

Figure 1. Hfq and bS21-2 influence production of T6SS proteins via different pathways. **(A)** Cells lacking bS21-2 have more Hfq. Bottom: Immunoblots probed with anti-VSV-G antibody. Whole cell lysates from bacteria containing Hfq-VSV-G and either with (WT) or without ($\Delta rpsU2$) bS21-2, in biological triplicate. Top: Quantification of immunoblots. Band intensities for each protein were normalized to total protein per lane on the membrane. **(B)** Loss of bS21-2 leads to more *hfq* translation. Relative fluorescence is reported for translational fusion reporters containing the 5' UTR of either *hfq* or *tul4* fused to *gfp* in cells with (WT) or without ($\Delta rpsU2$) bS21-2, in biological triplicate. Values relative to WT for each 5' UTR are shown. **(C)** Only some of the T6SS proteins are influenced by loss of Hfq. Bottom: Immunoblots probed with antibodies to indicated T6SS proteins in lysates of WT cells, cells lacking bS21-2 ($\Delta rpsU2$), or cells lacking Hfq (Δhfq). Top: Quantification of immunoblots. Band intensities for each protein were normalized to total protein per lane on the membrane. **(D)** Hfq does not influence translation of the T6SS protein PdpA. Relative fluorescence for translational fusion reporters containing the 5' UTR of either *pdpA* or *tul4* fused to *gfp* in WT cells, cells lacking bS21-2 ($\Delta rpsU2$), or cells lacking Hfq (Δhfq), in biological triplicate. **(E)** Hfq is a negative regulator of T6SS gene transcript abundance. Quantitative real-time PCR was used to determine the relative transcript abundance for indicated FPI-encoded genes in WT cells, cells lacking bS21-2 ($\Delta rpsU2$), or cells lacking Hfq (Δhfq), normalized to the *tul4* gene. The *rpoA1* and *bfr* genes are included as additional negative controls, as their expression is not meaningfully influenced by bS21-2. (A-E) Error bars represent 1 SD. Experiments were repeated at least twice and data from a representative experiment are shown. (A-D) * $p < 0.05$ after Bonferroni correction. (E) * $p < 0.005$ after Bonferroni correction.

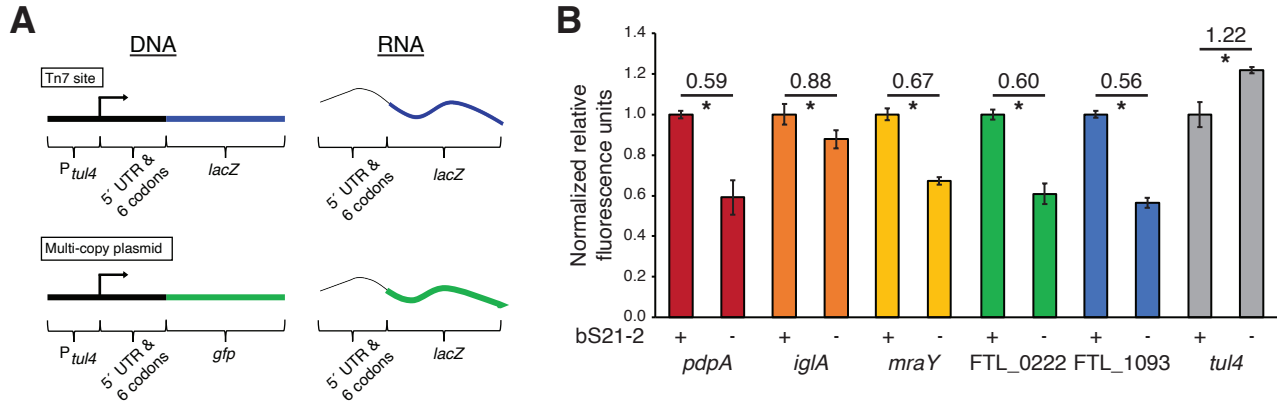


Figure 2. 5' UTRs are sufficient to lead to bS21-2-mediated changes in translation. (A) Diagrams of the translational reporter fusions used. Reporters used the *tul4* promoter to drive expression of the tested 5' UTR, including the first 6 codons of the gene, and are in frame with either *lacZ* at the Tn7 site of the genome or *gfp* on a multi-copy plasmid. **(B)** Relative fluorescence is reported 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 Table 1. 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.

