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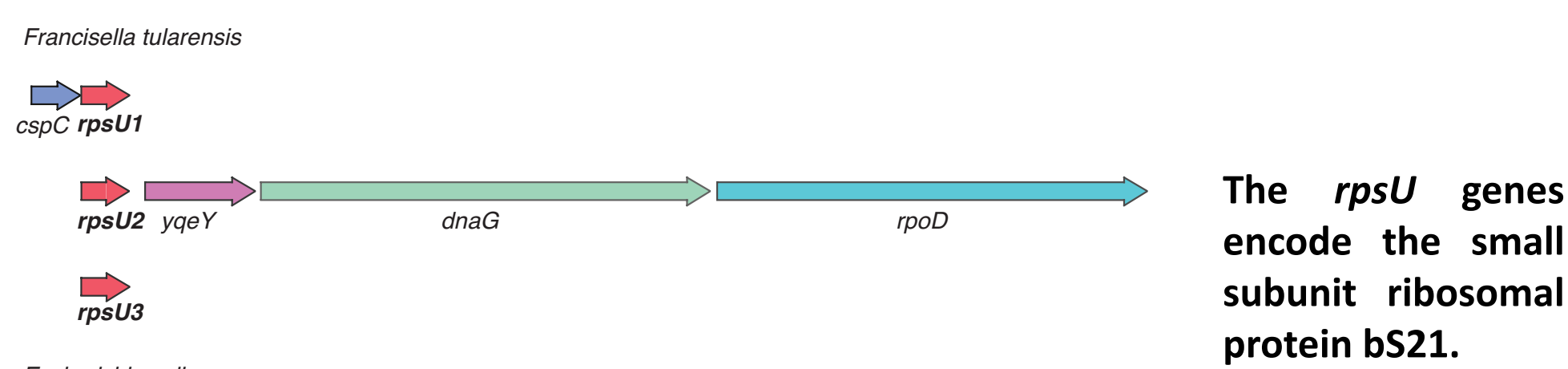
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*Contributed equally to this work

