

Table S1. Oligonucleotides and primers used in this study.

Target (construct, gene, or mutation)	Process	Oligonucleotide Sequence 5' to 3'
His-RlmN	Cloning in pET15b	GATACC ATGGGT <u>CATCATCACCATCACCATT</u> TCTGAACAATTAGTCACACCTGAA GATAGG ATCCTT ATCAGACCGCTTTAATGTCGATG
tRNA ^{Gln} _{UUG}	Cloning in pUC19	GATAG AATTC aattaatacgactcactatagGGGGGTATCGCCAAGCGGTA GATAGG ATCCT GGCTGGGGTACCTGGATTC
tRNA ^{Arg} _{ACG}	Cloning in pUC19	GATAG AATTC aattaatacgactcactatagGGCATCCGTAGCTCAGCTGGATAG GATAGG ATCCT GGTGCATCCGGGAGGATTC
tRNA ^{Chimera} _{UUG}	Cloning in pBSKma	GCCCGGATAGCTCAGTCGGTAGAGCA CCGGTTTTTGATACCGG GGGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCA GATAG AATTC GCCCGGATAGCTCAGTCGGTAG* GATA CTGCAGTGGCG CCCGAACAGGGACT*
A37C	Cloning in pBSKma	GCCCGGATAGCTCAGTCGGTAGAGCA CCGGTTTTTGCTACCGG GGGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCA
U35A and U35G	Cloning in pBSKma	GCCCGGATAGCTCAGTCGGTAGAGCA CCGGTTTTRGATACCGG GGGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCA
G36U and G36A	Cloning in pBSKma	GCCCGGATAGCTCAGTCGGTAGAGCA CCGGTTTTTWATACCGG GGGTCCAGGGTTCAAGTCCCTGTTCGGGCGCCA
Δ rlmN	Mutation checking, PCR	TCTTTGGCGAAGGCGAAGTGTG + <i>kan</i> primer (Datsenko & Wanner, 2000)
Δ miaA	Mutation checking, PCR	GAAGCAGTCCGTATTCGAACCTG + <i>kan</i> primer (Datsenko & Wanner, 2000)
Δ mnmE	Mutation checking, PCR	TCGTCAGCAACCTGGTAACCA + <i>kan</i> primer (Datsenko & Wanner, 2000)
Δ mnmA	Mutation checking, PCR	GGGTCAGCGCCGAAGAA + <i>kan</i> primer (Datsenko & Wanner, 2000)
<i>rpsL222</i>	Mutation checking, PCR & Sequencing	CTTGACACCTTTTCGGCATCGCC** CGTTTGGCCTTACTTAACGGAGAACC
<i>rpsD12</i>	Mutation checking, PCR & Sequencing	GATGTGACTCGATCCCTCATAACG** CTCTGTCACAGAACCCTGCATTGTG
tRNA ^{Chimera} _{UUG}	Quantitative PCR	GCCCGGATAGCTCAGTCGGTAG TGGCGCCCGAACAGGGACT
tRNA ^{Lys} _{UUU}	Quantitative PCR	GGGTCGTTAGCTCAGTTGGTAG TGGTGGGTCGTGCAGGATTCG

Bold sequences represent restriction targets for cloning in the corresponding plasmids; underlined sequences define the His-tag carried by recombinant RlmN; lowercase sequences define consensus T7 promoter; bold and underlined sequences define the Anticodon Stem Loop of tRNA^{Gln}_{UUG} fused to D, T and acceptor arms of the tRNA scaffold encoded in pBSKma plasmid. Nucleotide variants are presented according to IUPAC nomenclature. Primers labeled with an asterisk were used for amplification of the larger oligonucleotide containing the tRNA^{Chimera}_{UUG} sequence and its variants carrying mutations in U35, G36, and A37. Primers labeled with a double asterisk were used for DNA sequencing.