

# Supporting Information

Sherwood et al. 10.1073/pnas.1424175112

	%GC	<-----StemI'----->	AG bulge	top StemI	<-----StemI'----->	Specifier	GAmotif3'
BsubGly	44	GAmotif5'	<-----StemI'----->	AG bulge	top StemI		
Bsub	44	AATTATTAATATGTTGAGAGAAGAGTAACTTGGCTTACCTCATGAAAGCGAACCTTAGGGGGTGTAAAGCTAAGGATGAG	-----	CACGCC	-----	AA_GAAAGGCCA_TTCATGAA	
Apar	46	ATTCAGATAAAAACCGGTTAAAGGGAC-AGTCATG-----TTCCTTCAGCCGCTT-CATAGCGACCCGA	GATGGTGAGAGTCGGGGATGCC	-----GGAAAT-----	CATGTAATCACA_CCTCGGAGCT-	CATGTAATCACA_CCTCGGAGCT-	
Rxyl	71	CAAAGCTTTCACAGCGGATGACCGGAACCGAGATACTTGGCTCCCGG-GGGAGCGACCGGGGACGGTGCAGACCGG	-----	-----	TCTGAAATCCTCCCGAGGCC	TCTGAAATCCTCCCGAGGCC	
Faln	73	CCCCACAGGGCAACGGCTCGACGGGACCGAGTACGG-----GGGGCCGAGCCCGG-GTGGTGGAAAGCCCGTGTGCGC	-----	-----	CGCGAAAGATCA_CCCCGAGGCC	CGCGAAAGATCA_CCCCGAGGCC	
Tfus	68	CTCAACCCAGGAACGGCTTGATGGACAGAGTACCG-----GGGGACACAGCC-ATGAGCGACCGGGAGCTGGGGATAGACCGGA	-----	-----	CGGGAAAGATCA_CCCCGAGGCC	CGGGAAAGATCA_CCCCGAGGCC	
Scoo	72	CTGGGGCACCGAACCGCATGGATGGGGACCGAGTAGCG-----GGCTCACAGCC-CAGAGCGACCCGGGACGGTGTGAGCCCGGGGGAGAGCGGA	-----	-----	CCTGAAAGATCA_CCCCGAGGCC	CCTGAAAGATCA_CCCCGAGGCC	
Mmul	55	TACAGTGGGGCACGGCGCTTGAACTGGGCGGCTTGATGTTAGCAACCT-----GGGTCTTAC-----GATTATGAGGTG-CCAACGAAAT	-----	-----	CT-CCCGG-GAATCA-CCCGGAGGCC	CT-CCCGG-GAATCA-CCCGGAGGCC	
Mcurl	55	GATAGTGGGAATCCGGCTTGTAACTCG-----TCC-----GGGTCTTAC-----GGGTCTTAC-----GATTATGAGGTG-CCAACGAAAT	-----	-----	GG-TATCG-CGGGAGGCC	GG-TATCG-CGGGAGGCC	
Mcurl2	55	GATAAATTGGGTACGGCTTGTATCCA-----CCG-----GGTTGTGCTGTG-----TGACATGAGTT	-----	-----	CGG-GTATCG-CGGGAGGCC	CGG-GTATCG-CGGGAGGCC	
Agra	55	ATAGTGGCATACGATTGACCGG-----CCG-----CACTC-----G-----	-----	-----	CGG-GTATCA-CGGGAGGCC	CGG-GTATCA-CGGGAGGCC	
Atur	57	GCTCATACATAACACCAACGGCTTGTATGAT-----CCC-----GGGCCACAC-----GGGCC	-----	-----	GGG-GTATCA-CGGGAGGCC	GGG-GTATCA-CGGGAGGCC	
Aodo	65	GCTACACTGGCTTACGAT-----CCC-----GGCTTAC-----GC-----	-----	-----	GGG-GTATCA-CGGGAGGCC	GGG-GTATCA-CGGGAGGCC	
A0310	66	-GCTACATGAGCTATCGCTACGAT-----CCC-----GGCTCA-----	-----	-----	GGG-GTATCA-CGGGAGGCC	GGG-GTATCA-CGGGAGGCC	
A0338	66	--GGTAGAATGGGGCTGTATGAT-----CCC-----GGCTCA-----	-----	-----	GGG-GCATCA-CGGGAGGCC	GGG-GCATCA-CGGGAGGCC	
Acol	50	TGTTATGAGTACAC-----CCC-----GTATGACTAACACC-----CACAC	-----	-----	TGG-GTATCA-CGGGAGGCC	TGG-GTATCA-CGGGAGGCC	
Auro	69	ACTGGCGGCAATCAGGACTGAC-----CCG-----CAC-----GGAC-CCTAC-----GGCTG	-----	-----	CGG-GTATCA-CGGGAGGCC	CGG-GTATCA-CGGGAGGCC	
A0400	66	-ACTCCAGCCGTCACAGGAACGAC-----CCG-----GGCGCCCTCCG-----CGGCACCT	-----	-----	CGG-GCATCA-CGGGAGGCC	CGG-GCATCA-CGGGAGGCC	
A0384	66	--ACTCCCGGGAGGCAAAGGGCTCGAC-----CCG-----	-----	-----	CCATCA-CGGGAGGCC	CCATCA-CGGGAGGCC	
A0386	66	--ACTCCCGGGAGGCAAAGGGCTCGAC-----CCG-----	-----	-----	CCATCA-CGGGAGGCC	CCATCA-CGGGAGGCC	
A0332	66	64CTGATAATAATTGGGGCTTGAC-----CCG-----	-----	-----	CTATCA-CGGGAGGCC	CTATCA-CGGGAGGCC	
Ahae	53	ACTAAATCTGAAACCTGGAAACG-----CCG-----	-----	-----	CAATCA-CGGGAGGCC	CAATCA-CGGGAGGCC	
Blon	60	TATGCACCGTTGTGAGGAGTGT-----CCG-----	-----	-----	TATCA-GCGGAGACT	TATCA-GCGGAGACT	
Arthr	65	GTAGAATGAGTACGCTTGTGAC-----CCG-----	-----	-----	CCATCA-CGGGAGCT	CCATCA-CGGGAGCT	
Lxyl	68	GAATGAGCAGCACAGATGAC-----CCG-----	-----	-----	CCATCA-CGGGAGCT	CCATCA-CGGGAGCT	