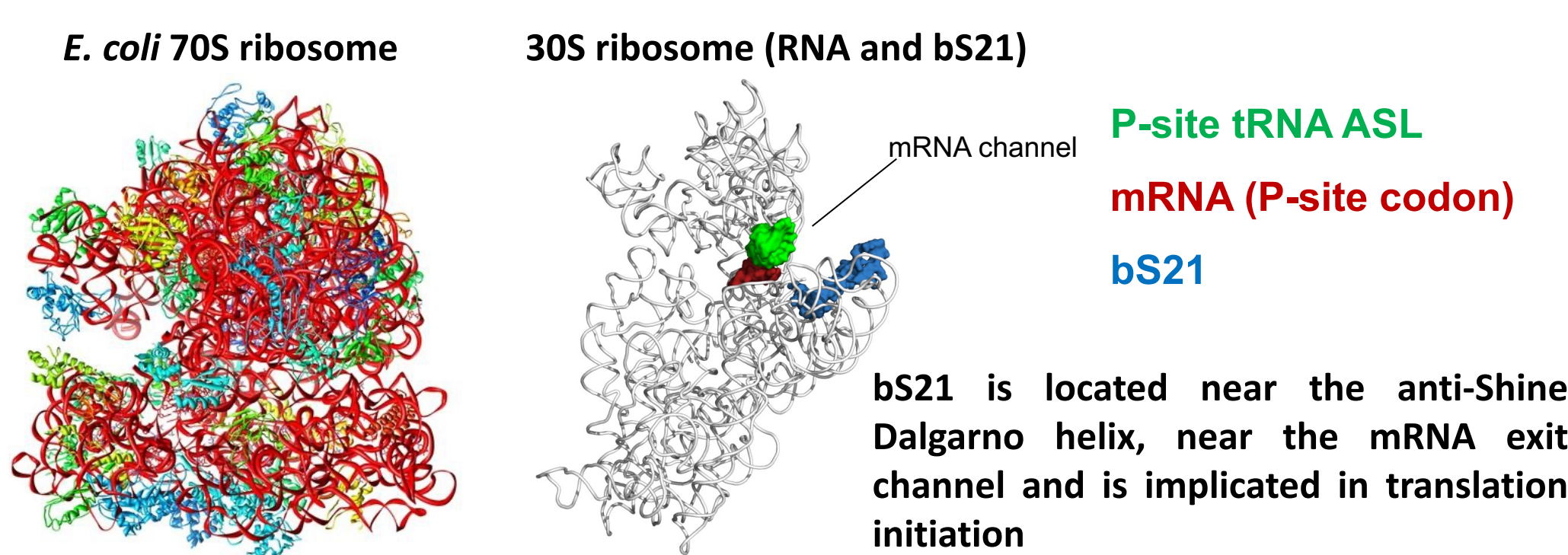
Introduction

Francisella sp. encode three bS21 homologs



The *rpsU* genes encode the small subunit ribosomal protein bS21.

bS21 is involved in translation initiation



bS21 is located near the anti-Shine Dalgarno helix, near the mRNA exit channel and is implicated in translation initiation

