Investigating the Regulation of bS21 Homologs in *Francisella tularensis*

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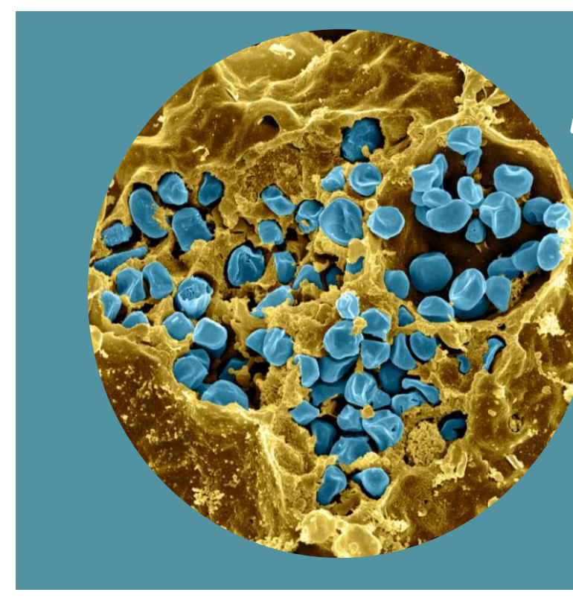
Introduction



Francisella tularensis

- Gram-negative
- Intracellular pathogen
- Causes tularemia
- Potential bioweapon

bS21: small subunit ribosomal protein involved in translation initiation

Three homologs in *F. tularensis*

- Leads to ribosome heterogeneity

Loss of bS21-2

- Influences virulence factors and intramacrophage growth

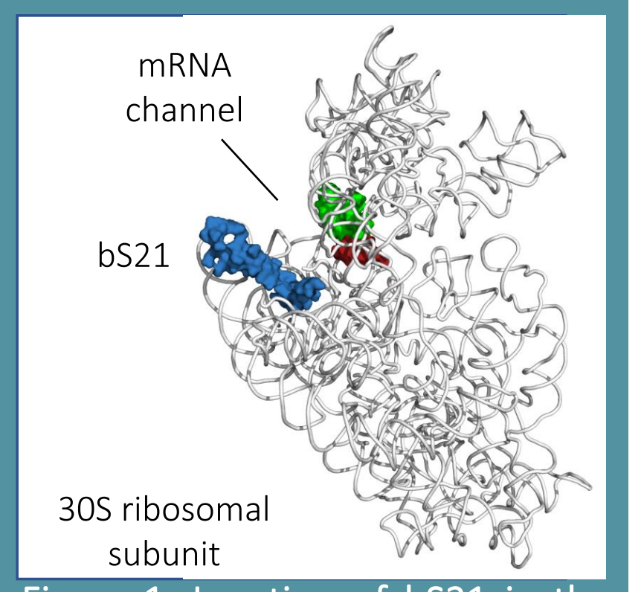
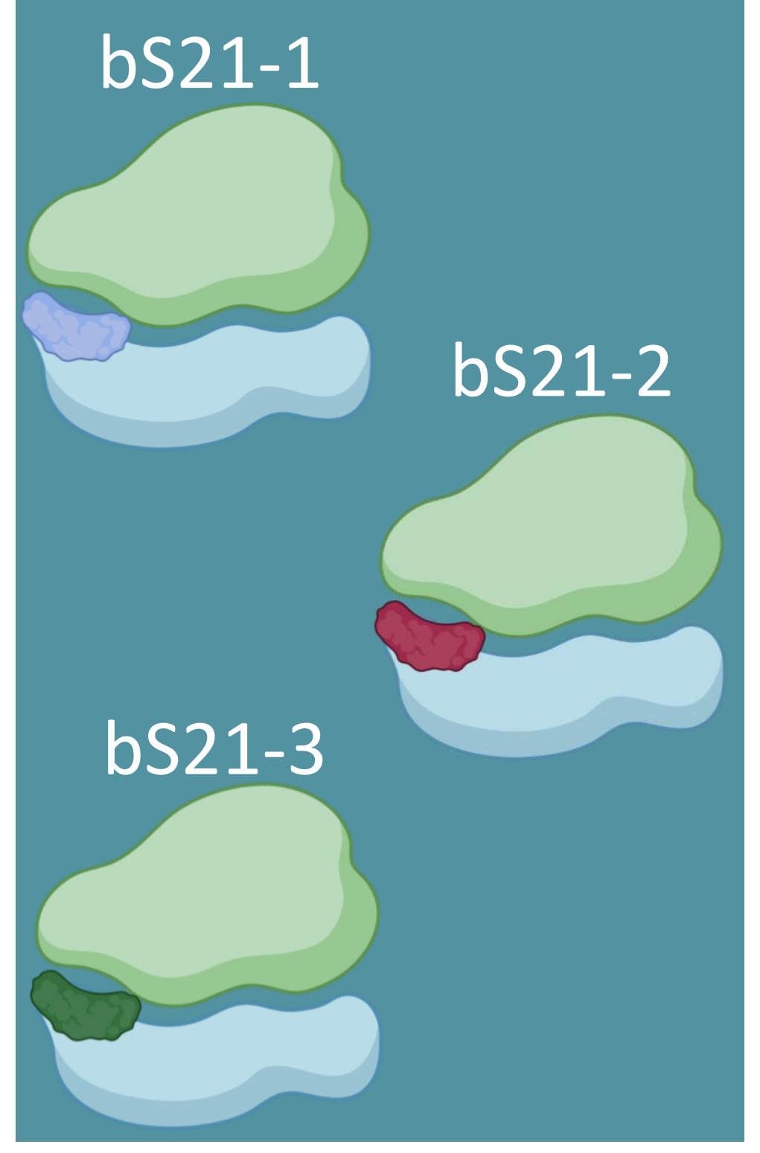