Blin	63	GATACTGGACGAGCAACGGCTTGAC-----CCG-----	-----	-----	CCATCA-CGGGAGCT	CCATCA-CGGGAGCT	
Krad	74	TAAGATGGTGACAGGGCTCGAT-----CCG-----	-----	-----	CCATCA-CGGGAGCT	CCATCA-CGGGAGCT	
Janib	68	AGACTGGGTGACAGGGCTTGAG-----CCG-----	-----	-----	CAATCA-CGGGAGCT	CAATCA-CGGGAGCT	
Ical	71	AGCTGGGAGACACA-----GGCTGCTGAACCC-----	-----	-----	CAATCA-CGGGAGCT	CAATCA-CGGGAGCT	
Spro	66	-GCATGTGCCCCACAACGGCTCAGACGGCT	-----	-----	CAATCA-CGGGAGCT	CAATCA-CGGGAGCT	
Derm	69	AGCATGTGGCAACGACGGCTGAC-----CCG-----	-----	-----	CATCA-GCGGAGTC	CATCA-GCGGAGTC	
Nocar	72	ACCTCTGGGACCCAAACGGCTTCAACGGCT	-----	-----	CATCA-GCGGAGTC	CATCA-GCGGAGTC	
NBroad	66	--CCCTCTTCTTACAACGGCTTCAACGGCT	-----	-----	CATCA-GCGGAGTC	CATCA-GCGGAGTC	
Pacn	60	TGCTTGGGACCCAAACGGCTGAC-----CCG-----	-----	-----	CGATCA-CGGGAGTC	CGATCA-CGGGAGTC	
Stro	70	GTGGTGGGACCCACGGGACGAC-----CCG-----	-----	-----	ATATCA-CGGGAGTC	ATATCA-CGGGAGTC	
Cdip	54	ATTTCGGGATAGACGACGCTGAT-----CCG-----	-----	-----	CTATCA-CGGGAGTC	CTATCA-CGGGAGTC	
Nfar	71	AGATCGAGGGAGACGGGACGGGAGT-----CCG-----	-----	-----	CCATCA-CGGGAGTC	CCATCA-CGGGAGTC	
Rhodo	67	GTATACGAGTTACGGGGAGGAT-----CCG-----	-----	-----	CCATCA-CGGGAGTC	CCATCA-CGGGAGTC	
Mtub	65	AGATCAGGGGACGGCTCGAT-----CCG-----	-----	-----	CGATCA-CGGGAGTC	CGATCA-CGGGAGTC	
		<-----StemII'----->		<-----StemII'----->	<-----StemIIa/b----->		
		S-turn5'		S-turn3'	Pseudoknot	F box	
BsubGly						TTTTAAAAAAAG	
Bsub		TCCTTGTG-GAAC				-----	CAAGAG
Apar		GCA-GTTCAACG-GTA				-----	CGAGTAA
Rxyl		CCCCG-CC-GAAA-GCG				-----	
Faln		GCC-GGAGAAC-GGG				-----	
Tfus		GCC-GGAGAAC-GCC				-----	
Scoo		GCC-GGAGAAA-GCC				-----	
Mmul		TCG-GAAAGAAC-AGC				-----	
Mcur430		TG-GAAAGAACGGTC				-----	
Mcur513		TG-GAAAGAACGGTC				-----	
Agra		TCC-GAAAGAACGGGGCTTACGATGGAGT-----AGCTCTAGTGT-----GGCTCTGTGCGAA-AACCGCTGCCCCCTAGTAGAAC	-----			-----	AATAAAGGCC
Atur		TTC-GAAAGAACGGGG				-----	
Aodo309		TCC-GAAAGAAA-GAG				-----	
A0310		TCC-GAAAGAAA-GAG				-----	
A0338		TCC-GAAAGAACGGG				-----	
Acol		TCT-GGAAGAACACGG-AAGTACG				-----	
Auro		CCC-GGAAGAACGGG				-----	
A0400		CCC-GGAAGAACGGG				-----	
A0384		TCC-GGAAGAACGGG				-----	
A0386		TCC-GGAAGAACGGG				-----	
A0332		CCC-GGAAGAACGGG-AAGGA-CATCGTCCCTGGCA-ATGAG				-----	
Ahae		TTC-GGAAGAACGG-GCC				-----	
Blon		TCC-GGAAGAAC-GCC				-----	
Arthr		TCC-GGAAGAAC-CTT				-----	
Lxyl		TTC-GGAAGAAC-GAT				-----	
Blin		TCC-GGAAGAACACGG-----TATTGACCTGACCAAT-----GTGCG-CAGGGGAG-GGG	-----			-----	
Krad		TCC-GGAAGAAC-GCC				-----	
Janib		TTC-GGAAGAACATG-----ACCCTCAC-----GGGT				-----	
Ical		TCC-GGAAGAAC-GGT				-----	
Spro		TGC-GGAAGAACCCC				-----	
Derm		CCC-GGAAGAACACGG				-----	
Nocar		TCC-GGAAGAAC-GGT				-----	
NBroad		CCG-GGAAGAAC-GCC				-----	
Pacn		GCT-GGAAGAAC-GGG				-----	
Stro		TCC-GGAAGAAC-GGG				-----	
Cdip		TTC-GGAAGAAC-CAC				-----	
Nfar		TTC-GGAAGAACGGG				-----	
Rhodo		TTC-GGAAGAAC-GCC				-----	
Mtub		TTC-GGAAGAAC-GCC				-----	

Fig. S1. (Continued)

<----StemIII5'-----> <----StemIII3'-----> <----AT5' or AASD5'-----> <----T box---> <----AT3' or AASD3'----->

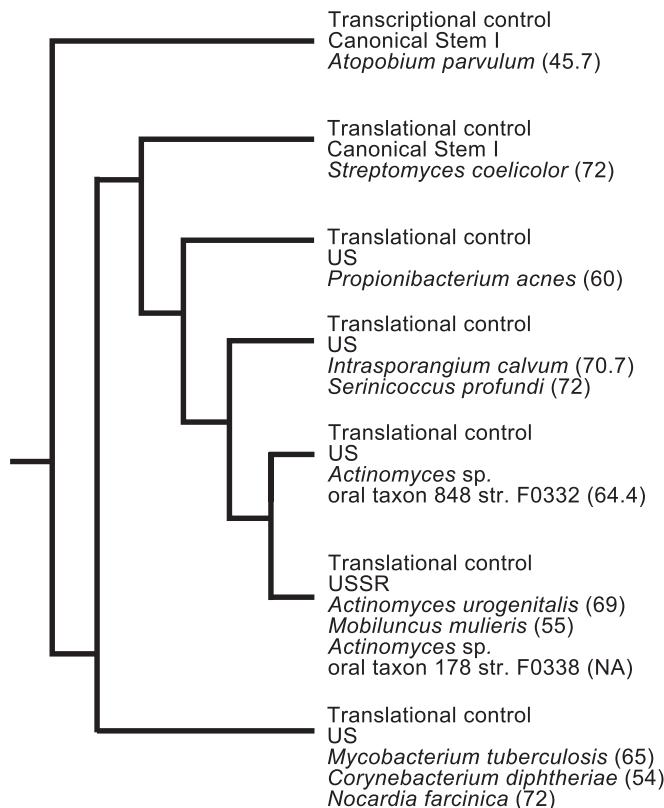
BsubGly	GCT-----GGGATTTTGTC	TCTC-----AGCAACTAGGGTGGAACCG-GCGGA-----GAAC-----TC TCGT-----CCCTA-
