

Supplemental Figures

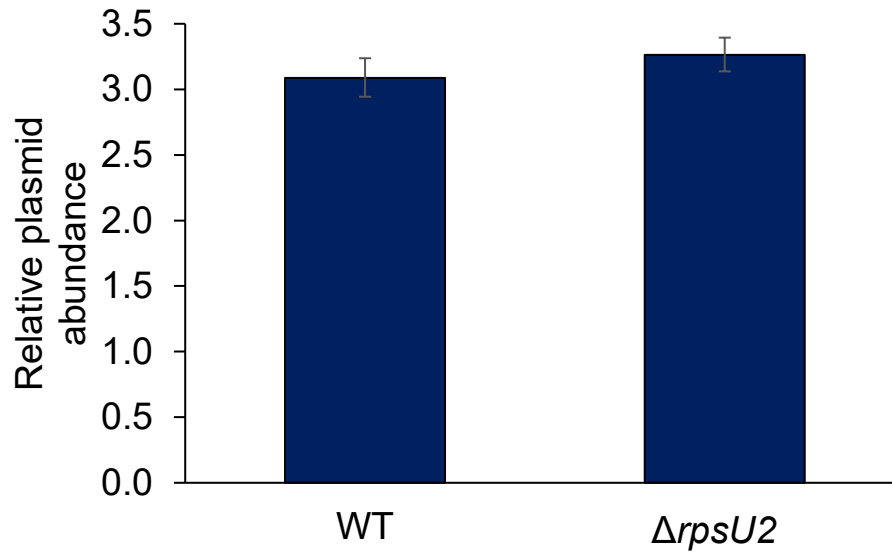


Figure S1. pF plasmid copy number is not affected by presence of bS21-2.

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.

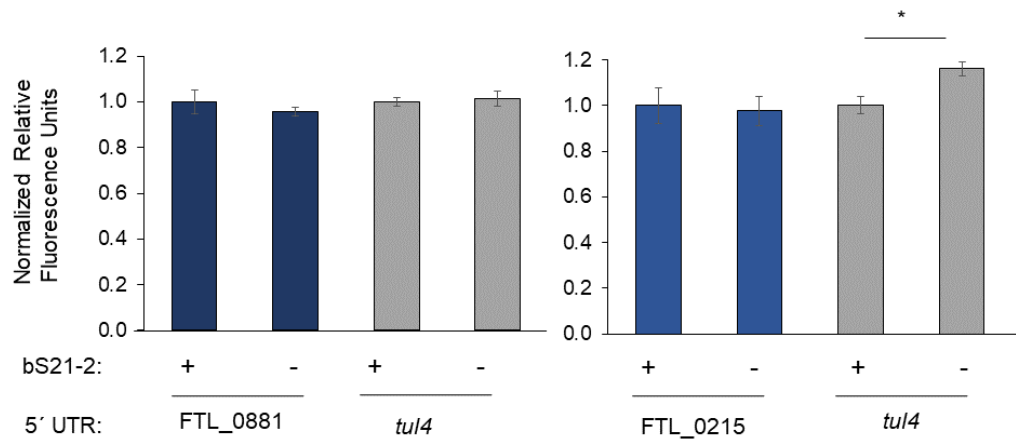


Figure S2. Predicted 5' UTR of some genes is not sufficient for bS21-2 to affect translation.

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. * $p < 0.05$.