Figure 1. Location of bS21 in the small subunit of the *E. coli* ribosome

Ribosomal Heterogeneity in *F. tularensis*



bS21-2 controls gene expression at the level of translation and influences abundance of its own transcript

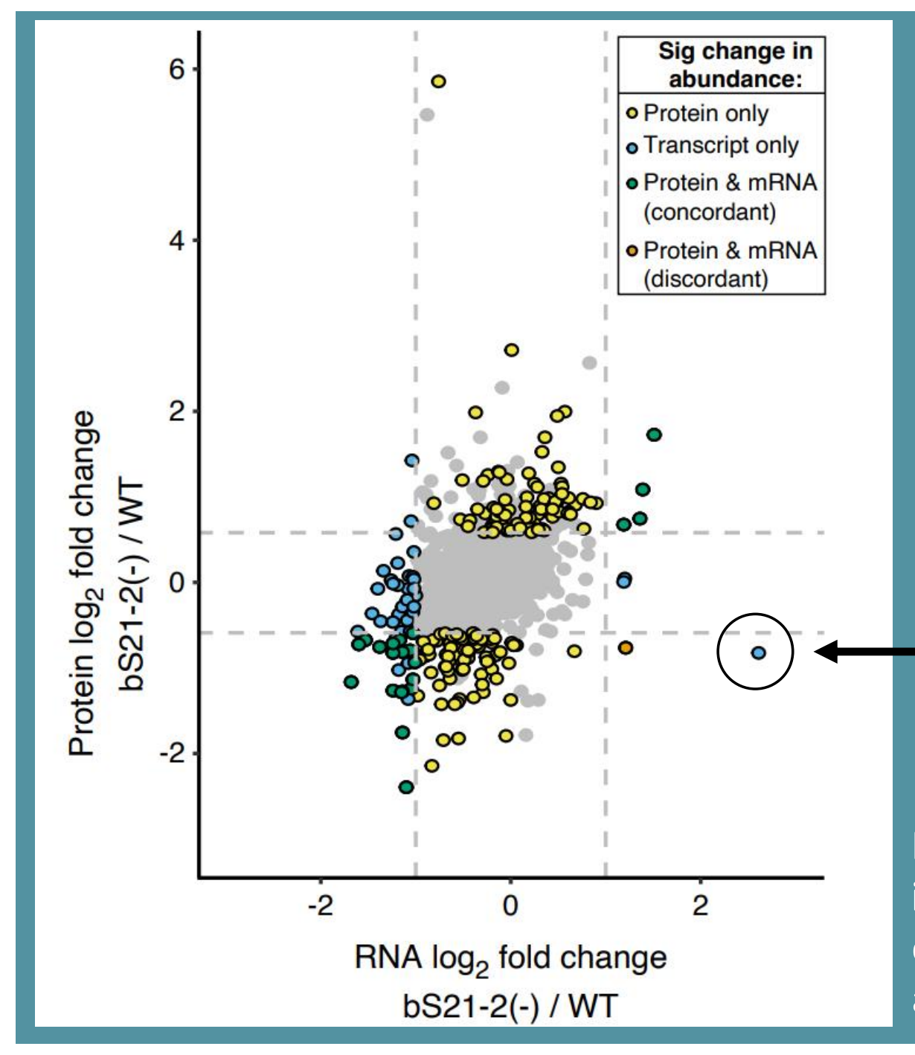
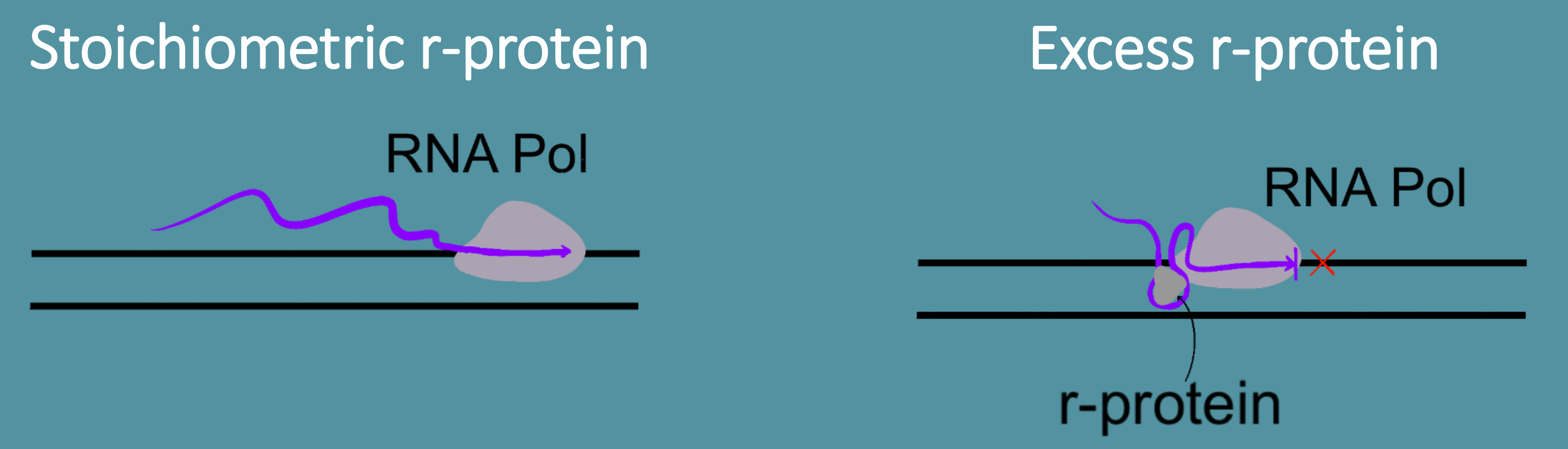