Bsub	GAA-----AAAGTGTATGCT	TGCTT-----TTCAAAGGGTGTGACCG-GAGA-----TAAGCTT-----TCTCC-----CCCTTA-
Apar	ACG-----GACATA-----TC-----CGTC-----GCTGGGTGGTACCAACGAGACTGT-----ATTGTG-----GCGCTCTG-----CCCTAC-	
Rxyl	GTC-----CGGGGGCGCGA	GCCCCTCG-----GCCAAGCACGGGTGGTACCAACGAGCCCTCTT-----GGAG-----AAAG-AG-GGCTCC-----CCCTGC-
Faln	GC-----CGCCCCACGGCCCGCTCGGCCG	GGATCGG-CGGAACCG-GGGGG-----TGCAGAACGGGTGGTACCAACGAGTA-----GCGGTAACCC-CGGG-----2-----CCGGTGAAGCGCTCT-----CCCTCC-
Tfus	GGG-----CGGGCGCGAAGGAGA	CGGGCGCG-----GCCAAGAGGGTGGTACCAACGAGG-----GCGG-GC-----GCT-----GCC TCG-----CCCTCC-
Scoe	GGC-----TCG-CGGAGAACACGAC	GACCGGCGG-----GCCAAGAGGGTGGTACCAACGAGG-----GCGG-GC-----GCT-----GCC TCG-----CCCTCC-
Mmul	GCT-----CGCTTCCAGAACCTGGGAGA3'-AAAAACG-AACCGGAGCGC	-AGCAAG-GGCTGGTACCAACGAGG-----GCGG-GC-----AAACA-----TGA-GGCGCT-----CCCTCG-
Mcur430	GCT-----CACTTGTGCGCTTCA-TAGTTG-----4	-CAGACTACGACAG-CACCAACGTC-----CGCAAGCGAGGGTGGTACCAACGAG-----GCGTACACCGACA-----AAG-----TGTGGA-A-GCTC-----CCCTCG-
Mcur513	GCT-----CACITGTGTCGGCTTCA-TAGTTG-----5	-CGGACTACGGCAAG-CATCACTG-----ACGAGAC-GGAGGTGGTACCAACGAG-----GCGTACACCGACA-----AAG-----TGTGGA-A-GCTC-----CCCTCG-
Agra	GCT-----TTGCT-GCTAGCT	-GCAAGCCCCCAC-----GGCTTTCGCGCTGCTGCTAGCT-----G-GCGCAAG-CGAGTGGTACCAACGAG-----GCGTACACCGACA-----AAG-----GCT-----GCC TCG-----CCCTCG-
Atur	GCT-----TTGCT-CCTCGCCGCGAC	-GATCG-CGGTGTGAG-----AGCAAG-CGGGGTGGTACCAACGAG-----GCGTACACCGACA-----GAA-----GCT-----GCC TCG-----CCCTCG-
Aodo309	GCC-----GCGGGCGGTGA-----GGG-AGC	-GGCAG-CGGGGTGGTACCAACGAG-----GCGTACACCGACA-----GAC-----GCGGTC-----CCCTCG-
A0310	GCC-----GCGCTTCTG-----GAGC	-GGCAAG-CGGGGTGGTACCAACGAG-----GCGTACACCGACA-----GAC-----GCGGTC-----CCCTCG-
A0338	GCC-----GGCGCA-----GGC	-GGCAAGGGGGTGGTACCAACGAG-----GCGTACACCGACA-----GTC-----GCGGTC-----CCCTCG-
Acol	GCT-----TTGTGAAAC-----TCCAGA	-AGCAACGGAGGAGTGGTACCAACGAG-----GCGGAGGAGAATACT-----ATAC-----CAGTA-CGCG-GAC-----CCCTCG-
Auro	GTC-----GCGCCCTCG-----GGC	-GGCAAGAGGTGGTACCAACGAG-----GCGGAGGAGAATACT-----ATAC-----CAGTA-CGCG-GAC-----CCCTCG-
A0400	GTC-----CGGGCGCTCTCGGACACT	-GGCGTGTGAC-AACCGG-----GGCAAGCGAGGGTGGTACCAACGAG-----GCGTGCCTCCCGG-----7-----CGG-GCGGGCGCTC-----CCCTCG-
A0384	GTC-----GTGCAACACCTGCTCTC	-GGGGCGCGGAC-----GGCAAGCGAGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----8-----GCTCGGAGGAGGAC-----GCT-----CCCTCG-
A0386	GTC-----GTGCAAGGT-CCTCCCGGT	-GGGGCGCG-----GGCAAGCGAGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----GCGGAGGCC-----GCT-----CCCTCG-
A0332	GCC-----ACCGCATCG-----GGC	-GGCAAGGGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----GCTCGGAGGCC-----GCT-----CCCTCG-
Ahae	GCC-----ACGCCACAGACTTACCCCCCG-----GGG-CAAAGTCT-TGGTTT	-GGCAAGCGAGGGTGGTACCAACGAG-----GCGTGAATA-CACT-----CCAA-----G-TGAGAC-----TCTC-----CCCTCG-
Blon	GTC-----GCGTAGGCT-CGGCGCTCCGGGACACT	-GGCAAGCGAGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----GCGG-CCTC-----TCTC-----CCCTCG-
Arthr	GCC-----GACGGCGTGTCTTCAAC-----GGGG-TCCGGCAAC	-GGTAAG-CGAGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----GCGG-CCTC-----TCTC-----CCCTCG-
Lxyl	GCC-----TCATCGGTGCTGTG-----GCCGAAGG	-GGCAAGGGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----GACAGGCTC-----GCT-----CCCTCG-
