Supporting Information

Sherwood et al. 10.1073/pnas.1424175112

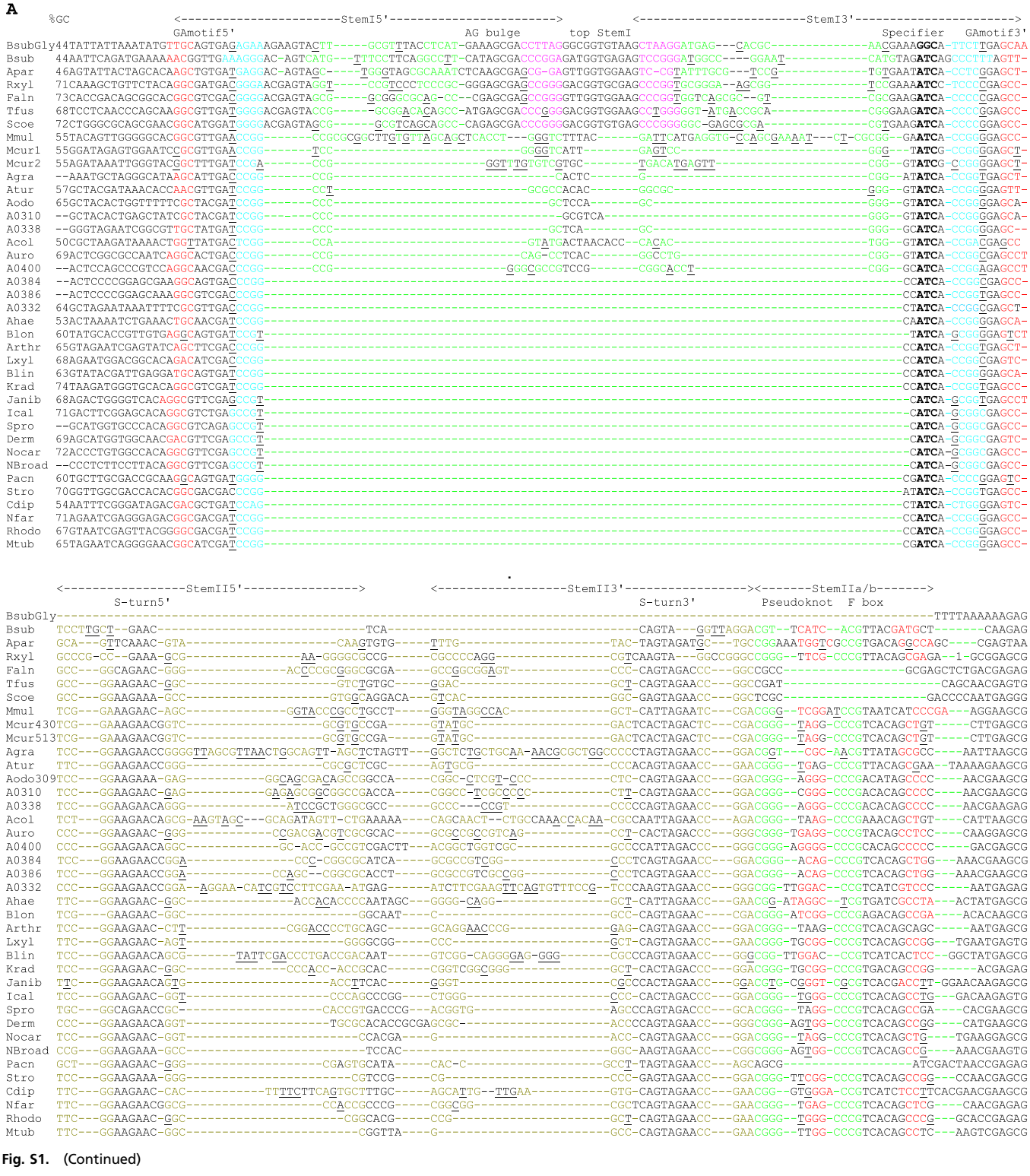

Fig. S1. (Continued)