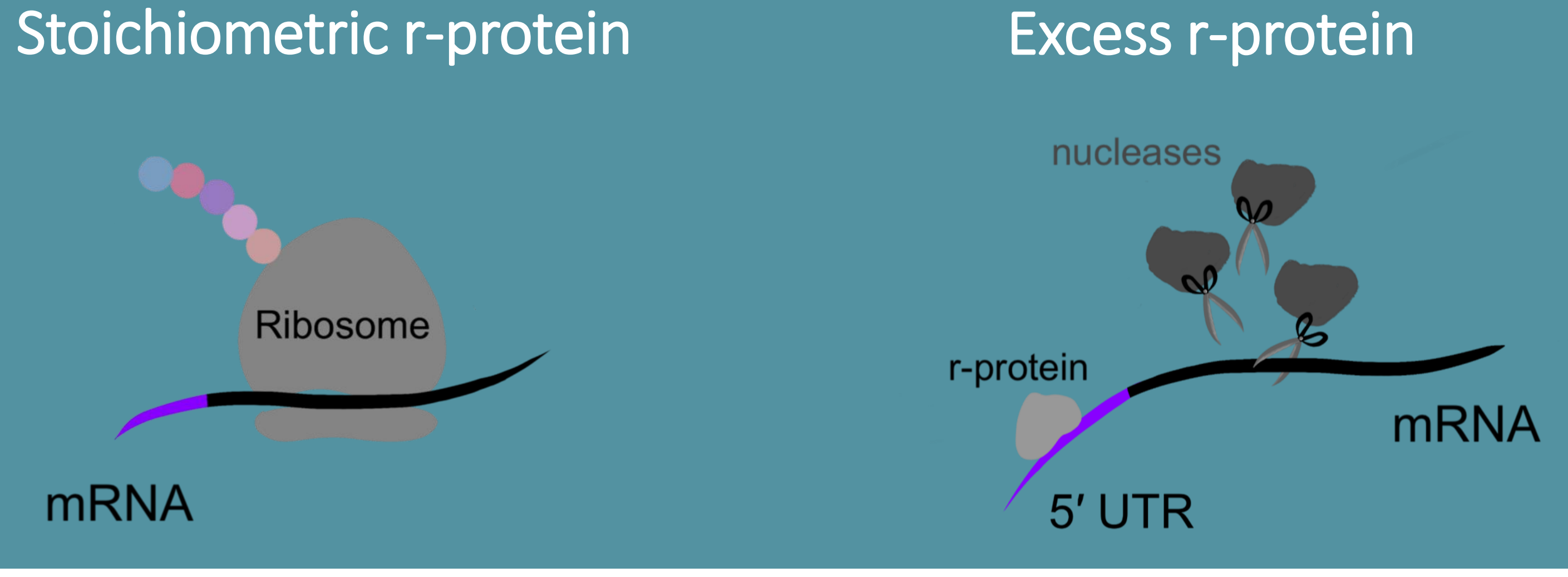
Figure 2. Cells lacking bS21-2 have changes in protein abundance that are not due to changes in transcript abundance. Each data point represents a gene and the axes represent log2 fold-change in either transcript (x-axis) or protein (y-axis) abundance, comparing wild-type cells and cells lacking bS21-2 (Trautmann & Ramsey, 2022).