Blin	ATC-----CTCCGAT-----CGTCTGTCATCC	-GACAGACGCTTACGGCG-----GATAAG-CGAGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----GACA-----TCTC-----CCCTCG-
Krad	GTC-----GTGCGCCAA-----CCCTCC	-GGGGAGGGCGGGTC-----GGCAAGCGAGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----GCGGGGTC-----CCCTCG-
Janib	GTT-----GTATCTCCAGTGT-----ACAGCA	-GGCAAGGGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----GCGGACCC-----GCC-----CCCTCG-
Ical	GTC-----GTATGCG-----TG-GCTCGTCTC	-GGGGTACGCGCGGCTGTG-----GGCAAGGGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----TCGGAG-----T-----CAGC-----G-TGAGAC-----TCTC-----CCCTCG-
Spro	GTT-----CGAGCACGCGGACCGATCCTA-----CGCG-----GGCGTCTC	-GGCAAGGGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----GCGGCGG-----T-----CAGC-----G-TGAGAC-----TCTC-----CCCTCG-
Derm	GTC-----ATGTCGCTCTCGTGC-----GCCCGCAC	-CGGGCGAGAGCGCGG-----TTGAGAT-----GGCAAGCGAGGGTGGTACCAACGAG-----GCGGCCAC-----CCGG-----ACAC-----GCGGCAC-----TCTC-----CCCTCG-
Nocar	GTC-----CCTTCG-----GG	-GGCAAGCGAGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----GCT-----CTCG-----CCCTCG-
NBroad	GCC-----CAGGCA-----TG	-GGCAAGCGAGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----GCA-----GAT-----TCTC-----CCCTCG-
Pacn	GC-----CGACGTCG-----TACGCTG-----GCC	-GGCAAGCGAGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----GAA-----TCCGG-TGTG-----TCTC-----CCCTCG-
Stro	GCC-----GGTCGATCCT-----GCC	-GGCAAGCGAGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----GCGGAG-----T-----CAGC-----GCGG-----T-----CCCTCG-
Cdip	GAG-----C-TTACATCAGATGCC-----TCTGGTGGAAATG	-GGCAAGCGAGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----GCGG-----T-----CAGC-----GCGG-----T-----CCCTCG-
Nfar	GTC-----CGGTGCGCTCGGA-----GCCG	-GGCAAGCGAGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----GAA-----TCCGG-TGTG-----TCTC-----CCCTCG-
Rhodo	GCG-----CCCCTCG-----GGG	-GGCAAGGGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----ACCGG-----GCGGCGA-----GCT-----CCCTCG-
Mtub	GCC-----GGCGCATCG-----GGGT	-GGCAAGGGGGTGGTACCAACGAG-----GCGTGCCTCCCG-----9-----ACC-----GCGGCT-----GCT-----CCCTCG-----A

	Terminator5' or ASD5'	ASD	Terminator3' or ASD3'	SD	ATG
BsubGly	-----CTC <del>T</del> CGTCCC <del>T</del> ATG <del>T</del> TTGC-----	GGCTG-----	GCAAGCATAGA <del>G</del> ACGGGAG <del>T</del> TTTTGGT		
Bsub	-----GCTT <del>T</del> CTCGTCCC-----	TTAT-----	-----GGGA <del>T</del> GAGAGGCC <del>T</del> TTTTTTATTT		
Apar	-----GCGCTTCG <del>T</del> CCC-----	AGCTGTGA-----	-----GGGACGG <del>C</del> CCGCTTATTATTT		
Rxyl	-AAAGAG-GGC <del>T</del> CCCG <del>T</del> CCC <del>T</del> GGGC-----	GGAGA-----	-----GGT <del>T</del> CCGCCGG <del>C</del> GGGAGCT <del>T</del> GGCTT		
Faln	-----GTG <del>T</del> CCCT <del>T</del> CGTG-----	GTCCGACGCCAGCCA-----	-----CGACCAGGG-AAGACGGGTCCTGCTACAG <del>T</del> ATG		
Tfus	-----CGT <del>C</del> CTT-CCT <del>T</del> CGAGTG-----	ACCAGCA-----	-----CCCTGAT <del>T</del> GA <del>A</del> AGGTACGCCAC <del>G</del> TG		
Scoe	-----CCTCCGACCGAAG-----	GCAGCA-----	-----CGT <del>T</del> CCGCCGG <del>G</del> GAAGGAC <del>T</del> CGCTG <del>T</del> ATG		
Mmul	-----CGTCCC <del>T</del> CG-----	TGACCAACACGACCAG-----	-----CGAGGACAA <del>T</del> ATG		