F. tularensis ribosomes are heterogeneous

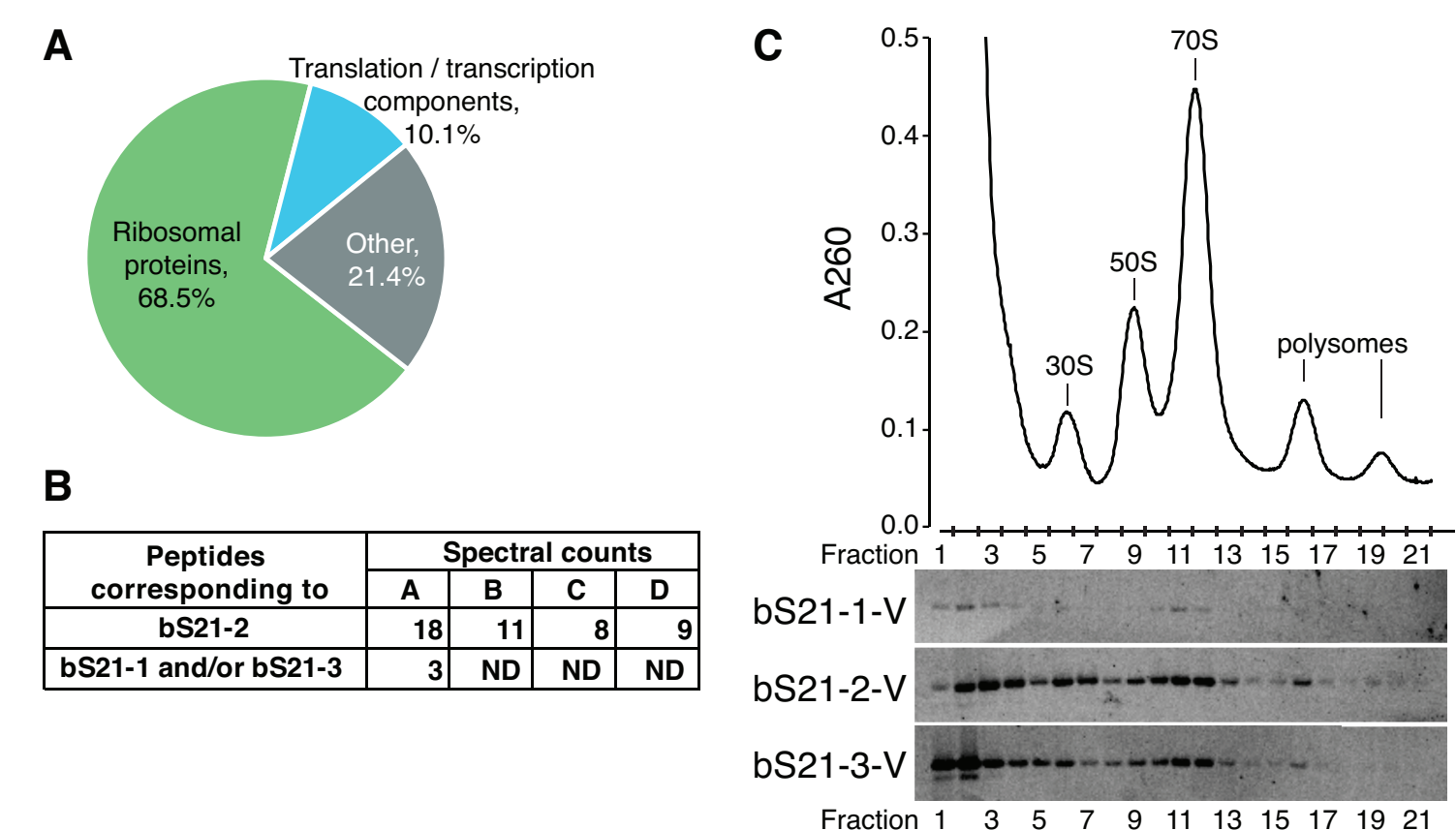


Figure 1. *F. tularensis* ribosomes are heterogeneous with respect to bS21. (A) Chart indicating purity of wild-type ribosomes. (B) Table with spectral counts corresponding to bS21 homologs, identified from individual ribosome purifications from wild-type cells. ND: not detected. (C) Each bS21 homolog can be incorporated into ribosomes. Top: Sucrose gradient sedimentation profile from actively-translating wild-type cells containing an empty vector. Bottom: Immunoblot analysis of fractions from sucrose gradient sedimentation performed on actively-translating cells ectopically expressing VSV-G-tagged bS21 homolog.