Models for R-Protein Regulation

MODEL 1: Attenuation



MODEL 2: Post-Transcriptional Control



Study Goals

Understand the regulation of the bS21 homologs

I. Transcript Abundance
   How do bS21-1, bS21-2, and bS21-3 affect their own production?
II. Protein Abundance
   What affects the translation of the bS21 coding transcript?

bS21 homologs repress bS21-2 transcript, *rpsU2*

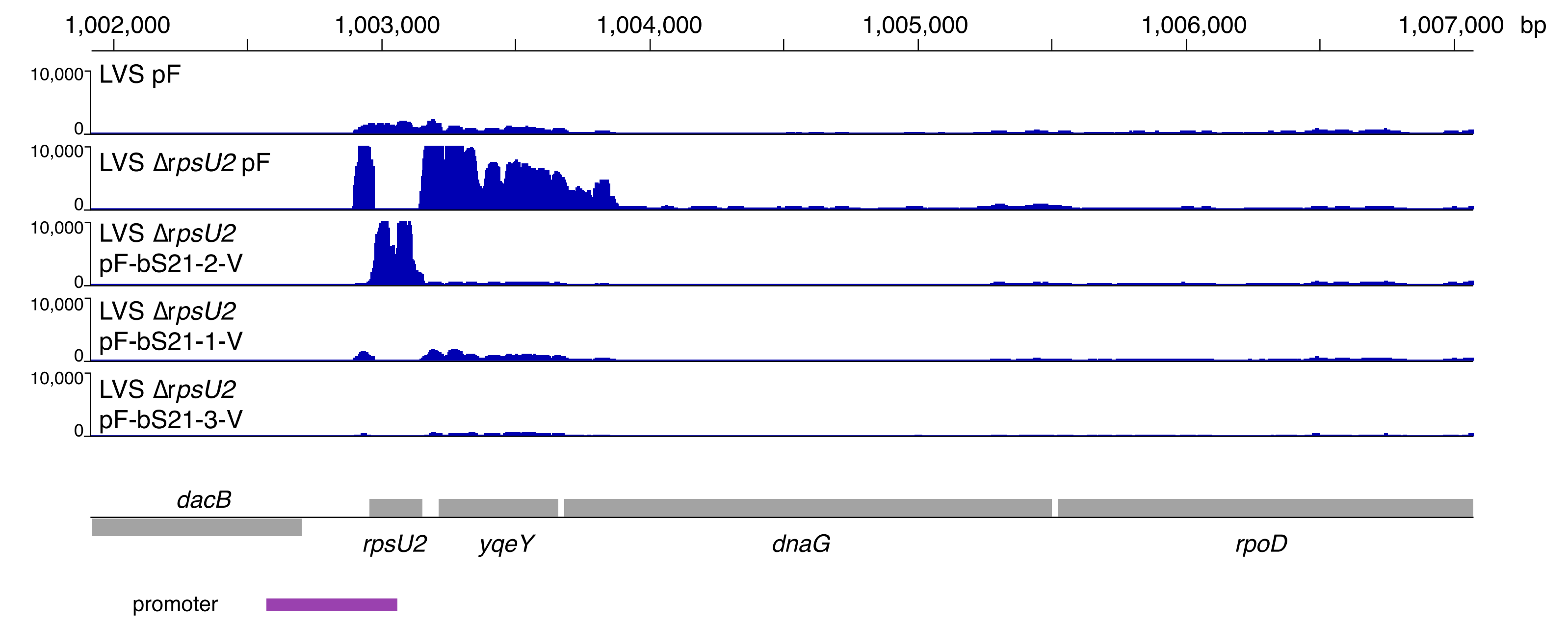


Figure 3. bS21-2 transcript abundance is controlled by all three bS21 homologs. RNA-Seq of wild-type (LVS) cells or cells lacking bS21-2 (Δ*rpsU2*) and either empty vector (pF) or vector producing indicated bS21 homolog. Y-axis is truncated at 10,000 for clarity. Note that reads corresponding to plasmid-encoded *rpsU2* map to the native operon. A subset of data are published (Trautmann & Ramsey, 2022).

Translational Fusion Constructs and Predictions

			Transcript Abundance		Protein Abundance	
			Cells **with** bS21-2 (WT)	Cells **without** bS21-2 (Δ*rpsU2*)	Cells **with** bS21-2 (WT)	Cells **without** bS21-2 (Δ*rpsU2*)
tul4 promoter	*tul4* 5′ UTR	*lacZ*	⊕⊕⊕	⊕⊕⊕	⊕⊕⊕	⊕⊕⊕
rpsU2 promoter	*rpsU2* 5′ UTR	*lacZ*	⊕	⊕⊕⊕⊕⊕	⊕	⊕
rpsU2 promoter	*tul4* 5′ UTR	*lacZ*	?	?	?	?
tul4 promoter	*rpsU2* 5′ UTR	*lacZ*	?	?	?	?