Mcur430	-----CGTCC-TCG <del>T</del> GAAT-----	GAACG-----	-----ATT <del>T</del> CGGATAAGGACGA <del>A</del> ATG		
Mcur513	-----CGTCC---TCG <del>T</del> GAAT-----	GAGCG-----	-----ATT <del>T</del> CG <del>T</del> ATAAGGACGA <del>A</del> ATG		
Agra	-----CCT <del>T</del> CTG <del>T</del> A-----	CAAGGATCG-----	-----TCA <del>A</del> GACG <del>C</del> AAA <del>A</del> ATG		
Atur	-----GT <del>T</del> CTCTG <del>T</del> CCCTGT-----	GAATCGA-----	-----ACAG-ACGAGGACAA <del>C</del> ATG		
Aodo309	-----GT <del>T</del> CTCTG <del>T</del> CC-----	TG <del>T</del> ATAGGCC-----	-----GAGACGAGGAC <del>T</del> CTG		
A0310	-----GT <del>T</del> CTCTG <del>T</del> CC-----	TG <del>T</del> ATAGGCC-----	-----GAGACGAGGACACT <del>T</del> ATG		
A0338	-----CGGT <del>T</del> CTG <del>T</del> CC <del>T</del> GC-----	ACG-----	-----GCCAA-CACGGGAC <del>T</del> GGCATG		
Acol	-----UGT <del>T</del> CTCGG-----	AAAAGAAA <del>G</del> ATA <del>A</del> AGC <del>C</del> AA <del>A</del> ACGCT <del>G</del> CA <del>G</del> GT <del>T</del> AA <del>C</del> AGCC <del>C</del> CC <del>C</del> TA <del>C</del> CC <del>G</del> AGG <del>A</del> GG <del>A</del> AA <del>A</del> AG <del>A</del> ATG			
Auro	-----GT-CCTCTG-----	AGACACGGTTGTT <del>T</del> CACGAGCACGA-----	-----CGC <del>G</del> AGGCCCC <del>A</del> ATG		
A0400	-----TC <del>T</del> CTCTG <del>T</del> CTG-----	GCATG <del>C</del> AGAT <del>T</del> TC <del>C</del> ACGAGT <del>G</del> GA-----	-----T <del>G</del> CCAGG- <del>C</del> GA <del>G</del> ACT <del>C</del> ATG		
A0384	-----GT <del>T</del> CTCTG <del>T</del> CTG-----	CCCGGGCACCCGG <del>C</del> GGGAGCCG <del>G</del> GA <del>G</del> GA <del>C</del> -----	-----GT <del>G</del> AGACGAGGAC <del>C</del> ACCG <del>G</del> ATG		
A0386	-----GT <del>T</del> CTCTG <del>T</del> CTG-----	AGT <del>T</del> CCGGG <del>C</del> ACCCGG <del>C</del> GGGGGG <del>G</del> GG <del>G</del> GA <del>G</del> AC <del>G</del> GT <del>T</del> GA-----	-----GAC <del>G</del> AG <del>G</del> AC-AC <del>C</del> ACCG <del>G</del> ATG		
A0332	-----GT <del>C</del> G-TCTCTG-----	CTCGGGG <del>G</del> AT <del>G</del> TA-----	-----TGAGGAG <del>C</del> AA <del>A</del> ATG		
Ahae	-----TCTCTG <del>T</del> CTG-----	CCAAACA-----	-----TCA- <del>T</del> GA <del>G</del> AG <del>T</del> TCT <del>T</del> CT <del>T</del> ATG		
Blon	-----C-AT <del>T</del> CTCTG <del>T</del> TAG <del>C</del> GGAA <del>G</del> CG <del>C</del> AGAC <del>G</del> GG <del>G</del> CT <del>T</del> GG <del>G</del> GG-----	GGAACA-----	-----CAGTC- <del>A</del> GT <del>T</del> CC- <del>G</del> CTG <del>G</del> AGG <del>G</del> CG <del>A</del> ATG		
Arthr	-----C <del>T</del> CTCTG <del>T</del> CAT <del>T</del> CTG <del>A</del> -----	TG <del>T</del> TC-----	-----ACT <del>A</del> CT <del>T</del> AA <del>C</del> CCAGG <del>A</del> GT <del>T</del> CGAG- <del>A</del> ATG		
Lxyl	-----TCC-GGC-G <del>T</del> CTCTG-----	GCAGAAC-----	-----GCG <del>A</del> AA <del>A</del> CT <del>G</del> CCAGGAGGAGA <del>A</del> ATG		
Blin	-----CAT <del>T</del> G <del>T</del> CTCTG <del>T</del> CA <del>G</del> AG <del>T</del> GA-----	CGCA-----	-----GT <del>G</del> ACT- <del>C</del> AA <del>A</del> GG <del>G</del> CC <del>T</del> ATG		
