Supplemental Figures

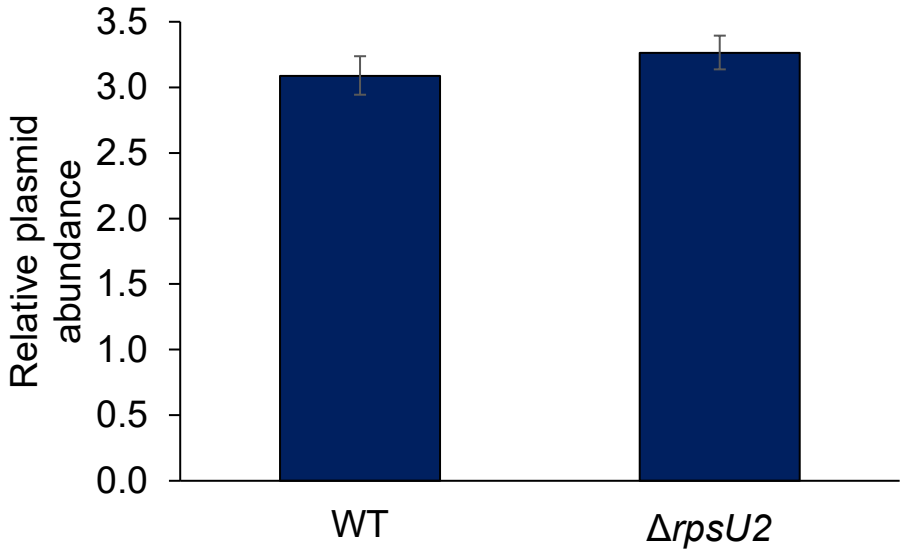


Figure S1. pF plasmid copy number is not affected by presence of bS21-2. Quantitative real-time PCR of total DNA from wild-type (WT) cells and cells lacking bS21-2 (Δ*rpsU2*) was used to assess the relative abundance of the multi-copy plasmid used in GFP experiments. An opening reading frame on the plasmid, ORF3, was amplified and normalized to chromosomally-encoded *tul4*, whose expression is not influenced by bS21-2. Error bars represent 1 SD from the mean value (calculated using the mean threshold cycle). Experiments were repeated three times and data from a representative experiment are shown.

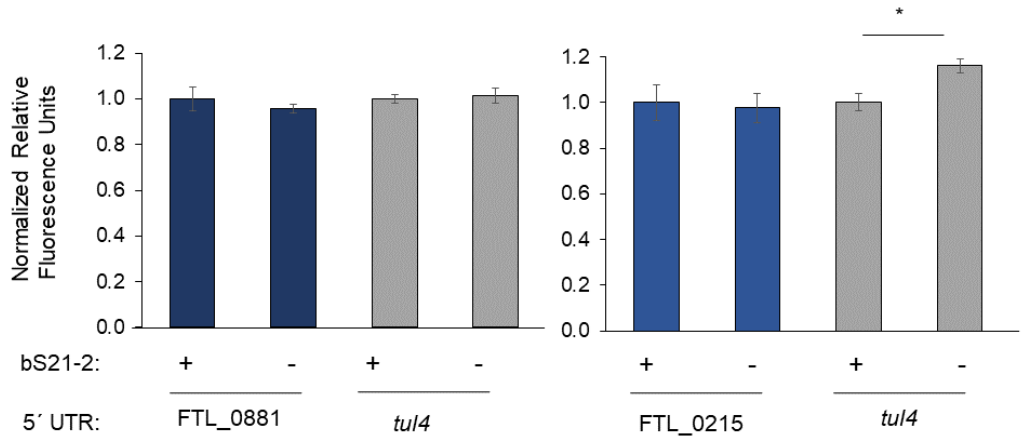


Figure S2. Predicted 5´ UTR of some genes is not sufficient for bS21-2 to affect translation.