Model

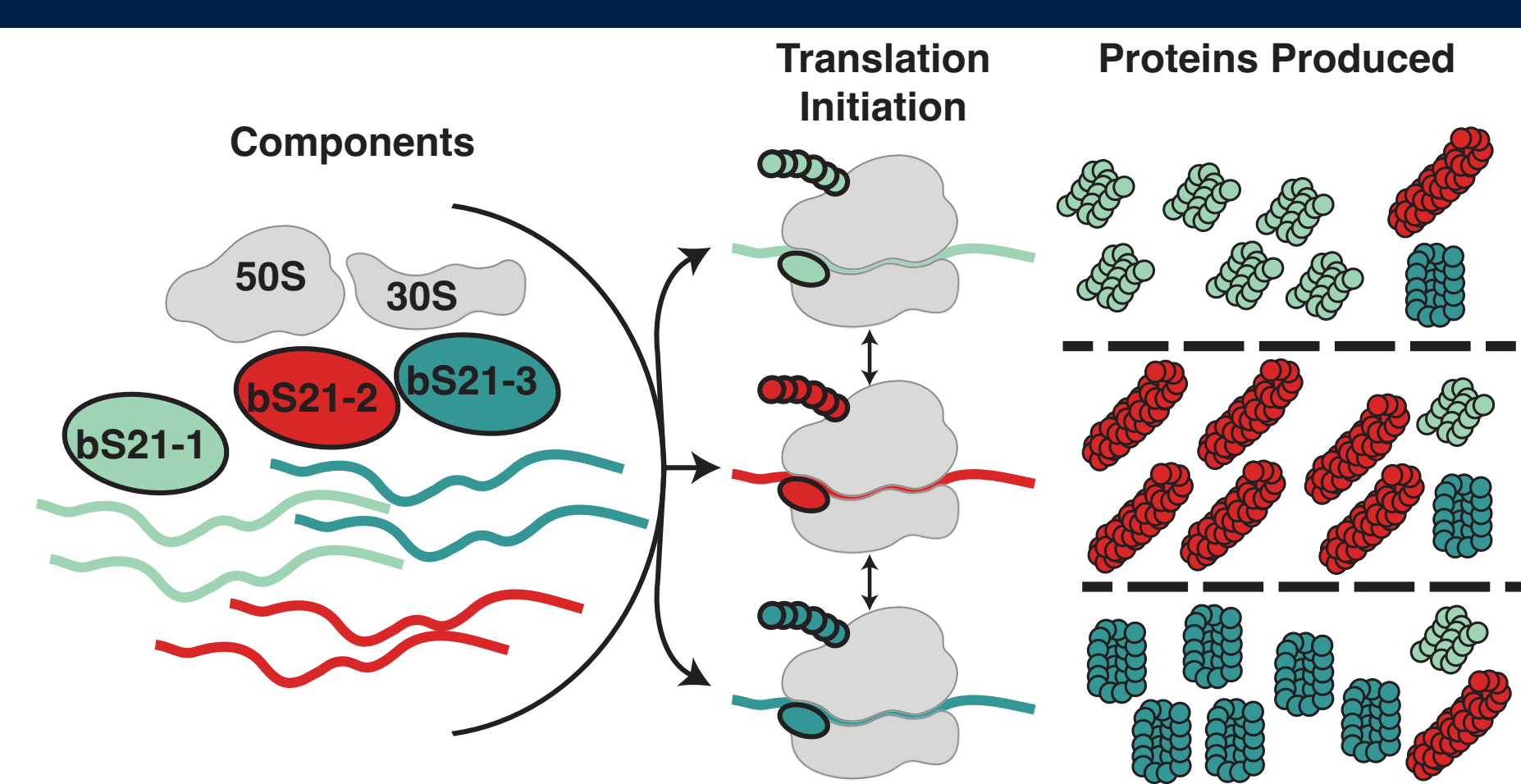


Figure 2. Incorporation of distinct bS21 homologs into ribosomes leads to preferential translation initiation to modulate protein abundance. One of three bS21 homologs may be incorporated into ribosomes and preferentially translate specific mRNAs in a sequence-dependent manner. bS21 homologs may be easily exchanged among assembled 70S ribosomes.

