

Figure 1. 5' UTRs are sufficient to lead to bS21-2-mediated changes in translation. (A) Diagrams of the translational reporter fusions used. Reporters used the *tul4* promoter to drive expression of the tested 5' UTR, including the first 6 codons of the gene, and are in frame with either *lacZ* at the Tn7 site of the genome or *gfp* on a multi-copy plasmid. **(B)** Relative fluorescence for indicated translational fusion reporters in cells with (+; WT) or without (-; $\Delta rpsU2$) bS21-2 in biological triplicate. The *tul4* reporter serves as a control. 5' UTR sequences can be found in Supplemental Table 1. **(C)** Relative β -galactosidase activity for indicated translational fusions in cells with (+; WT) or without (-; $\Delta rpsU2$) native bS21-2, or with ectopically expressed bS21-2 from a multicopy plasmid, pF-nat (pF-bS21-2). Strains without ectopically expressed bS21-2 contained an empty vector (pF). Error bars represent 1 SD. * $p < 0.05$ by t-test. Experiments were repeated at least twice and data from a representative experiment are shown.

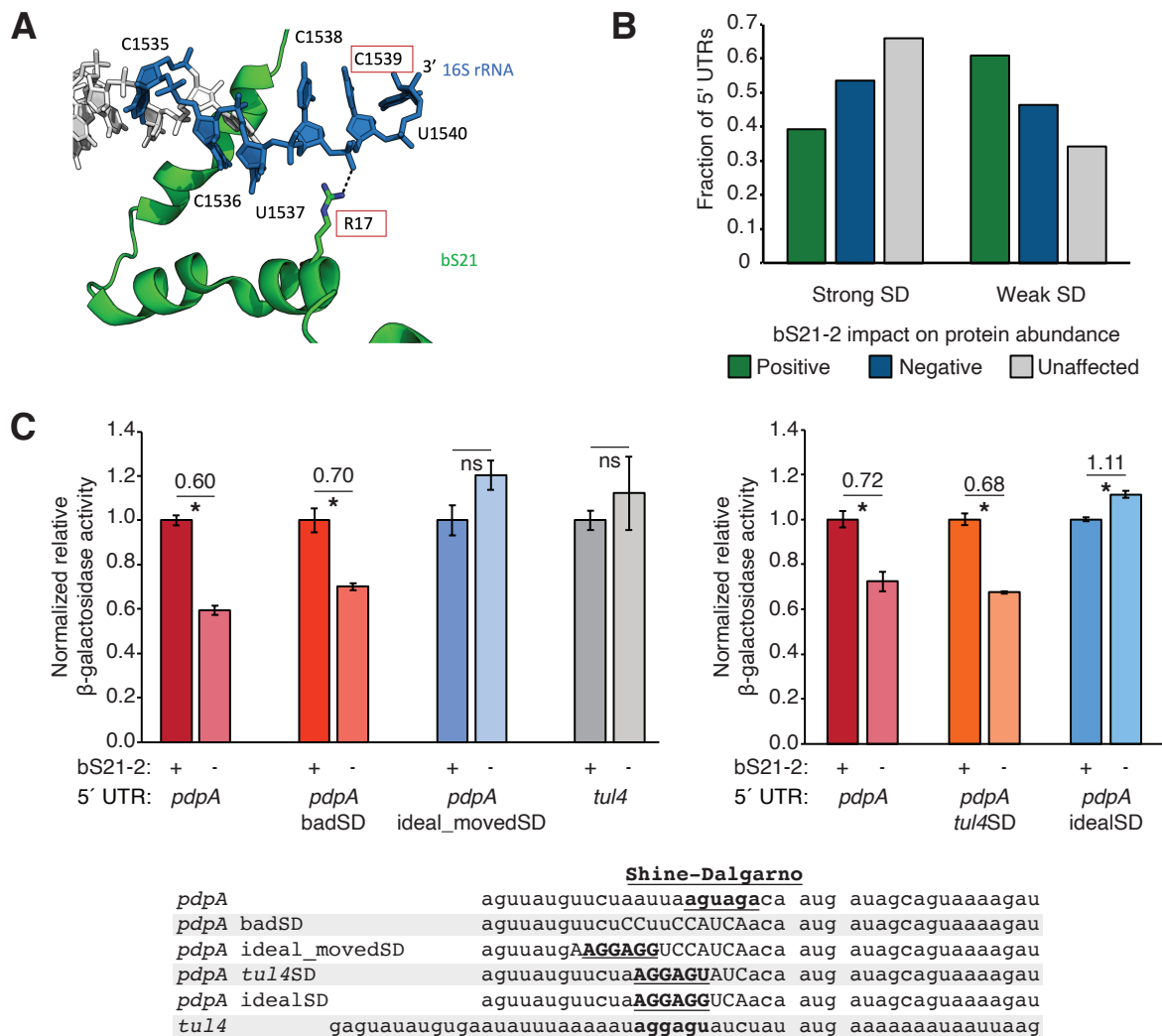
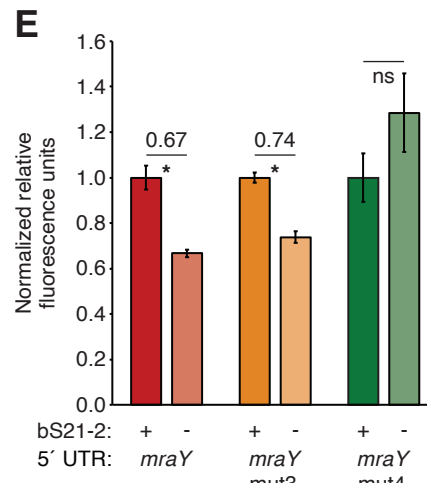
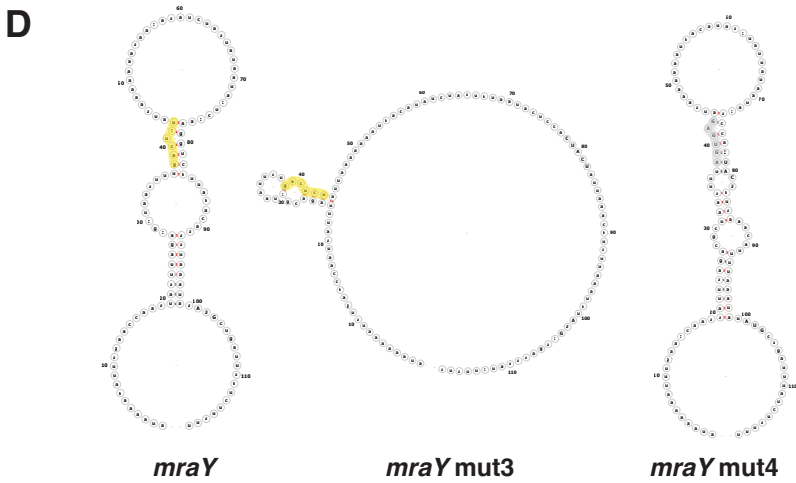
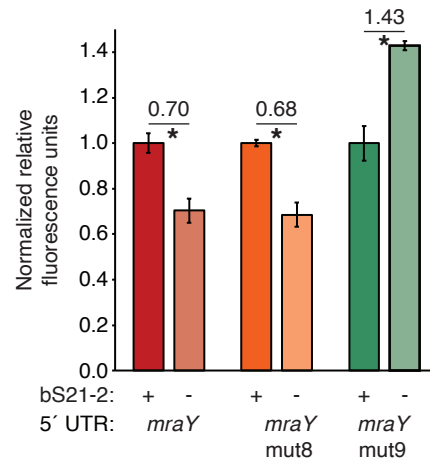
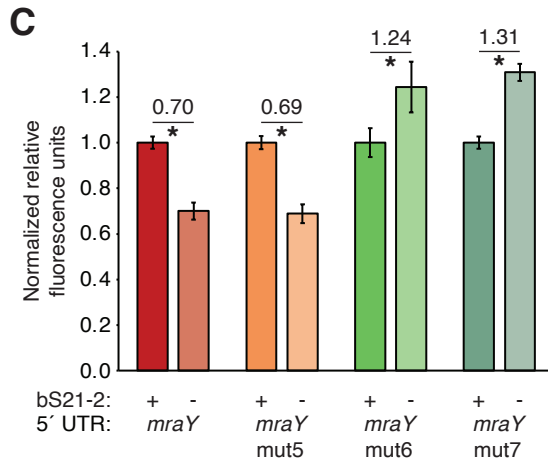
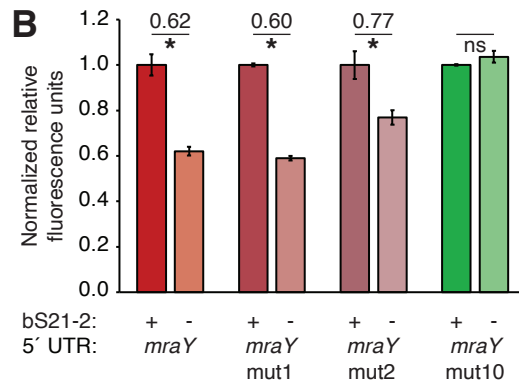
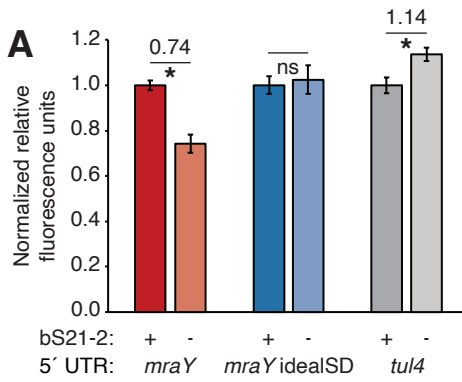


