



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 (-; $\Delta rpsU2$) 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.