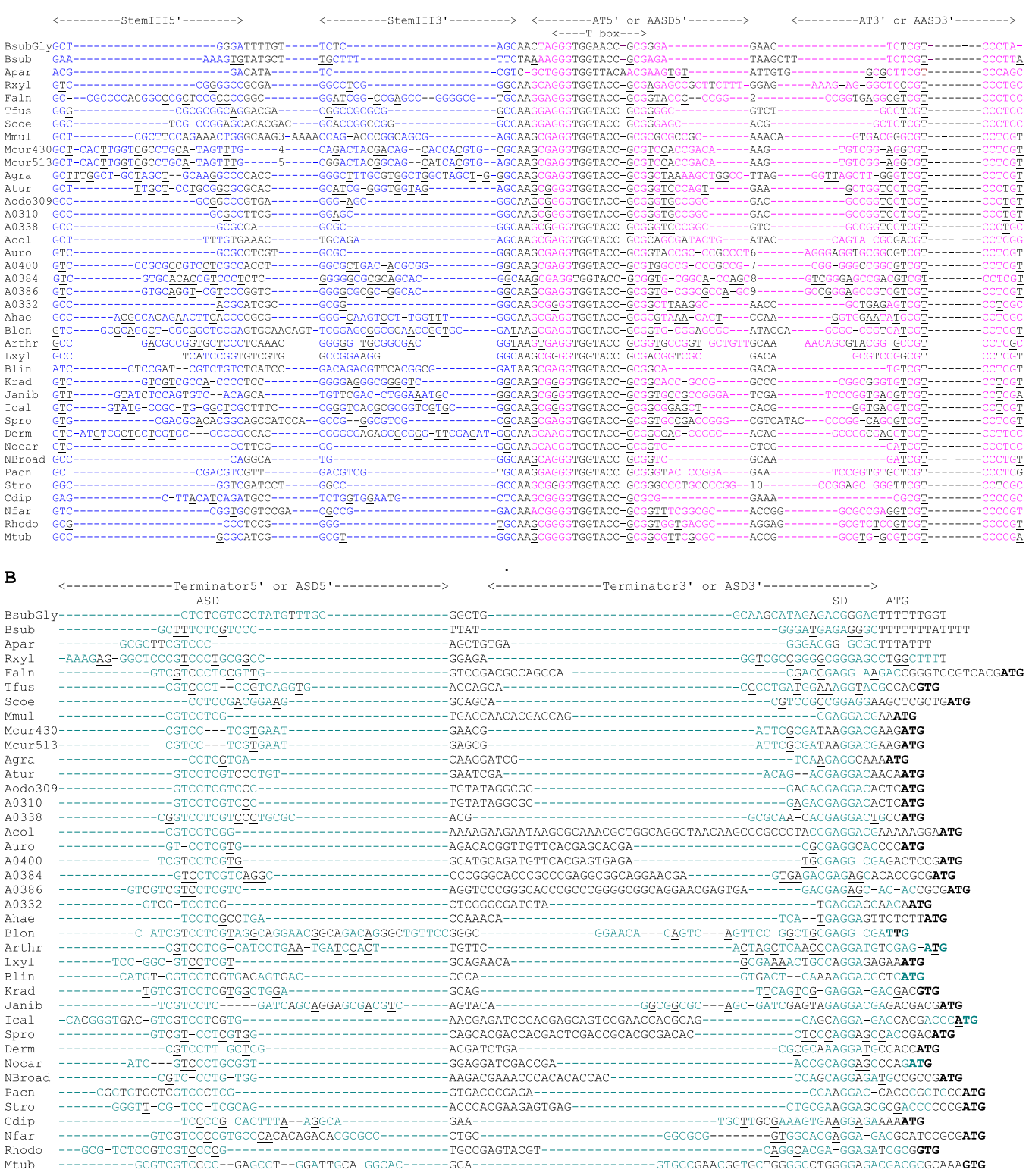

Fig. S1. Alignment of Actinobacteria *ileS* leader regions that represent various classes of structure and possible regulatory mechanisms. The *B. subtilis glyQS* and *ileS* sequences are shown for comparison. (*A*) Alignment of sequences that extend from 15 nt 5′ of the GA motif through the antisequestrator helix (or antiterminator). (*B*) Alignment of sequences that form the sequestrator helix (or terminator) extending through the ATG for translationally controlled genes (or the run of T's for transcriptionally controlled genes). The 3′ side of the antisequestrator helix (or antiterminator) in *A* is partially repeated within the 5′ side of the sequestrator helix (or terminator) in *B*. Dashed arrows at the top indicate helical regions, and the same colored sequences indicate paired regions. Helical regions are aligned at each end of the paired region with a spacer, and colored dashes are inserted for alignment based on conserved sequences. A black base within a helical area means that this base does not have a counterpart on the opposite side of the helix, whereas a black underlined base means that this base does have a counterpart on the opposite side of the helix. A black dash in a helix means there is an extra base opposite to this location in the other side of the helix. In Rxyl, there is an extra helix after the pseudoknot: GGGTGTGCCGCCGCGCACCC; in Faln, there is an additional sequence at the top of the