Figure 2. Genes with ideal Shine-Dalgarno (SD) sequences are not responsive to bS21-2. (A) bS21 interacts with the anti-Shine Dalgarno (ASD) sequence. In *E. coli*, amino acid R17 of the sole bS21 protein (green) directly interacts with C1539 of 16S, which is part of the ASD (blue). Measured distance is 2.7Å (PDB 6o7k; Kaledhonkar et al. 2019). **(B)** The absence of strong SD-ASD interactions is correlated with bS21-2 influencing translation. Fraction of genes that are positively-impacted (n=74), negatively-impacted (n=84), or unaffected (n=82) by bS21-2, categorized by strength of SD. “Strong” SD: 4 or more nts complementary to ASD; “weak” SD: 3 or fewer complementary nts. **(C)** Introduction of an ideal SD in the *pdpA* leader leads to loss of bS21-2 responsiveness. Top: Relative β-galactosidase activity for indicated *lacZ* translational fusions in cells with (+; WT) or without (-; Δ*rpsU2*) bS21-2, in biological triplicate. Error bars represent 1 SD. **p*<0.05 by t-test. ns=not significant. Experiments were repeated at least twice and data from a representative experiment are shown. Bottom: Alignment of modifications to *pdpA* 5' UTR. Capital letters: altered from WT; bold and underlined: predicted SD sequences; unnormalized β-galactosidase activity can be found in Fig S3.



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Sequences of 5' UTRs in Panel

	-100	-90	-80	-70	-60	-50	-40	-30	-20	-10	-1	+1	+9	+18	
<i>mraY</i>	auaaaaaaaaauuuu	gacccaauuuuuu	agacgcuaauuuu	gacucuaauuuuuu	aaaaaaaaaaaa	aacauauuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	AUG	cugauuuuuuuuuu
<i>idealSD</i>	auaaaaaaaaauuuu	gacccaauuuuuu	agacgcuaauuuu	gacucuaauuuuuu	aaaaaaaaaaaa	aacauauuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	AUG	cugauuuuuuuuuu
<i>mraY</i>	auaaaaaaaaauuuu	gacccaauuuuuu	agacgcuaauuuu	gacucuaauuuuuu	aaaaaaaaaaaa	aaaauaacauuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	AUG	cugauuuuuuuuuu
<i>mut1</i>	auaaaaaaaaauuuu	gacccaauuuuuu	agacgcuaauuuu	gacucuaauuuuuu	aaaaaaaaaaaa	CCCCGCCG	aaauaacauuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	AUG	cugauuuuuuuuuu
<i>mut2</i>	auaaaaaaaaauuuu	gacccaauuuuuu	agacgcuaauuuu	gacucuaauuuuuu	aaaaaaaaaaaa	UAU	aaauaacauuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	AUG	cugauuuuuuuuuu
<i>mut10</i>								auuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	AUG	cugauuuuuuuuuu
<i>mraY</i>	auaaaaaaaaauuuu	gacccaauuuuuu	agacgcuaauuuu	gacucuaauuuuuu	aaaaaaaaaaaa	aacauauuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	AUG	cugauuuuuuuuuu
<i>mut5</i>								gacgcuaauuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	AUG	cugauuuuuuuuuu
<i>mut8</i>								GCCGGGG	gacucuaauuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	AUG	cugauuuuuuuuuu
<i>mut9</i>								AGUAGA	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	AUG	cugauuuuuuuuuu
<i>mut7</i>								AGA	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	AUG	cugauuuuuuuuuu
<i>mut6</i>									uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	AUG	cugauuuuuuuuuu
<i>mraY</i>	auaaaaaaaaauuuu	gacccaauuuuuu	agacgcuaauuuu	gacucuaauuuuuu	aaaaaaaaaaaa	aacauauuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	AUG	cugauuuuuuuuuu
<i>mut3</i>	auaaaaaaaaauuuu	gacccaauuuuuu	agacgcuaauuuu	gacucuaauuuuuu	aaaaaaaaaaaa	aacauauuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	AUG	cugauuuuuuuuuu
<i>mut4</i>	auaaaaaaaaauuuu	gacccaauuuuuu	agacgcuaauuuu	gacucuaauuuuuu	aaaaaaaaaaaa	AGUGAG	aaauaacauuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	uuuuuuuuuuuu	AUG	cugauuuuuuuuuu