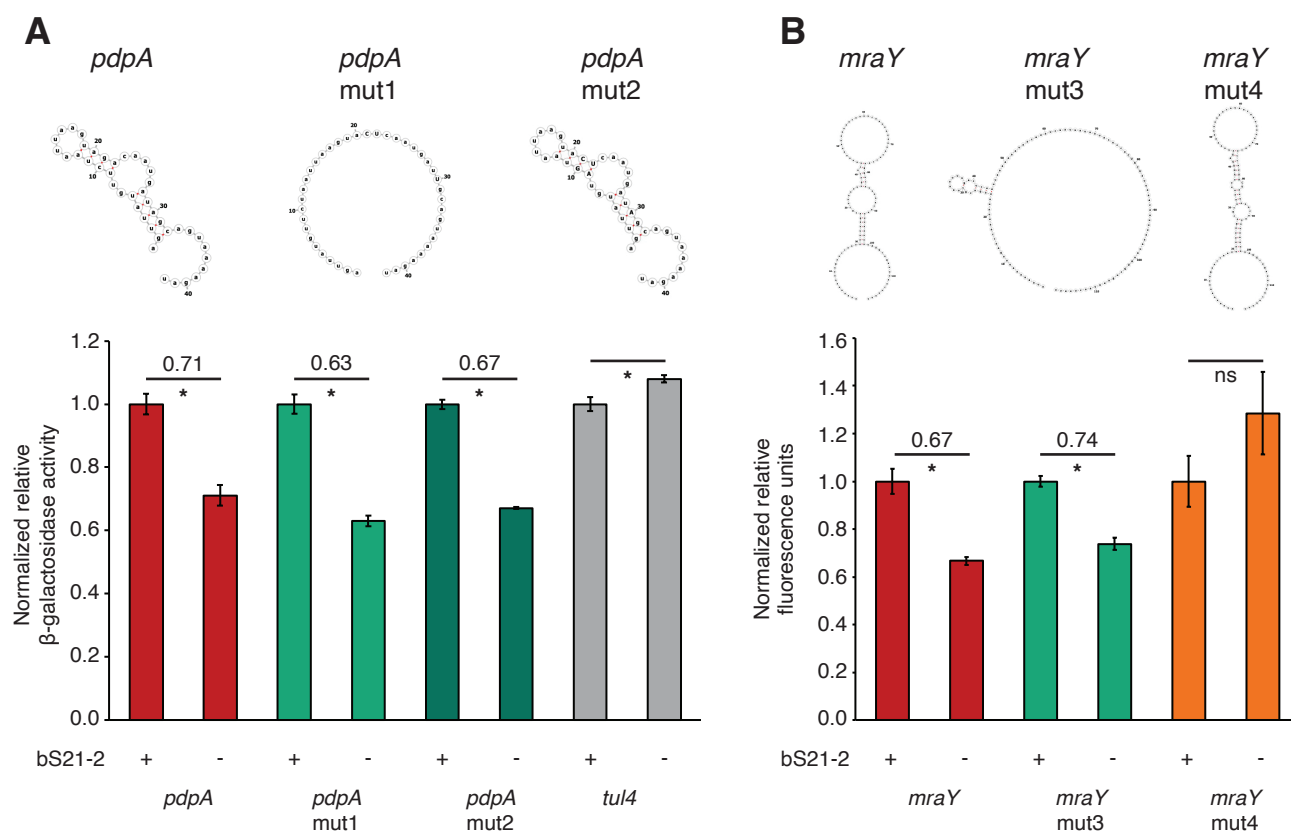


Figure 5. Predicted secondary structure plays no clear role in bS21-2- responsive translation.

(A) Changing the *pdpA* secondary structure does not impact responsiveness to bS21-2. Top: Predicted secondary structures for wild-type and modified *pdpA* 5' UTRs, from MXFold2. Sequence modifications in Table 1. Bottom: Relative β -galactosidase activity from indicated 5' UTR fused to *lacZ*, in cells with (+; WT) or without (-; $\Delta rpsU2$) bS21-2, in biological triplicate. *tul4* is included as a control. **(B)** Changing the *mraY* secondary structure does not impact responsiveness to bS21-2. Top: Secondary structure predictions of wild-type and modified *mraY* 5' UTRs, from MXFold2. Sequence modifications in Table 1. Bottom: Relative fluorescence is reported for indicated translational fusion reporters in cells with (+; WT) or without (-; $\Delta rpsU2$) bS21-2, in biological triplicate. (A-B) Error bars represent 1 SD. * $p < 0.05$ by t-test. Experiments were repeated twice and data from a representative experiment are shown.