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antisequestrator helix: TCCCGGGCGGGACG-CGG-CGGCGGGCAGGCCCGTCGGCCGTCGTCACGC–GCGG; in Mmul, there are two extra helices in stem III: TTCGGG ACT-TGAAACCTTTGCCAGGGAAACC-GGCAAGCACCTCATGAACCCGAAACCAGATGGGCACGGGACATGAACGCCGCCGGGAATACCGGGTGGCGCCTCATGAACTC–ACCATC; in Mcur43063, there is an additional sequence at the top of stem III: ACCGCACCGGATAGGTCGG–CGCC; in Mcur51333, there is an additional sequence at the top of stem III: ACCGCACCGGATAGGTCGG–CGCC; in Auro, there is an additional sequence at the top of the antisequestrator helix: CG-GCC-TGCCAGGGCACG; in A0400, there is an additional sequence at the top of the antisequestrator helix: TCGTCGGCCACCGGCCGGCG; in A0384, there is an additional l sequence at the top of the antisequestrator helix: CGGGCACCAGGCCCG; in A0386, there is an additional sequence at the top of the antisequestrator helix: CGG-ACGCCAGTCCCG; in Stro, there is an additional sequence at the top of the antisequestrator helix: CACGCCGACGGCGT-. Acol, *Actinomyces coleocanis* Difco Sporulation Media (DSM) 15436; Agra, *Actinomyces graevenitzi* C83; Ahae, *Arcanobacterium haemolyticum* DSM 20595; Aodo309, *Actinomyces odontolyticus* F0309; A0310, *Actinomyces* sp. oral taxon 180 str. F0310; A0332, *Actinomyces* sp. oral taxon 848 str. F0332; A0338, *Actinomyces* sp. oral taxon 178 str. F0338; A0384, *Actinomyces* sp. oral taxon 175 str. F0384; A0386, *Actinomyces* sp. oral taxon 170 str. F0386; A0400, *Actinomyces* sp. oral taxon 448 str. F0400; Apar, *Atopobium parvulum* DSM 20469; Arthr, *Arthrobacter* sp. FB24; Atur, *Actinomyces turicensis* ACS-279-V-Col4; Auro, *Actinomyces urogenitalis* DSM 15434; Blin, *Brevibacterium linens* BL2; Blon, *Bifidobacterium longum*; Bsu, *B. subtilis*; Cdip, *Corynebacterium diphtheriae*; Derm, *Dermacoccus* sp. Ellin 185; Faln, *Frankia alni* ACN14a; Ical, *Itrasporangium calvum* DSM 43043; Janib, *Janibacter* sp. HTCC2649; Krad, *Keneococcus radiotolerans* SRS30216; Lxyl, *Leifsonia xyli* subsp. xyli str. CTCB07; Mcur430, *Mobiluncus curtisii* American Type Culture Collection (ATCC) 43063; Mcur513, *Mobiluncus curtisii* ATCC 51333; Mmul, *Mobiluncus mulieris* FB024-16; Mtub, *M. tuberculosis*; NBroad, Nocardioidaceae bacterium Broad-1; Nfar, *N. farcinica* IFM 10152; Nocar, *Nocardioides* sp. JS614; Pacn, *Propionibacterium acnes*; Rhodo, *Rhodococcus* sp. DK17; Rxyl, *Rubrobacter xylanophilus* DSM 9941; Scoe, *S. coelicolor* A32; Spro, *Serinicoccus profundi* Marine Culture Collection of China (MCCC) 1A05965; Stro, *Salinispora tropica* CNB-440; Tfus, *Thermobifida fusca* YX.