Green fluorescence was measured for indicated 5´ UTR reporter constructs in cells with (+; WT) or without (-; Δ*rpsU2)* bS21-2 and normalized to OD600 and a nonfluorescent strain. FTL_0881 and FTL_0215 were found to be less abundant at the protein level in cells lacking bS21-2 in a proteomics screen (Trautmann & Ramsey, 2022). *tul4* 5´ UTR is included as a control. Error bars represent 1 SD. Experiments were repeated twice and data from representative experiments are shown. * $p<0.05$.

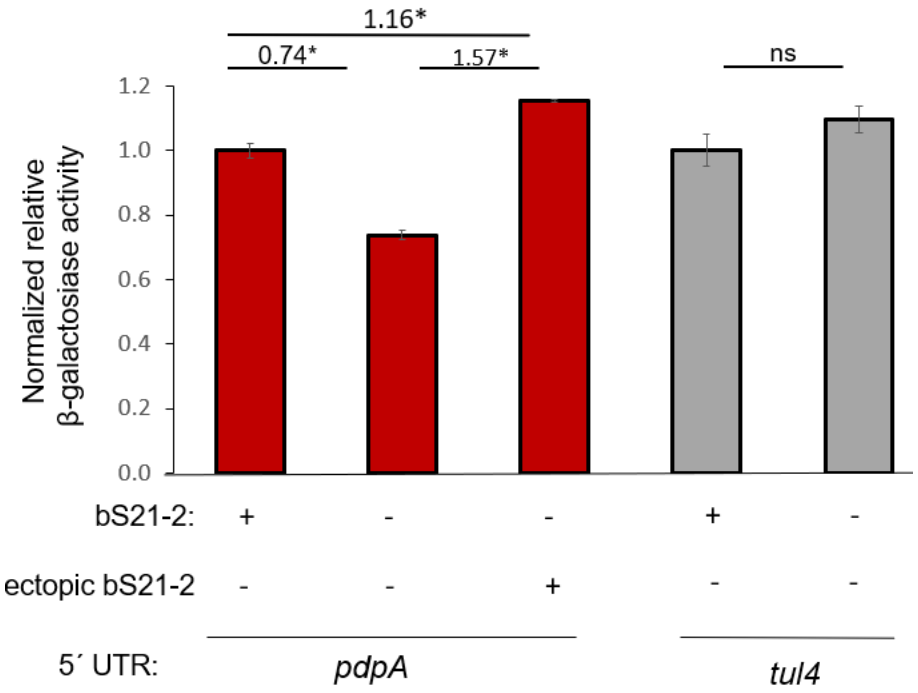


Figure S3. Defects in protein production can be complemented by ectopic expression of bS21-2.

Relative β-galactosidase activity of *pdpA* or *tul4* 5´ UTRs in cells with (+; WT) or without (-; Δ*rpsU2)* native bS21-2, or with ectopically expressed bS21-2. β-galactosidase reporters were located at the Tn7 site on the chromosome and ectopic expression of bS21-2 was from a multicopy plasmid, pF-nat. Strains without ectopically expressed bS21-2 contained an empty vector. β-galactosidase activity was normalized to OD600 and a nonfluorescent strain. Error bars represent 1 SD. * $p<0.05$. ns = not significant.