bS21-2 Transcriptional Control

rpsU2 5′UTR is sufficient for regulation by bS21

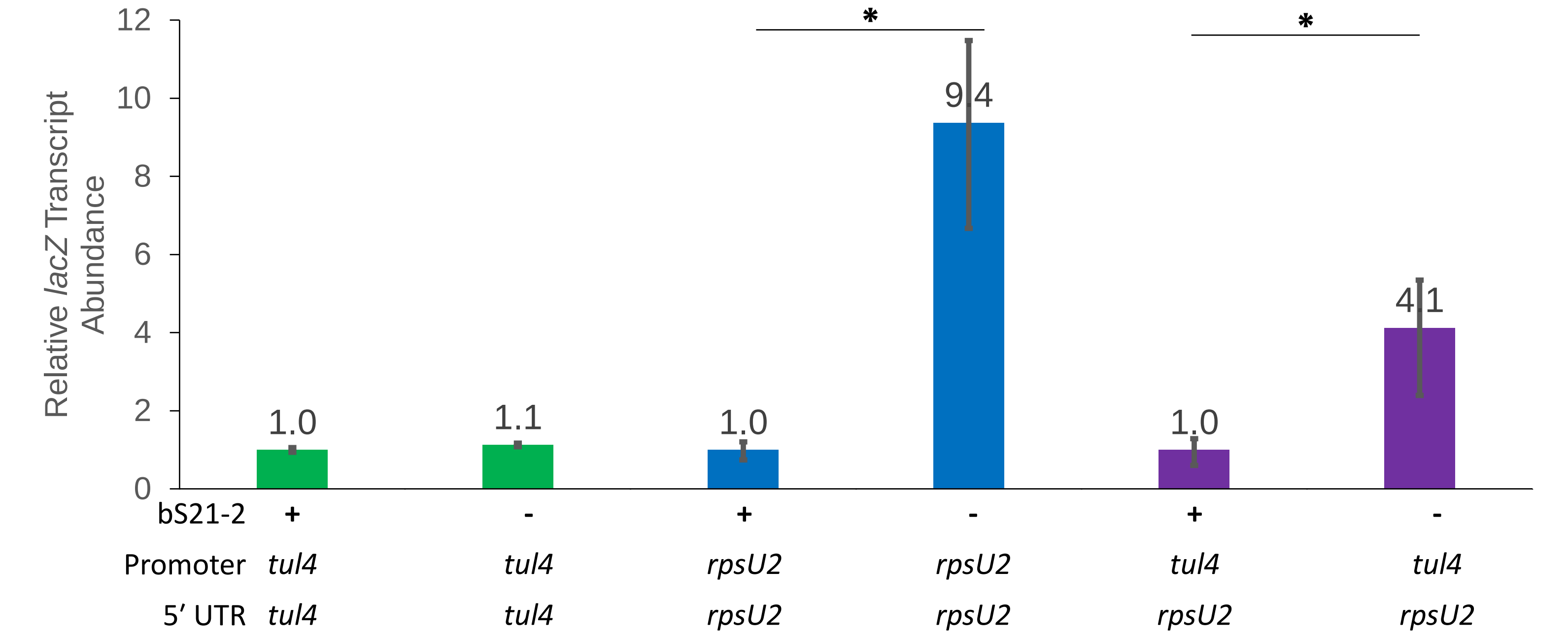


Figure 4. The leader sequence of rpsU2 is sufficient for autoregulation by bS21-2. The indicated translational fusions, in cells with or without bS21-2, were assessed by qPCR for relative abundance of *lacZ*. Results from reporter fusions containing the promoter and 5′ UTR of either *tul4* only or *rpsU2* only reveal that the fusions recapitulate regulation of the native genes. Error bars are 1 SD, * p<0.05 by t-test.