Krad	-----TGT <del>T</del> CTCTG <del>T</del> CTG <del>G</del> GT <del>G</del> -----	GCAG-----	-----T <del>C</del> ACT <del>G</del> CG- <del>G</del> AGGA <del>G</del> -GAC <del>G</del> AC <del>T</del> G		
Janib	-----TGT <del>T</del> CTCTG <del>T</del> CTG <del>G</del> GT <del>G</del> -----	GAT <del>T</del> CA <del>G</del> AGGAG <del>G</del> CC <del>A</del> CTC-----	-----AGT <del>A</del> CA-----	-----GGC <del>G</del> GGC <del>G</del> - <del>A</del> GC-GAT <del>G</del> AG <del>T</del> AG <del>G</del> AG <del>G</del> AC <del>G</del> AC <del>G</del> ATG	
Ical	-----CAGGGTGAC-G <del>T</del> CTCTG <del>T</del> CTG-----	AAC <del>G</del> AGAT <del>C</del> CC <del>A</del> GG <del>G</del> AG <del>T</del> CC <del>G</del> AA <del>C</del> AC <del>G</del> CG-----	-----CAGCAGGA-----	-----GAC <del>C</del> AC <del>G</del> AC <del>C</del> AC <del>G</del> ATG	
Spro	-----G <del>T</del> CT <del>T</del> C <del>T</del> CTG <del>G</del> -----	CAC <del>G</del> AGGAC <del>C</del> AC <del>G</del> ACT <del>G</del> CG <del>A</del> CG <del>G</del> AC <del>A</del> -----	-----CT <del>C</del> CC <del>A</del> GGAGG <del>C</del> AC <del>C</del> CG <del>A</del> ATG		
Derm	-----G <del>T</del> CT <del>T</del> CTT-G <del>T</del> CTG-----	ACGAT <del>T</del> CTG-----	-----CC <del>G</del> CG <del>A</del> AA <del>G</del> GT <del>T</del> CC <del>A</del> AC <del>T</del> G		
Nocar	-----ATC-G <del>T</del> CTCTG <del>T</del> CGGT-----	GAGGAGT <del>C</del> AC <del>G</del> CGA-----	-----ACCC <del>G</del> AGG <del>G</del> CC <del>A</del> AC <del>T</del> G		
NBroad	-----GTC <del>T</del> CCTG-TGG-----	AAGAC <del>G</del> AA <del>A</del> CC <del>C</del> ACAC <del>A</del> CCAC-----	-----CCAC <del>G</del> AGGAG <del>A</del> GT <del>T</del> CC <del>G</del> CG <del>A</del> ATG		
Pacn	-----GGT <del>T</del> GT <del>T</del> CTG <del>T</del> CCCTG-----	GTGAC <del>G</del> CC <del>G</del> AGA-----	-----CGAAG <del>G</del> AC-CAC <del>C</del> CG <del>T</del> CG <del>A</del> ATG		
Stro	-----GGGT <del>T</del> -CG-T <del>C</del> C-T <del>C</del> CGAG-----	ACCC <del>G</del> AGAAG <del>G</del> GT <del>T</del> GAG-----	-----CT <del>G</del> GG <del>A</del> AGG <del>G</del> CC <del>G</del> AC <del>C</del> CC <del>G</del> ATG		
Cdip	-----T <del>C</del> CCCG-CACT <del>T</del> TA- <del>A</del> GG <del>G</del> CA-----	GAA-----	-----TGCT <del>T</del> GC <del>A</del> AA <del>G</del> GT <del>A</del> AGGA <del>G</del> AAA <del>A</del> ATG		
Nfar	-----GTC <del>T</del> CCCGT <del>T</del> CCCTG-----	CTGC-----	-----GGC <del>G</del> CG-----	-----GT <del>G</del> GC <del>A</del> AGG <del>G</del> GA <del>G</del> AC <del>G</del> GT <del>T</del> CC <del>G</del> CG <del>A</del> ATG	
Rhodo	-----GCG-T <del>T</del> CCCGT <del>T</del> CCCTG-----	TG <del>T</del> CCGAGTAC <del>G</del> T-----	-----CAGC <del>G</del> ACCA-----	-----CAGC <del>G</del> ACCA-GAGA <del>T</del> CG <del>G</del> GT <del>T</del> G	
Mtub	-----GCGT <del>T</del> CTG <del>T</del> CCC-GAGCCT- <del>G</del> ATT <del>G</del> CA-G <del>G</del> AC-----	GCA-----	-----GT <del>G</del> CC <del>G</del> AA <del>C</del> GG <del>T</del> GT <del>G</del> CT <del>G</del> GG <del>G</del> CT <del>G</del> GG <del>G</del> AGAC <del>G</del> AC <del>G</del> GC <del>G</del> CAA <del>A</del> ATG		