Role of bS21-2 in vivo

bS21-2 is important for intramacrophage growth

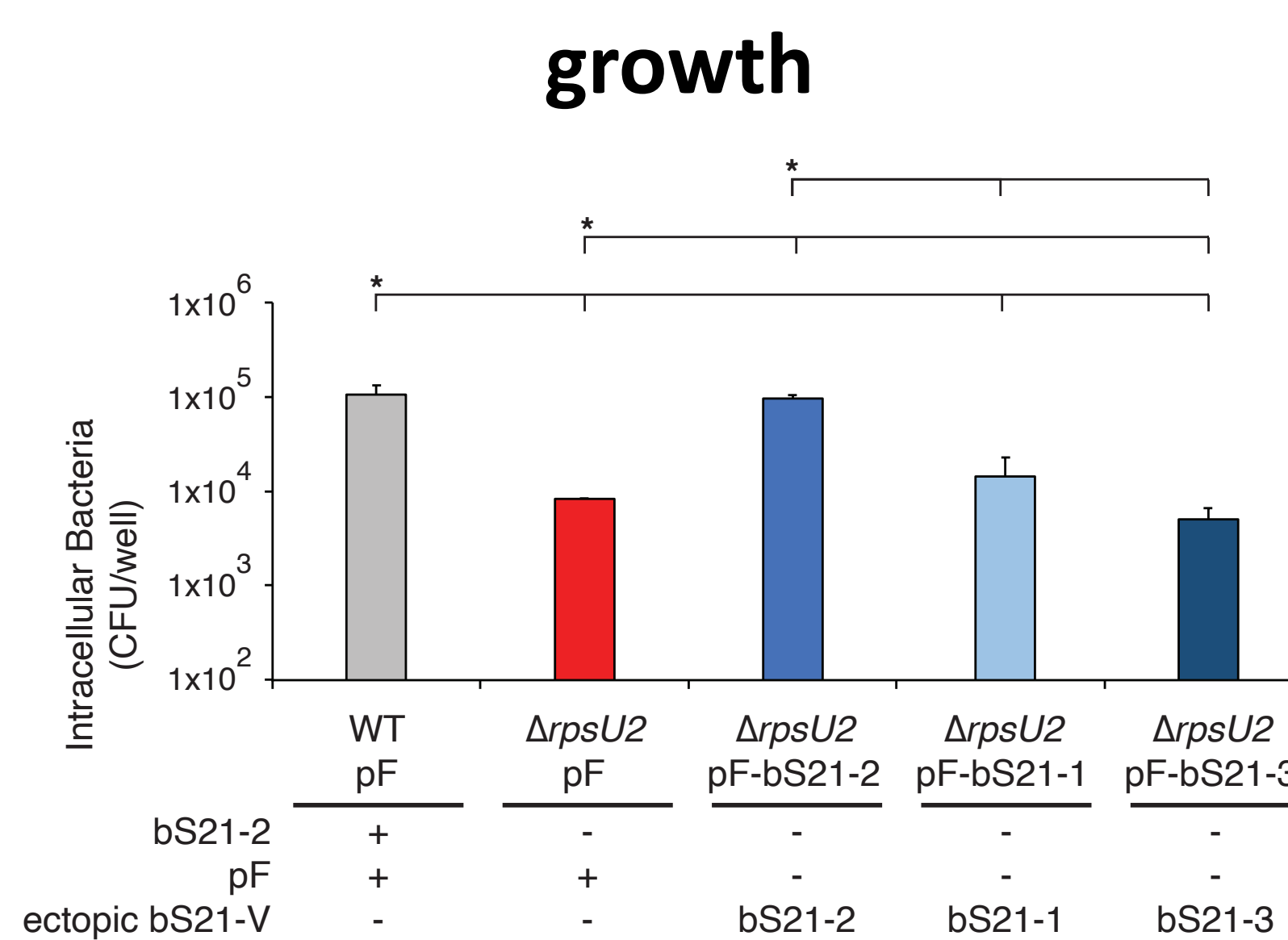


Figure 3. Cells without bS21-2 have an intramacrophage growth defect, which can be complemented by ectopic expression of bS21-2. Growth of *F. tularensis* LVS cells within J774A.1 cells, infected at MOI of 5 – 10. J774A.1 cells were lysed and bacteria were plated for enumeration (CFU) 24 hours post-infection. *p < 0.05 using Benjamini-Hochberg correction.

