Supplemental Material

Table S1. Primers, plasmids and strains used in experiments

Primers	Sequence
BsnifZ-PciI5	5′-GCGGACATGTTATATTTAGATAATAG-3'
BsnifZ-BamHI3	5′-TAAAGGATCCATGTTCTACCTCATC-3'
BsnifZ′-BamHI3	5'-CTCGGATCCCTTAATTTTTTGATGCC-3'
BsthiI-PciI5	5'-CACATGTATTACGATCATATATTAATTCG-3'
BsthiI-BglII3	5'-CGAGATCTACAAAGCTTTCAAAGTG-3'
BsthiI-NheI5	5'-CATGGCTAGCGATCATATATTAATTCG-3'
BsthiI-KpnI3	5'-CTGAGGTACCTCCTTGTGAGTATAGAATGTGTC-3'
BssufC-NcoI3	5'-TACCATGGCTGCTTCAACATTAAC-3'
BssufC-BamHI5	5'-CAGGATCCGGAGTGATGTAG-3'
Bssuf-pro5	5'-GAGTCGACGTATCTGAATAAGACTGAAAC-3'
Bssuf-pro3	5'-GTCCCGGGTGAAGCAGCCATAT-3'
*Ec*tRNA^{Met}5	5′-AATTCCTGCAGTATACGACTCACTATAGGCTACGTA GCTCAGTTGGTTAGAGCACATCACTCATAATGATGGG GTCACAGG -3′
*Ec*tRNA^{Met}3	5'-mUmGGTGGCTACGACGGGATTCGAACCTGTGAC CCATCATTATG-3'

Plasmids	Relevant Gene Cloned	Vector	Reference
pDS13	*nifZ* PCR product using *BsnifZ*-PciI5 and *BsnifZ*-BamHI3 primers. It places PciI and BamHI sites at 5′ and 3′ sites flanking nifZ coding sequence (codons 2-382)	pCR2.1 TOPO	This work
pDS18	*thiI′* PCR product using *Bsthi*I-PciI5 and *Bsthi*I-BglII3 primers. It places PciI and BglII sites at 5′ and 3′ sites flanking *thiI′* coding sequence (codons 1-358)	pCR2.1 TOPO	This work
pDS19	908 bp HindIII *′thiI′* fragment (198-1101 bp) from pDS18 into the HindIII site of pMutin4 plasmid.	pMutin4	This work
pDS20	1.1 kb PciI-BglII fragment of *thiI′* ligated into NcoI-BglII sites of pAra13	pAra13	This work
pDS21	1.15 kb PciI and BamHI *nifZ* fragment from pDS13 ligated *nifZ* gene into NcoI-BglII sites of pAra13.	pAra13	This work
pDS47	*nifZ′* PCR product using *BsnifZ*-PciI5 and *BsnifZ′*-BamHI3 primers. It places PciI and BamHI sites at 5′ and 3′ sites of *nifZ* (codons 2-380).	pCR2.1 TOPO	This work
pDS54	650 bp BglII-BamHI *′nifZ′* fragment (codons 130-380) ligated in forward orientation into BamHI-digested pMutin4 plasmid.	pMutin4	This work
pDS71	2.2 kB *nifZ-thiI′* PCR product amplified with primers *BsnifZ*-PciI5 and *Bsthi*I-BglII3.	pCR2.1 TOPO	This work
pDS80	2.2 kb StuI-KpnI *′nifZ-thiI′* fragment ligated into StuI-KpnI sites of pDS21	pAra13	This work
pDS101	*thiI* PCR product using *Bsthi*I-NheI5 and *Bsthi*I-KpnI3 primers. It places NheI and KpnI sites at 5′ and 3′ sites of *thiI* (codons 1-403, and additional 90 bp downstream).	pCR2.1 TOPO	This work
pDS108	976 bp NruI-KpnI *′thiI* fragment from pDS101 ligated into NruI-KpnI sites of pDS20	pAra13	This work
pDS115	976 bp NruI-KpnI *′thiI* fragment from pDS101 ligated into NruI-KpnI sites of pDS80.	pAra13	This work
pBH113	*Escherichia coli thiI*	pet15b	(3)
pDB943	*Azotobacter vinelandii iscS*	pT7-7	(6)

Strain	Relevant Genotype	Reference
B. subtilis PS832	wild type strain	(1)
B. subtilis DD5	*'nifZ*::pMutin4	This work
B. subtilis DD14	*'nifZ'*::pMutin4	This work
B. subtilis 1A603	*thiA*84::Tn197	(4)
E. coli MG1655	wild type strain	Laboratory stock
E. coli CL100	*ΔiscS*	(2)
E. coli JLD26501	*thiI::Kan*^{R}	P1(VJS2890)→TL524[a]

[a] Constructed by phage P1-mediated transduction of strain TL524 (5) to kanamycin resistance using stain VSJ2890 (3) as P1 donor