**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 antisequestor helix (or antiterminator). (B) Alignment of sequences that form the sequestor 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 antisequestor helix (or antiterminator) in A is partially repeated within the 5' side of the sequestor 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: GGGTGTGCCGCCGCACCC; in Fahn, there is an additional sequence at the top of the

Legend continued on following page

antisequestrator helix: TCCCGGGCGGGACG-CGG-CGGCGGGCAGGGCCGTGGCCGTCGTCAACGC-GCGG; in Mmul, there are two extra helices in stem III: TTGGG ACT-TGAAACCTTGCCAGGGAAACC-GGCAAGCACCATGAACCCGAAACCAGATGGGCAACGGGACATGAACGCCGCCGGAAATACCGGGTGGCGCCTCATGAACTC-ACCATC; in Mcur43063, there is an additional sequence at the top of stem III: ACCGCACCGGATAGGTGG-CGCC; in Mcur51333, there is an additional sequence at the top of stem III: ACCGCACCGGATAGGTGG-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: CGGGCACAGGCCG; in A0386, there is an additional sequence at the top of the antisequestrator helix: CGG-ACGCCAGTCCG; 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, *Intrasporangium calvum* DSM 43043; Janib, *Janibacter* sp. HTCC2649; Krad, *Keneococcus radiotolerans* SRS30216; Lxyl, *Leifsonia xyli* subsp. *xyli* str. CTCB07; Mcur430, *Mobiluncus curtisi* American Type Culture Collection (ATCC) 43063; Mcur513, *Mobiluncus curtisi* 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
<i>Acidimicrobium</i>	<i>Atopobium</i>	<i>Actinomyces</i>	<i>Actinomyces</i>
<i>Acidothermus</i>	<i>Catenulispora</i>	<i>Mobiluncus</i>	<i>Actinosynnema</i>
<i>Aeromicrobium</i>	<i>Collinsella</i>		<i>Amycolatopsis</i>
<i>Blastococcus</i>	<i>Coriobacterium</i>		<i>Amycolicoccus</i>
<i>Candidatus</i>	<i>Frankia</i>		<i>Arcanobacterium</i>
<i>Conexibacter</i>	<i>Nocardiopsis</i>		<i>Arthrobacter</i>
<i>Cryptobacterium</i>	<i>Olsenella</i>		<i>Beutenbergia</i>
<i>Eggerthella</i>	<i>Propionibacterium</i>		<i>Bifidobacterium</i>
<i>Geodermatophilus</i>	<i>Rubrobacter</i>		<i>Brachybacterium</i>
<i>Modestobacter</i>	<i>Streptomyces</i>		<i>Brevibacterium</i>
<i>Patulibacter</i>	<i>Streptosporangium</i>		<i>Cellulomonas</i>
<i>Segniliparus</i>	<i>Thermobifida</i>		<i>Clavibacter</i>
<i>Slackia</i>	<i>Thermobispora</i>		<i>Corynebacterium</i>
<i>Tropheryma</i>	<i>Thermomonospora</i>		<i>Dermacoccus</i>
	<i>Kitasatospora</i>		<i>Dietzia</i>
			<i>Gardnerella</i>
			<i>Gordonia</i>
			<i>Intrasporangium</i>
			<i>Janibacter</i>
			<i>Jonesia</i>
			<i>Kineococcus</i>
			<i>Kocuria</i>
			<i>Kribbella</i>
			<i>Kytococcus</i>
			<i>Leifsonia</i>
			<i>Microbacterium</i>
			<i>Micrococcus</i>
			<i>Micromonospora</i>
			<i>Mycobacterium</i>
			<i>Nakamurella</i>
			<i>Nocardia</i>
			<i>Nocardioidaceae</i>
			<i>Nocardioides</i>
			<i>Parascardovia</i>
			<i>Propionibacterium</i>
			<i>Pseudonocardia</i>
			<i>Renibacterium</i>
			<i>Rhodococcus</i>
			<i>Rothia</i>
			<i>Saccharomonospora</i>
			<i>Salinispora</i>
			<i>Sanguibacter</i>
			<i>Scardovia</i>
			<i>Stackebrandtia</i>
			<i>Tsukamurella</i>
			<i>Verrucosispora</i>
