein
DVU2938	-15.539		DUF39, predicted homocysteine formation from aspartate semialdehyde