References

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3. **Mueller, E. G., C. J. Buck, P. M. Palenchar, L. E. Barnhart, and J. L. Paulson.** 1998. Identification of a gene involved in the generation of 4-thiouridine in tRNA. Nucleic Acids Res. **26:**2606-2010.
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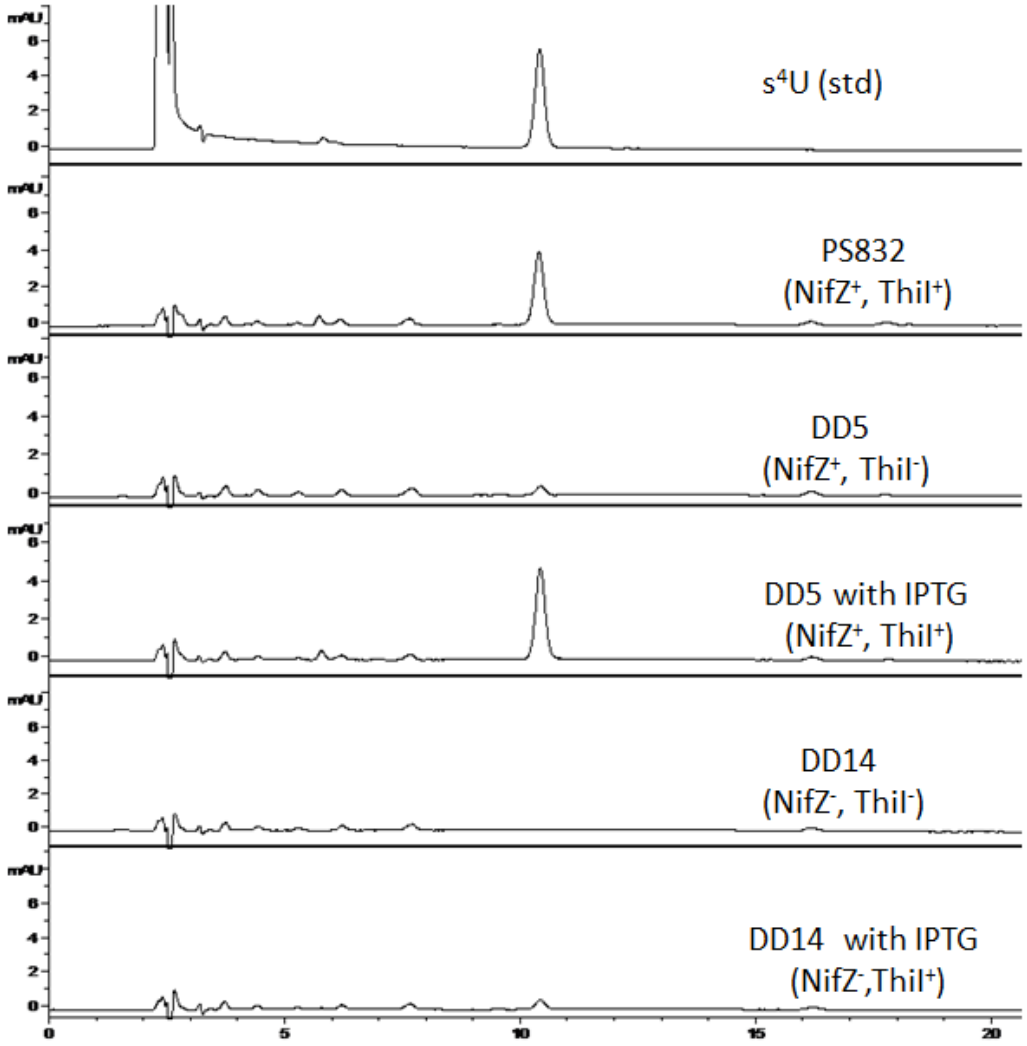
Figure S1. Detection of s^4U8 in tRNA nucleosides isolated from cells lacking NifZ and/or ThiI. (**A**) HPLC-MS chromatograms at Abs_{330nm} of tRNA nucleosides isolated from *B. subtilis* PS832, DD5 in the presence (*nifZ*$^+$, *thiI*$^+$) and absence of IPTG (*nifZ*$^+$, *thiI*$^-$) and DD14 culture in the presence (*nifZ*$^-$, *thiI*$^+$) and absence (*nifZ*$^-$, *thiI*$^-$) of IPTG. The peak corresponding to s^4U was compared to the standard (shown on top), and to pseudouridine which was used as an internal control (not shown). (B) Negative mode mass spectrum of the peak corresponding to s^4U.

Figure S2. Growth of *B. subtilis* strains in minimum medium in the presence (filled symbols) and in the absence (empty symbols) of 0.8 µg/mL thiamine. Cells were cultured in Spizizen's medium at 37^{o}C, 300 RPM for PS832 (○) and DD14 in the absence (□) and presence (△) of IPTG. *B. subtilis* 1A603 (thiA84::Tn917) was cultured in Spizizen medium supplemented with tryptophan (◇). Growth patterns were monitored for 14 hours and optical density at 600 nm (A_{600nm}) was recorded as indicated.

Figure S1

A


mAU
s4U (std)
PS832
(NifZ+, ThiI+)
DD5
(NifZ+, ThiI-)
DD5 with IPTG
(NifZ+, ThiI+)
DD14
(NifZ-, ThiI-)
DD14 with IPTG
(NifZ-,ThiI+)
0
5
10
15
20

B

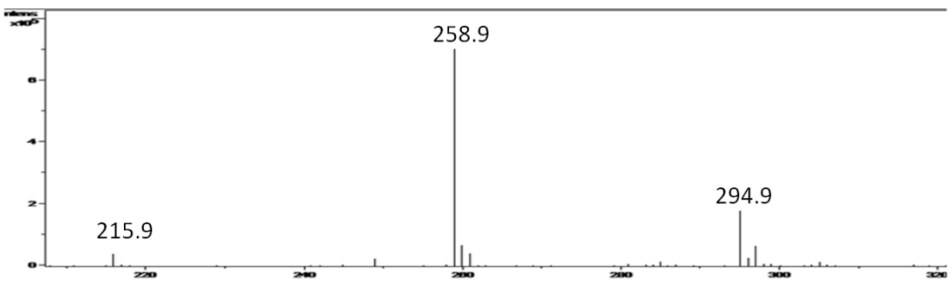

258.9
294.9
215.9

Figure S2