bS21-2 Translational Control

Evidence of regulated bS21-2 translation

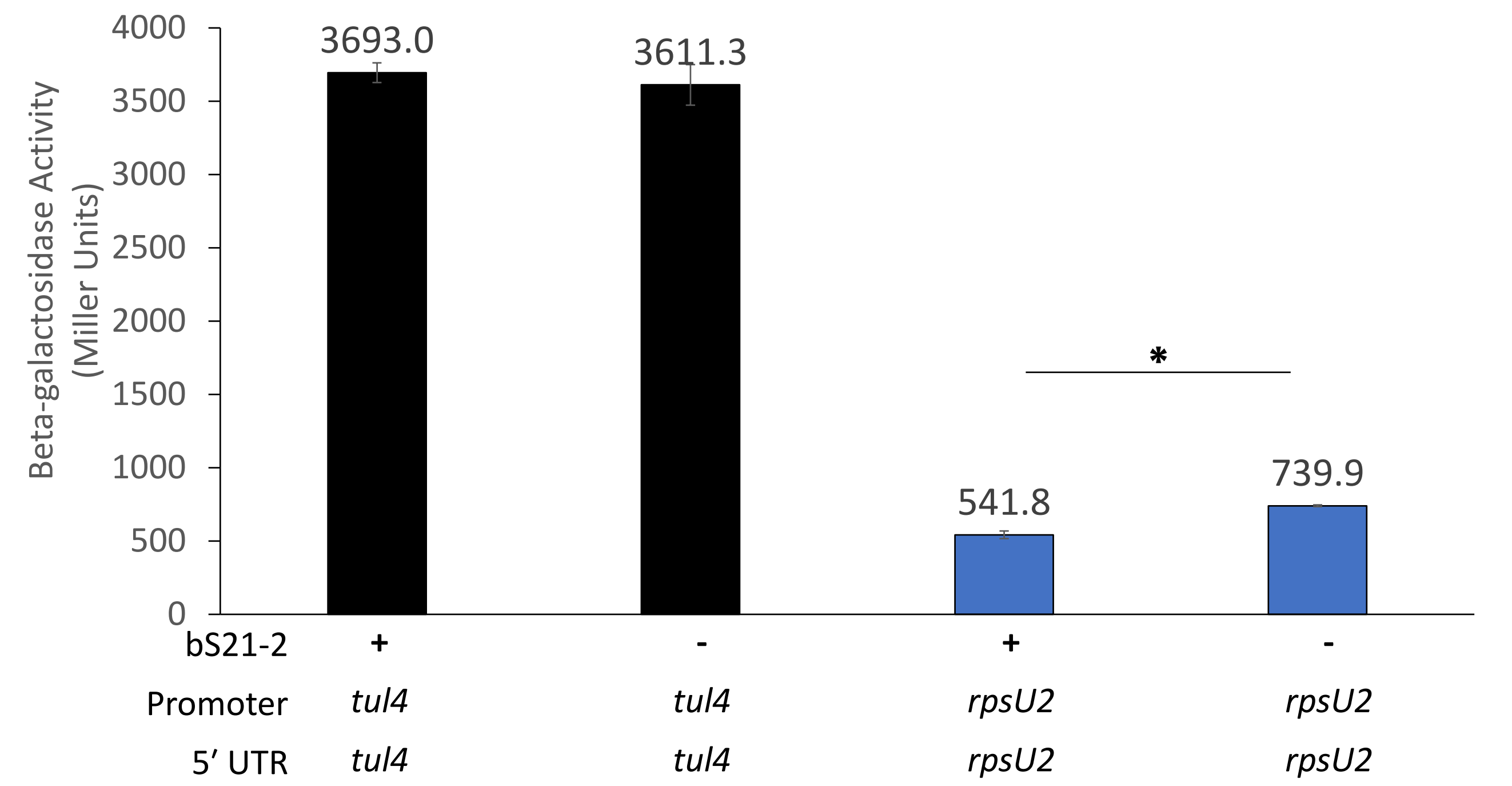


Figure 5. The protein abundance of bS21-2 is not correlated with changes in its transcript abundance. β-galactosaidase activity, in Miller Units (y-axis), of indicated translational fusions in cells with or without bS21-2. Error bars are 1 SD, * p<0.05 by t-test.