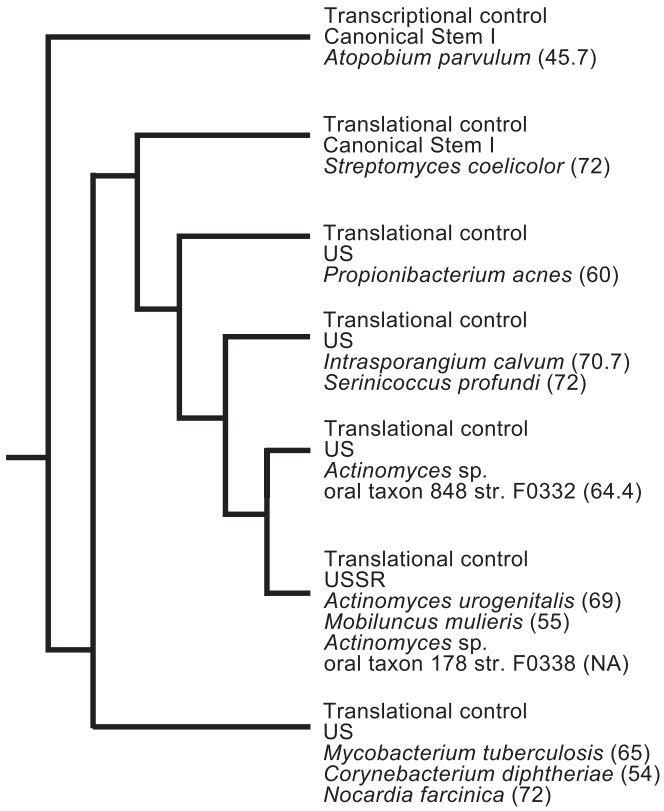

Fig. S2. Modified phylogenetic tree of species from the phylum Actinobacteria labeled with types of Stem I found in the *ileS* leader sequences (1, 2). Numbers in parentheses are the percentage of G + C genome contents (1–4).

1. Gao B, Gupta RS (2012) Phylogenetic framework and molecular signatures for the main clades of the phylum *Actinobacteria*. *Microbiol Mol Biol Rev* 76(1):66–112.
2. Gillespie JJ, et al. (2011) PATRIC: The comprehensive bacterial bioinformatics resource with a focus on human pathogenic species. *Infect Immun* 79(11):4286–4298.
3. Glavina Del Rio T, et al. (2010) Complete genome sequence of *Intrasporangium calvum* type strain (7 KIP T). *Stand Genomic Sci* 3(3):294–303.
4. Xiao J, Luo Y, Xu J (2011) Genome sequence of *Serinicoccus profundi*, a novel actinomycete isolated from deep-sea sediment. *J Bacteriol* 193(22):6413.

Table S1. Actinobacteria genera grouped by Stem I class of the T box riboswitch found in the *ileS* gene

No T box riboswitches	Canonical T box riboswitches	USSR T box riboswitches	US T box riboswitches
Acidimicrobium	*Atopobium*	*Actinomyces*	*Actinomyces*
Acidothermus	*Catenulispora*	*Mobiluncus*	*Actinosynnema*
Aeromicrobium	*Collinsella*		*Amycolatopsis*
Blastococcus	*Coriobacterium*		*Amycolicicoccus*
Candidatus	*Frankia*		*Arcanobacterium*
Conexibacter	*Nocardiopsis*		*Arthrobacter*
Cryptobacterium	*Olsenella*		*Beutenbergia*
Eggerthella	*Propionibacterium*		*Bifidobacterium*
Geodermatophilus	*Rubrobacter*		*Brachybacterium*
Modestobacter	*Streptomyces*		*Brevibacterium*
Patulibacter	*Streptosporangium*		*Cellulomonas*
Segniliparus	*Thermobifida*		*Clavibacter*
Slackia	*Thermobispora*		*Corynebacterium*
Tropheryma	*Thermomonospora*		*Dermacoccus*
	Kitasatospora		*Dietzia*
			Gardnerella
			Gordonia
			Intrasporangium
			Janibacter
			Jonesia
			Kineococcus
			Kocuria
			Kribbella
			Kytococcus
			Leifsonia
			Microbacterium
			Micrococcus
			Micromonospora
			Mycobacterium
			Nakamurella
			Nocardia
			Nocardioidaceae
			Nocardioides
			Parascardovia
			Propionibacterium
			Pseudonocardia
			Renibacterium
			Rhodococcus
			Rothia
			Saccharomonospora
			Salinispora
			Sanguibacter
			Scardovia
			Stackebrandtia
			Tsukamurella
			Verrucosispora
			Xylanimonas