			<i>Xylanimonas</i>

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

DNA template	Generation of plasmid vectors	PCR primers
<i>N. farcinica ileS</i>	PCR using ligation reaction as a template and 5'-TTTATATGATCATTACGAGGGAGACGGCAGCATCCGCCA- TC-3' and 5'-TAGCTGCGACGCTGTCGCCATCGGGATGCGCCTCCT-3' as primers PCR using <i>B. subtilis</i> chromosomal DNA as a template and 5'-ATTAATCTAGATTACGAAGAATTCGGATTGTA-3' and 5'-GCCGTCTCCCTCGTAAATGATCATATAAAAAGATGGACC-3' as primers PCR using products from both of the PCR above as template and 5'-ATTAATCTAGATTACGAAGAATTCGGATTGTA-3' and 5'-TAGCTGCGACGCTGTCGCCATCGGGATGCGCCTCCT-3' as primers Insert PCR product into plasmid pFG328 using XbaI, SalI No plasmid generated	<b>tRNA-binding reactions:</b> 5'-TTCTCGAATTCTAATACGACTCACTATAGGCAC- GATCCGGCCATCAC-3' 5'-ACGGGACGGCCTCGCGCCGGTGC-3'  <b>Primer extension reactions:</b> 5'-TTCTCGAATTCTAATACGACTCACTATAGGCAC- GATCCGGCCATCAC-3' 5'-TGCAGGGCCTTCGCTATTACGCCA-3'  <b>RNase H cleavage assays:</b> 5'-CTTAATGCAGGAGTCGCATAAGGG-3' 5'-CGATTAAGTTGGTAACGCCAGG-3'
A8G <i>N. farcinica ileS</i>	No plasmid generated	5'-ATATAATACGACTCACTATAGGCACGGTCCGGC- CATCACGGGGAGCCT-3' 5'-ACGGGACGGCCTCGCGCCGGTGC-3' 5'-TTCTCGAATTCTAATACGACTCACTATAGGCAC- CGATCCGGCCATCAC-3' 5'-ACGGGACGGCCTCGCGCCGGTGC-3'
A16U <i>N. farcinica ileS</i>	No plasmid generated	
<i>N. farcinica tRNA<sup>Ile</sup></i>	Insert ligation reaction product into plasmid pGEM7-S4P-tRNQ using XmaI, NcoI Insert ligation reaction product into pGEM4 plasmid using XbaI, PvuII	5'-GAGCGAGGAAGCGGAAGAGCGGCC-3' 5'-TGGTGGGCCTAGGAGGA-3' 5'-TAATACGACTCACTATAGGCCTATAGCTCAG-3' 5'-GGGCCTAGGAGGACTTGAA-3' 5'-TAATACGACTCACTATAGGCCTATAGCTCAG-3' 5'-GTGGTGGGCCTAGGAGGA-3' 5'-TAATACGACTCACTATAGGCCTATAGCTCAG-3'
<i>N. farcinica tRNA<sup>Ile</sup>ΔACCA</i>	No plasmid generated	
<i>N. farcinica tRNA<sup>Ile</sup>Ex1C</i>	No plasmid generated	
<i>N. farcinica U36A tRNA<sup>Ile</sup></i>	Site-specific oligomutagenesis of the plasmid pGEM7-S4P-tRNQ with <i>N. farcinica tRNA<sup>Ile</sup></i> template insert using 5'-GGTAGAGCGCTCGCTGAAACGAAGAGGTGGAGG-3' and 5'-CCTCCGACCTCTCGTTTCAGCGAAGCGCTAAC-3' oligonucleotides	5'-TGGTGGGCCTAGGAGGA-3'
<i>Actinomyces</i> sp. <i>ileS</i>	Insert ligation reaction product into pGEM4 plasmid using XbaI, PvuII	5'-TAATACGACTCACTATAGG-3' 5'-GCAGGGACGAGGACCGG-3'
<i>Actinomyces</i> sp. <i>tRNA<sup>Ile</sup></i>	Insert ligation reaction product into pGEM4 plasmid using XbaI, PvuII	
<i>S. coelicolor ileS</i>	No plasmid generated	
<i>S. coelicolor tRNA<sup>Ile</sup></i>	No plasmid generated	
<i>B. subtilis tRNA<sup>Gly</sup></i>	No plasmid generated	
<i>B. subtilis tRNA<sup>Val</sup></i>	PCR using <i>B. subtilis</i> chromosomal DNA as a template and 5'-AATATTAATACGACTCACTATAGTCGGTAGCTCAGCTGG-3' 5'-ATAATTCTAGAGCAATGCATGGTAGTCGACTGGCTCGAAC-3' as primers Insert into pGEM4 plasmid using XbaI, PvuII	5'-TAATACGACTCACTATAGGCATGGATGGGACG-3' 5'-GGAGGGACGAGAGCCGTGCT-3' 5'-ATATAATACGACTCACTATAGGGCTATAGCTCA- GTTGGTTAGA-3' 5'-TGGTGGGCCTAACAGGATTGAAAC-3' 5'-TAATACGACTCACTATAGCGGAAGTAGTCAGTGG-3' 5'-TGGAGCGGAAGACGGGATTGAAAC-3'

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'-CGATTAAGTTGGTAACGCCAGG-3'