



**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  $\beta$ -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.