

Table S1. Effects of 16S rRNA mutations isolated in the genetic screen on translation from ACG, AUC and AUG.

16S allele ^a	β-galactosidase activity ^b			Relative translation level ^c	
	ACG	AUC	AUG	ACG/AUG	AUC/AUG
Control	3.1 ± 0.2	8.0 ± 0.4	2300 ± 80	0.0014 ± 0.0001	0.0035 ± 0.0002
U13G	6.6 ± 0.1	13 ± 0.6	1500 ± 60	0.0043 ± 0.0002	0.0087 ± 0.0005
U789C	5.0 ± 0.2	9.9 ± 0.1	500 ± 30	0.0092 ± 0.0006	0.018 ± 0.0009
A790G	7.2 ± 0.1	13 ± 0.1	500 ± 60	0.014 ± 0.002	0.025 ± 0.003
G886A	8.6 ± 0.1	16 ± 0.1	1800 ± 50	0.0047 ± 0.0002	0.0088 ± 0.0003
U1083C	4.5 ± 0.1	11 ± 0.2	1700 ± 80	0.0026 ± 0.0001	0.0062 ± 0.0003
A1092G	4.3 ± 0.1	11 ± 0.2	1800 ± 30	0.0023 ± 0.0001	0.0058 ± 0.0001
G1094A	4.3 ± 0.1	9.1 ± 0.4	1000 ± 100	0.0043 ± 0.0004	0.0089 ± 0.001
C1389U	5.0 ± 0.03	12 ± 0.1	1900 ± 90	0.0026 ± 0.0001	0.0059 ± 0.0003
A1408G	5.0 ± 0.01	12 ± 0.7	1600 ± 100	0.0031 ± 0.0002	0.0075 ± 0.0006
A1410G	8.5 ± 0.1	15 ± 0.5	1300 ± 30	0.0067 ± 0.0002	0.012 ± 0.0005
C1411U	4.2 ± 0.03	8.9 ± 0.3	1200 ± 10	0.0034 ± 0.0001	0.0071 ± 0.0003
U1414C	7.7 ± 0.5	15 ± 0.1	1900 ± 100	0.0041 ± 0.0003	0.0080 ± 0.0004
G1486A	4.9 ± 0.1	9.7 ± 0.1	2200 ± 20	0.0022 ± 0.0001	0.0045 ± 0.0001

^aEach 16S allele contains the specialized SD* sequence to allow specific translation of the *lacZ* reporter gene.

^bSpecialized 16S rRNA (without or with mutations as indicated) was expressed in indicator strains KLF2522, KLF2526 and KLF2528 (harboring SD*-ACG-*lacZ*, SD*-AUC-*lacZ*, or SD*-AUG-*lacZ*, respectively) and specific β-galactosidase activity (based on cleavage of CPRG) was measured. Values represent the mean ± SEM (arbitrary units) of at least 3 independent experiments.

^cValues represent the quotient of two means ± standard error.

Table S2. Effects of engineered mutations in h44 on translation of *lacZ* from ACG, AUC and AUG.

16S allele ^a	β-galactosidase activity ^b			Relative translation level ^c	
	ACG	AUC	AUG	ACG/AUG	AUC/AUG
Control	3.1 ± 0.2	8.0 ± 0.4	2300 ± 80	0.0014 ± 0.0001	0.0035 ± 0.0002
A1413C	8.6 ± 0.7	21 ± 0.5	1600 ± 200	0.0055 ± 0.0007	0.014 ± 0.002
A1413G	1.7 ± 0.6	7.0 ± 0.7	2000 ± 100	0.0009 ± 0.0003	0.0035 ± 0.0004
U1414G	1.9 ± 0.5	5.2 ± 0.6	800 ± 200	0.0023 ± 0.0008	0.0064 ± 0.001
G1486C	1.5 ± 0.03	3.3 ± 0.5	1100 ± 200	0.0014 ± 0.0003	0.0029 ± 0.0007
G1487U	5.5 ± 0.6	12 ± 0.2	1800 ± 100	0.0030 ± 0.0004	0.0063 ± 0.0004
G1487A	0.7 ± 0.2	1.7 ± 0.2	1700 ± 100	0.0004 ± 0.0001	0.0010 ± 0.0001
U1490C	4.7 ± 0.5	9.9 ± 0.06	550 ± 100	0.0086 ± 0.002	0.018 ± 0.004
U1414G-G1486C	5.0 ± 0.5	14 ± 0.6	2000 ± 200	0.0024 ± 0.0004	0.0067 ± 0.0008
A1410G-G1490C	2.6 ± 0.6	6.1 ± 0.4	1500 ± 300	0.0017 ± 0.0005	0.0039 ± 0.0009

^aEach 16S allele contains the specialized SD* sequence to allow specific translation of the *lacZ* reporter gene.

^bSpecialized 16S rRNA (without or with mutations as indicated) was expressed in indicator strains KLF2522, KLF2526 and KLF2528 (harboring SD*-ACG-*lacZ*, SD*-AUC-*lacZ*, or SD*-AUG-*lacZ*, respectively) and specific β-gal activity (based on cleavage of CPRG) was measured. Values represent the mean ± SEM (arbitrary units) of at least 3 independent experiments.

^cValues represent the quotient of two means ± standard error.