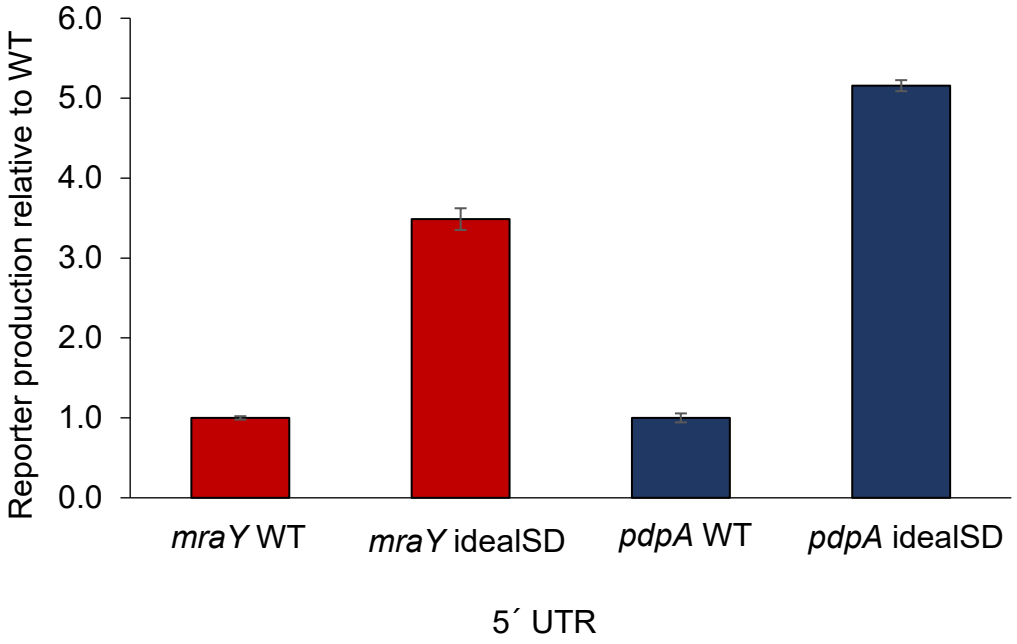
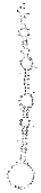
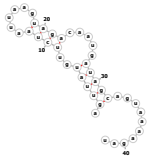
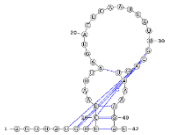


Figure S4. Modifications adding ideal Shine-Dalgarno sequences increase reporter protein production relative to unmodified 5´ UTRs.

Total β-galactosidase activity or green fluorescence of *pdpA* or *mraY* 5´ UTRs, respectively, in cells with bS21-2. Reporter production is relative to unmodified (WT) 5´ UTR for each gene. Production was normalized to OD600 (both constructs) and a nonfluorescent strain (GFP only). Error bars represent 1 SD. Experiments were repeated twice and data from representative experiments are shown.

5´ UTR	Sequence	MXFold2	Ufold	ViennaRNA
mraY WT	auaaaaaauuugaaccaauuauu uagacgcuaauuuugacucuauu aaaaaaauaacauaucuauuaua auacuccaaggucauuaaacauu uuaaauauAUGcugauuuaucu uuuu			
mraY mut3	auaaaaaauuugaaccaauuauu uagacgcuaauuuugacucuauu aaaaaaauaacauaucuauuaua auacuccaCUACUauuaaacau uuuaaauau AUG cugauuuaucuuuuu		Server unavailable	
mraY mut4	auaaaaaauuugaaccaauuauu uagacgcuaauuuuAGUGAGa uuaaaaaaauaacauaucuauua uaauacuccaCUACUauuaaac auuuuaaauau AUG cugauuuaucuuuuu		Server unavailable	
pdpA WT	aguuauguucuaauuaaguagac aaugauagcaguaaaagau			
pdpA mut1	aguuauguucuaauuaaguaCU caaugauUgcaguaaaagau			

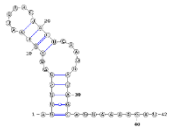
5´ UTR	Sequence	MXFold2	Ufold	ViennaRNA
pdpA mut2	aguuauguAGuaauuaaguaCU caaugauAgcaguaaaagau			

Figure S5. Comparison of secondary structure prediction software.

Predicted structures of wild-type or modified *mraY* and *pdpA* 5´ UTRs from three secondary structure prediction programs: MxFold2 (Sato et al. 2021), Ufold (Fu et al. 2022), and ViennaRNA (Lorenz et al. 2011).