Table S2. Generation of the plasmid vectors and DNA oligonucleotides used in PCR

DNA template	Generation of plasmid vectors	PCR primers
N. farcinica ileS	PCR using ligation reaction as a template and 5′-TTTATATGATCATTTACGAGGGAGACGGCGACGATCCGGCCA-TC-3′ and 5′-TAGCTGTCGACGCTGTCGTCCGCCATCGCGGATGCGCCTCCT-3′ as primers	tRNA-binding reactions: 5′-TTCTCGAATTCTAATACGACTCACTATAGGCGAC-GATCCGGCCATCAC-3′ 5′-ACGGGGACGGCCTCGGCGCCCGGTGC-3′
	PCR using *B. subtilis* chromosomal DNA as a template and 5′-ATTAATCTAGATTACGAAGAATATTCGGGATTGTA-3′ and 5′-GCCGTCTCCCTCGTAAATGATCATATAAAAAGATGGACC-3′ as primers	Primer extension reactions: 5′-TTCTCGAATTCTAATACGACTCACTATAGGCGAC-GATCCGGCCATCAC-3′ 5′-TGCGGGCCTCTTCGCTATTACGCCA-3′
	PCR using products from both of the PCR above as template and 5′-ATTAATCTAGATTACGAAGAATATTCGGGATTGTA-3′ and 5′-TAGCTGTCGACGCTGTCGTCCGCCATCGCGGATGCGCCTCCT-3′ as primers	RNase H cleavage assays: 5′-CCTAATGCAGGAGTCGCATAAGGG-3′ 5′-CGATTAAGTTGGGTAACGCCAGG-3′
	Insert PCR product into plasmid pFG328 using XbaI, SalI	
A8G *N. farcinica ileS*	No plasmid generated	5′-ATATAATACGACTCACTATAGGCGACGGTCCGGC-CATCACCGGGGAGCCT-3′ 5′-ACGGGGACGGCCTCGGCGCCCGGTGC-3′
A16U *N. farcinica ileS*	No plasmid generated	5′-TTCTCGAATTCTAATACGACTCACTATAGGCGA-CGATCCGGCCTTCAC-3′ 5′-ACGGGGACGGCCTCGGCGCCCGGTGC-3′
N. farcinica $tRNA^{Ile}$	Insert ligation reaction product into plasmid pGEM7-S4P-tRNQ using XmaI, NcoI	
	Insert ligation reaction product into pGEM4 plasmid using XbaI, PvuII	5′-GAGCGAGGAAGCGGAAGAGCGCCC-3′ 5′-TGGTGGGCCTAGGAGGA-3′
N. farcinica $tRNA^{Ile}$ΔACCA	No plasmid generated	5′-TAATACGACTCACTATAGGGCCTATAGCTCAG-3′ 5′-GGGCCTAGGAGGACTTGAA-3′
N. farcinica $tRNA^{Ile}$Ex1C	No plasmid generated	5′-TAATACGACTCACTATAGGGCCTATAGCTCAG-3′ 5′-GTGGTGGGCCTAGGAGGA-3′
N. farcinica U36A $tRNA^{Ile}$	Site-specific oligomutagenesis of the plasmid pGEM7-S4P-tRNQ with *N. farcinica* $tRNA^{Ile}$ template insert using 5′-GGTTAGAGCGCTTCGCTGAAAACGAAGAGGTCGGAGG-3′ and 5′-CCTCCGACCTCTTCGTTTTCAGCGAAGCGCTCTAACC-3′ oligonucleotides	5′-TAATACGACTCACTATAGGGCCTATAGCTCAG-3′ 5′-TGGTGGGCCTAGGAGGA-3′
Actinomyces sp. *ileS*	Insert ligation reaction product into pGEM4 plasmid using XbaI, PvuII	5′-TAATACGACTCACTATAGG-3′ 5′-GCAGGGACGAGGACCGG-3′
Actinomyces sp. $tRNA^{Ile}$	Insert ligation reaction product into pGEM4 plasmid using XbaI, PvuII	
S. coelicolor ileS	No plasmid generated	5′-TAATACGACTCACTATAGGCATGGATGGGGACG-3′ 5′-GGAGGGACGAGAGCCGTGCT-3′
S. coelicolor $tRNA^{Ile}$	No plasmid generated	5′-ATATAATACGACTCACTATAGGGGCTATAGCTCA-GTTGGTTAGA-3′ 5′-TGGTGGGGCTAACAGGATTTGAACC-3′
B. subtilis $tRNA^{Gly}$	No plasmid generated	5′-TAATACGACTCACTATAGCGGAAGTAGTTCAGTGG-3′ 5′-TGGAGCGGAAGACGGGATTCGAAC-3′
B. sublitis $tRNA^{Val}$	PCR using *B. subtilis* chromosomal DNA as a template and 5′-AATATTAATACGACTCACTATAGATTCCGTAGCTCAGCTGG-3′ 5′-ATAATTCTAGAGCAATGCATGGTGATTCCGACTGGGCTCGAACC-3′ as primers Insert into pGEM4 plasmid using XbaI, PvuII	

NcoI, PvuII, SalI, XbaI, and XmaI are restriction enzymes purchased from New England Biolabs, Inc.

Table S3. DNA oligonucleotides used in primer extension reactions

Oligonucleotide name	Sequence
310–330	5′-CGCTATTACGCCAGCTGGCG-3′
267–289	5′-CGATTAAGTTGGGTAACGCCAGG-3′