rpsU2 5' UTR is sufficient for regulation

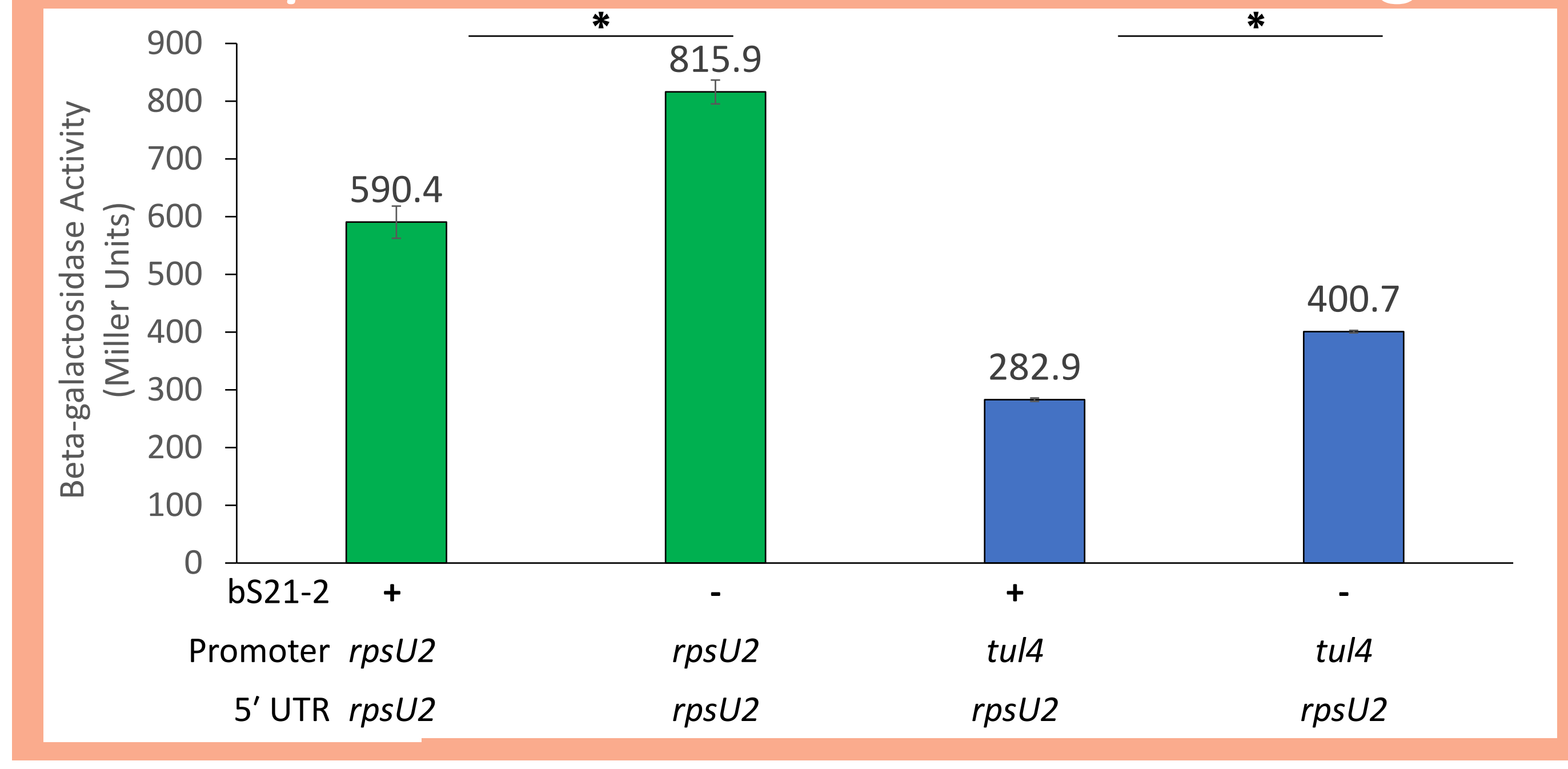


Figure 6. The 5′ UTR is sufficient to prevent increased translation of the *rpsU2* transcript. β-galactosidase activity of indicated translational fusions in cells with or without bS21-2. Error bars are 1 SD, * p<0.05 by t-test.

bS21-1 and bS21-3

bS21-1 and bS21-3 do not autoregulate

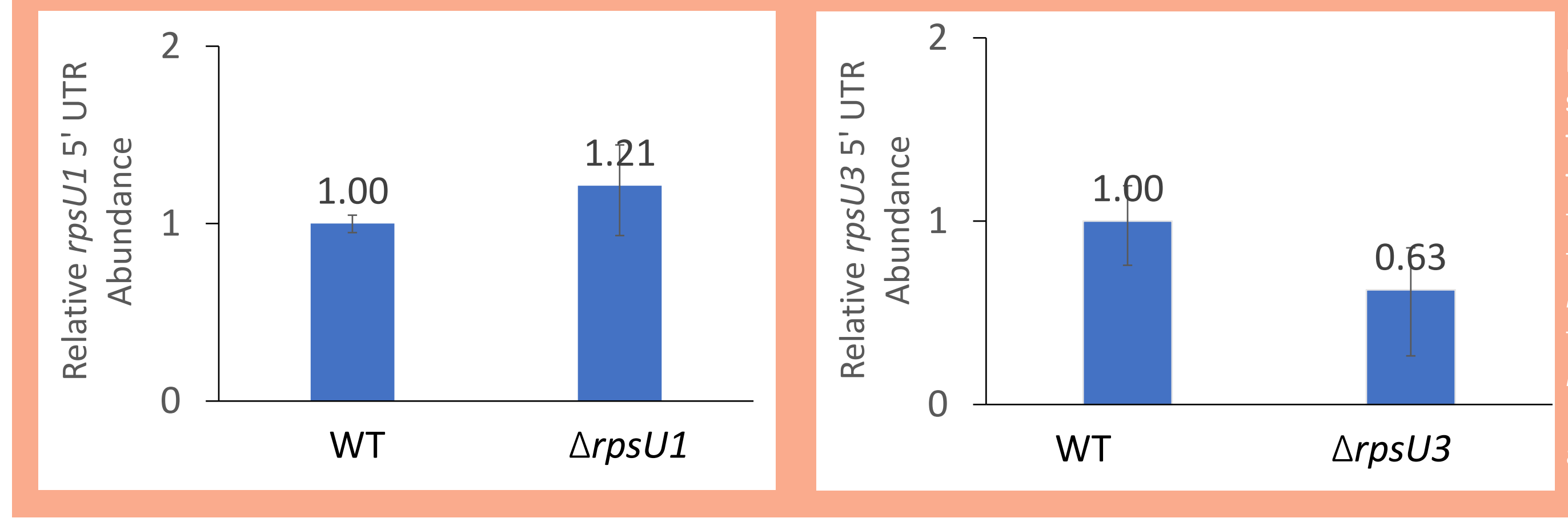


Figure 7. bS21-1 and bS21-3 do not significantly influence the abundance of their transcripts. The relative transcript abundance of the 5′ UTR of either *rpsU1* or *rpsU3* in wild-type cells or cells lacking *rpsU1* or *rpsU3*, respectively. Error bars are 1 SD.

Conclusions

- bS21-2 transcript abundance is repressed by all three bS21 homologs
- The 5' UTR is sufficient for control of *rpsU2* transcript abundance and translation
- Transcript regulation is not equivalent to protein regulation

Future Directions

- Determine if transcript abundance is due to changes in stability or termination during transcription
- Assess how the 5′ UTR structure of the *rpsU2* transcript impacts translation

Reference
Trautmann HS, Ramsey KM. A Ribosomal Protein Homolog Governs Gene Expression and Virulence in a Bacterial Pathogen. *J Bacteriol*. 2022 Oct 18;204(10):e0026822.

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