bS21-2 controls key virulence factors, type VI secretion system proteins

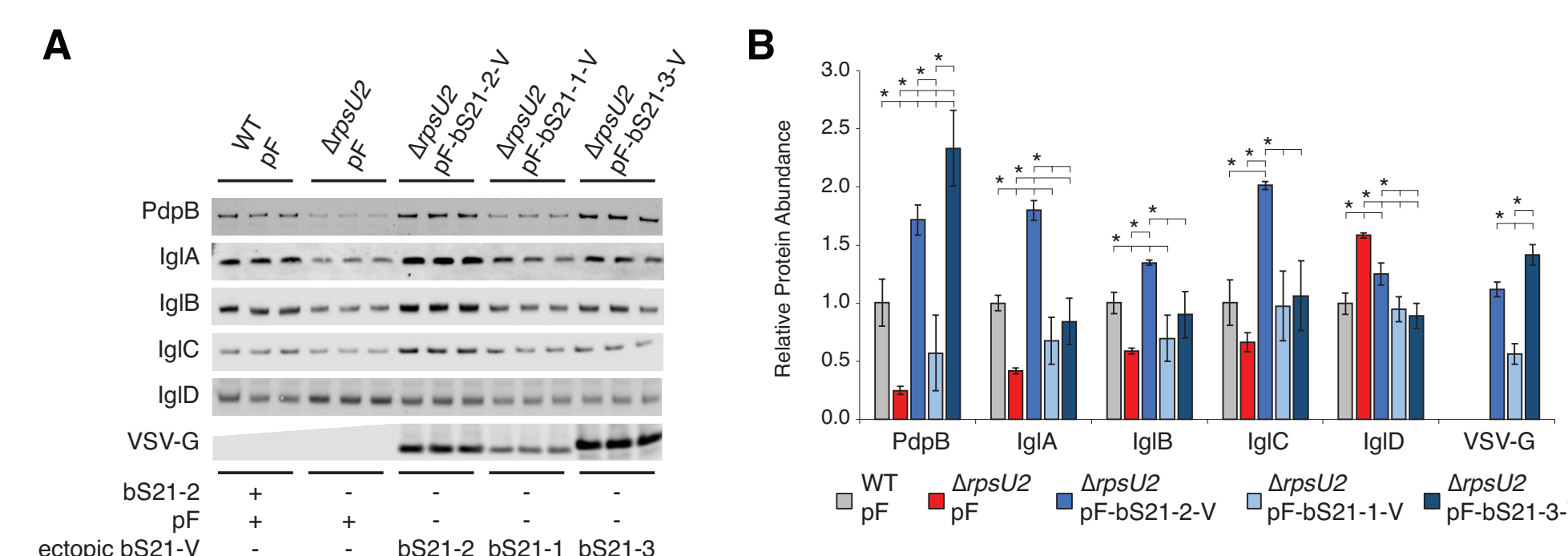


Figure 4. bS21-2 is a regulator of T6SS component protein abundance. (A) Immunoblot analysis of indicated T6SS protein abundance. Immunoblot against VSV-G was included to demonstrate production of bS21-2-V. (B) Quantification of immunoblots from A. Band intensities for each protein was normalized to total protein on the membrane. *p < 0.05 using Benjamini-Hochberg correction.