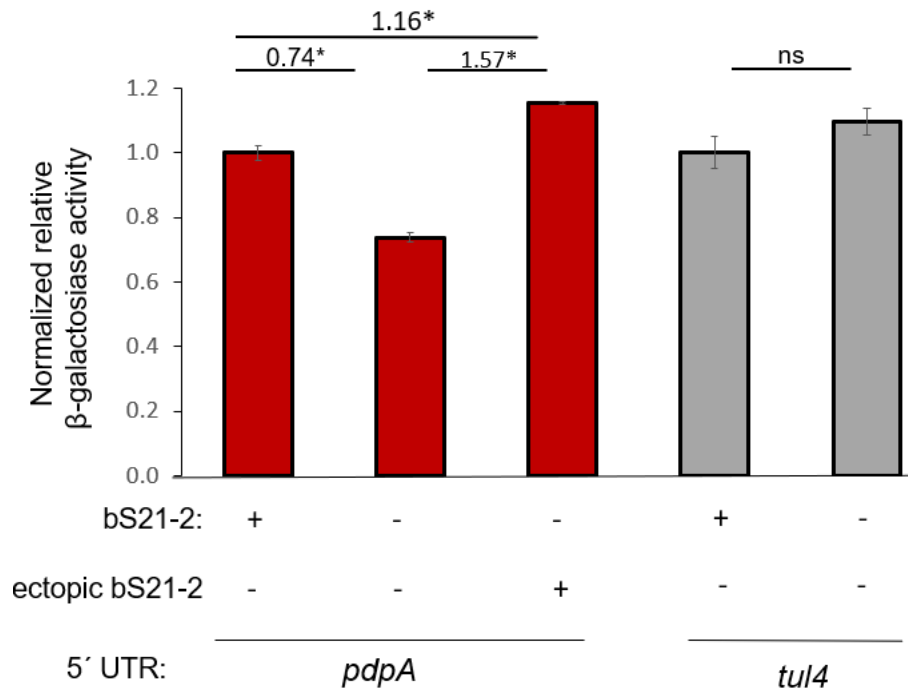


Figure S3. Defects in protein production can be complemented by ectopic expression of bS21-2.

Relative β-galactosidase activity of *pdpA* or *tul4* 5' UTRs in cells with (+; WT) or without (-; $\Delta rpsU2$) 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.

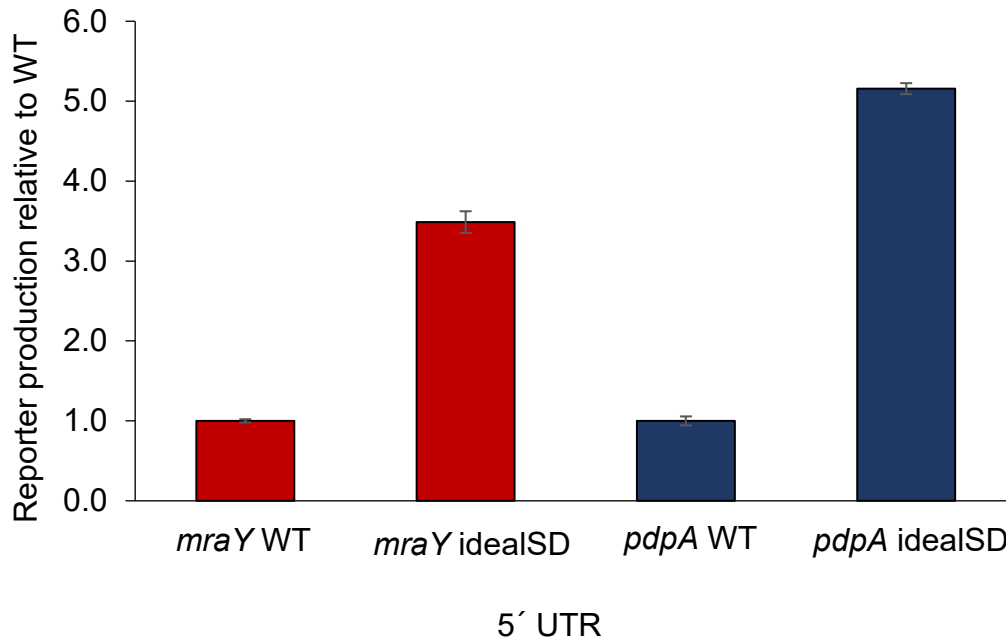


Figure S4. Modifications adding ideal Shine-Dalgarno sequences increase reporter protein production relative to unmodified 5' UTRs.

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.