

Supplemental Figures

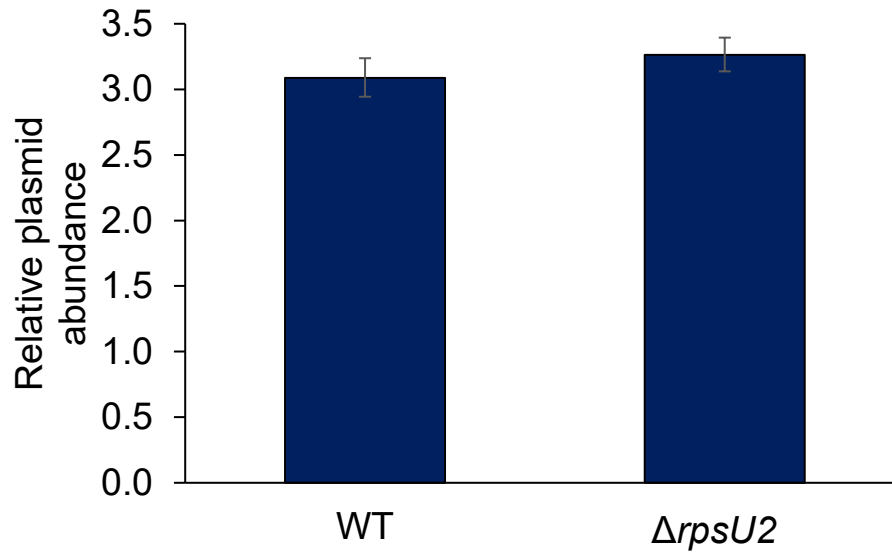


Figure S1. pF plasmid copy number is not affected by presence of bS21-2.

Quantitative real-time PCR of total DNA from wild-type (WT) cells and cells lacking bS21-2 ($\Delta rpsU2$) 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.

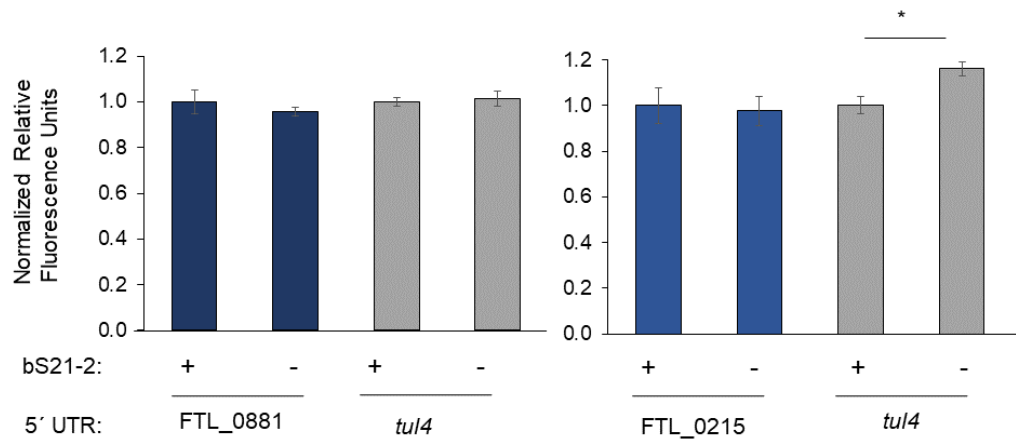


Figure S2. Predicted 5' UTR of some genes is not sufficient for bS21-2 to affect translation.

Green fluorescence was measured for indicated 5' UTR reporter constructs in cells with (+; WT) or without (-; $\Delta rpsU2$) 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. * $p < 0.05$

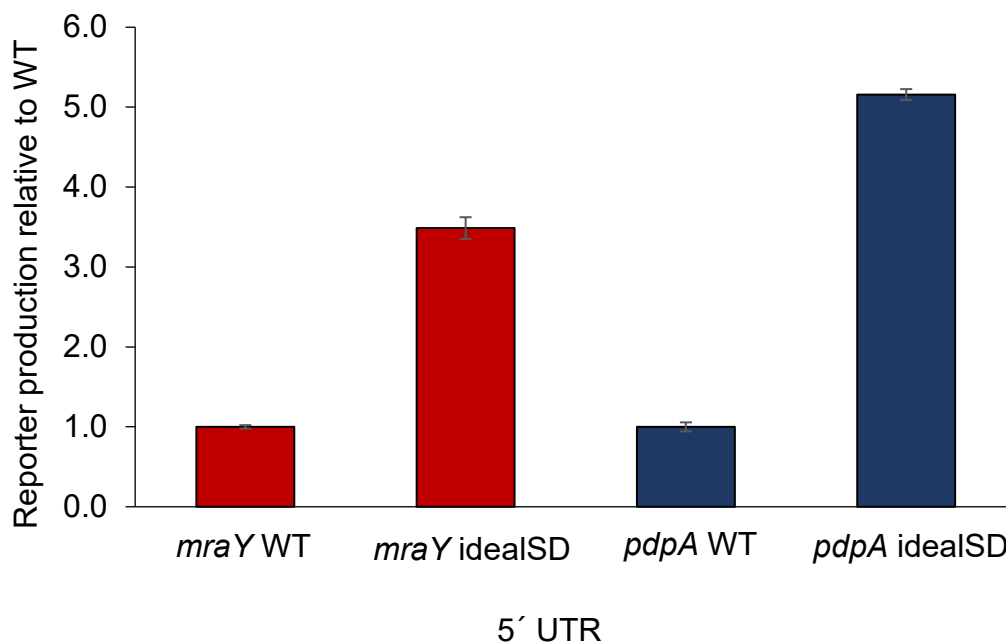







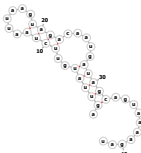
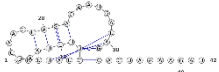


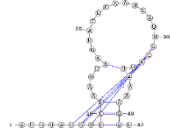



Figure S3. Modifications adding ideal Shine-Dalgarno sequences increase reporter protein production relative to unmodified 5' UTRs.

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
<i>mraY</i> WT	auaaaaaaaauuugaaccaauuuuu uagacgcuaauuuuugacucuauu aaaaaaaaaaacaauaucuauuuau auacuccaaggucuuuaacauu uaaaauauAUGcugauuuaucu uuuu			
<i>mraY</i> mut3	auaaaaaaaauuugaaccaauuuuu uagacgcuaauuuuugacucuauu aaaaaaaaaaacaauaucuauuuau auacuccaCUACUauuuaaacau uuuuuuuau AUG cugauuuaucuuuuu		Server unavailable	
<i>mraY</i> mut4	auaaaaaaaauuugaaccaauuuuu uagacgcuaauuuuuAGUGAGa uaaaaaaaaaacaauaucuauua uauacuccaCUACUauuuaaac auuuuuuuuuau AUG cugauuuaucuuuuu		Server unavailable	
<i>pdpA</i> WT	aguuauguucuaauuaaguagac aaugauagcaguaaaagau			
<i>pdpA</i> mut1	aguuauguucuaauuaaguaCU caaugauUgcaguaaaagau			

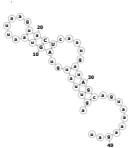
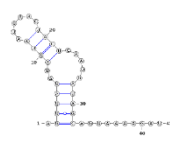

5' UTR	Sequence	MXFold2	Ufold	ViennaRNA
<i>pdpA</i> mut2	aguuauguAGuaauuaaguaCU caaugauAgcaguaaaaagau			

Figure S4. 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).