Control by bS21-2 is leader sequence-dependent

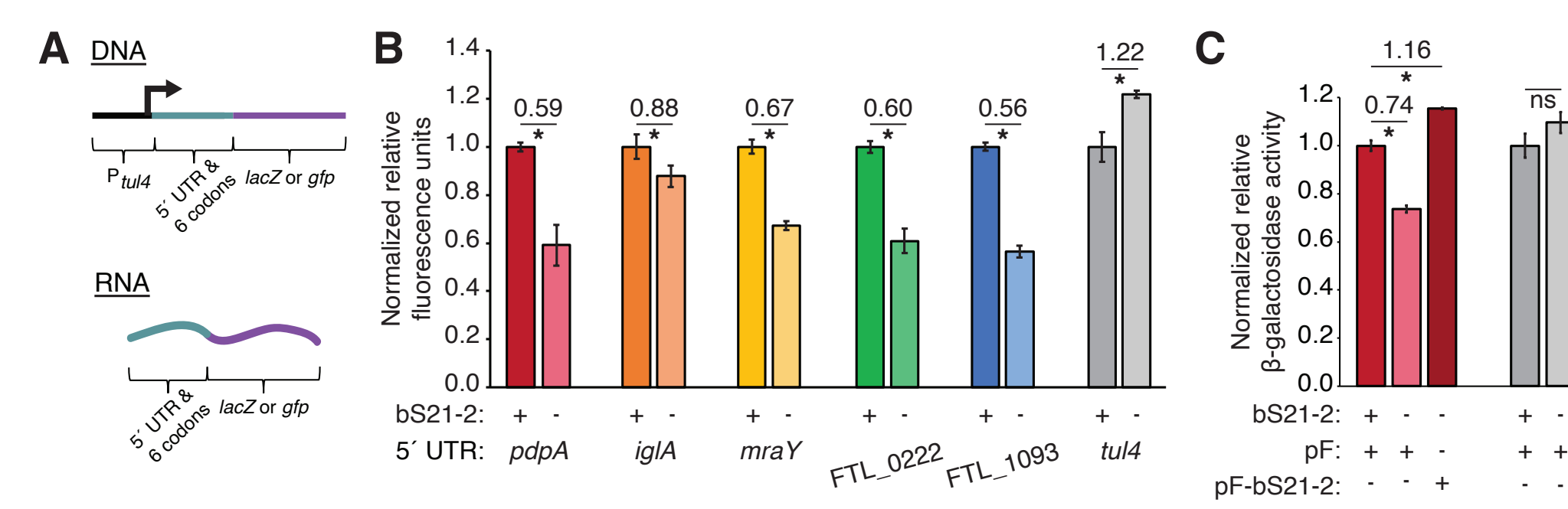
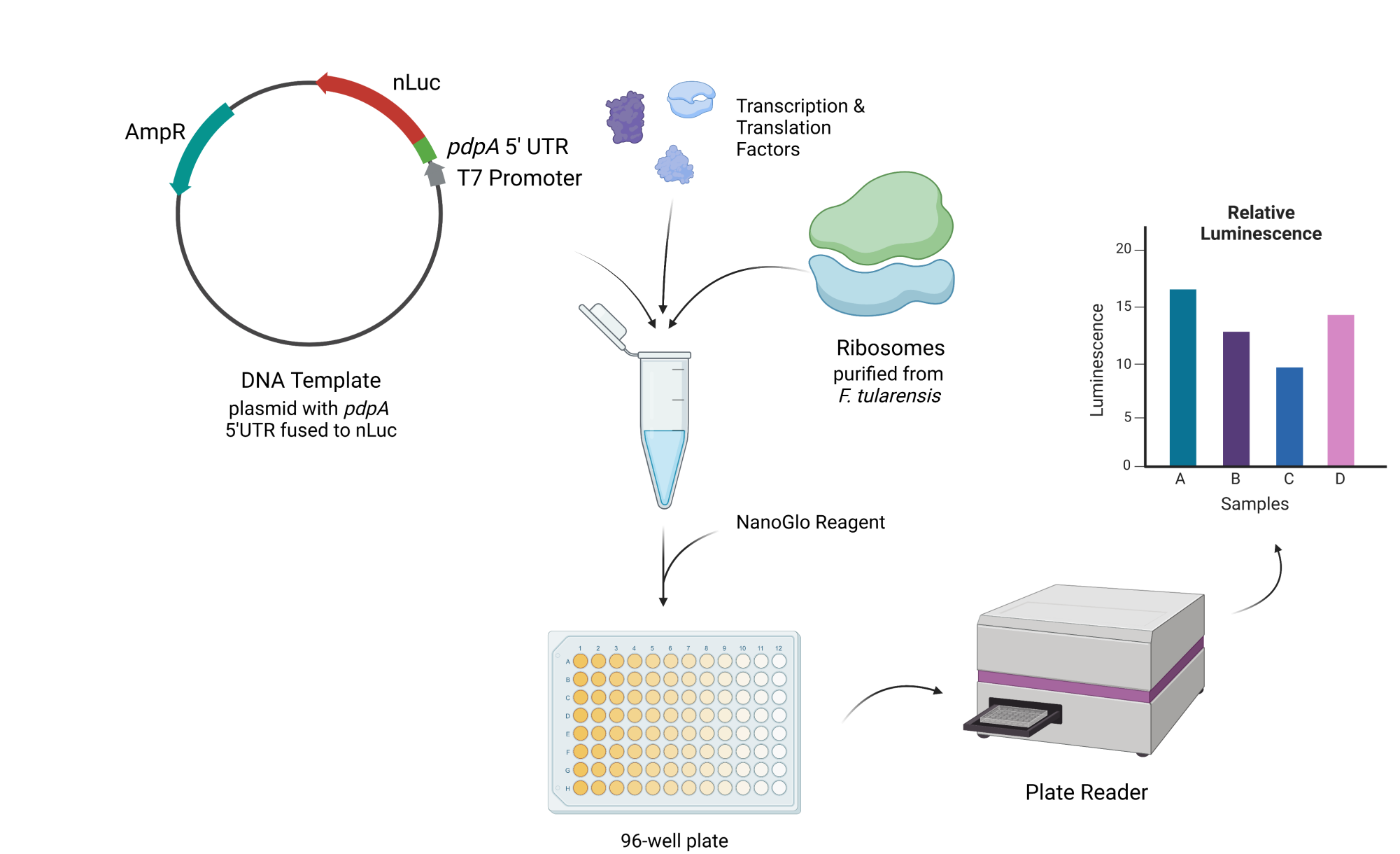


Figure 5. 5' UTRs are sufficient to lead to bS21-2-mediated changes in translation. (A) Diagram of the translational reporter fusions used. (B) Relative fluorescence for indicated *gfp* translational fusion reporters in cells with (+; WT) or without (-; ΔrpsU2) bS21-2. (C) Relative β-galactosidase activity for indicated *lacZ* translational fusions in cells with (+; WT) or without (-; ΔrpsU2) native bS21-2, or with ectopically expressed bS21-2 from a plasmid (pF-bS21-2). Strains without ectopically expressed bS21-2 contained an empty vector (pF). *p < 0.05. ns = not significant.

in vitro translation

Overview of in vitro translation assay



Creation of a sensitive, easily-modified reporter

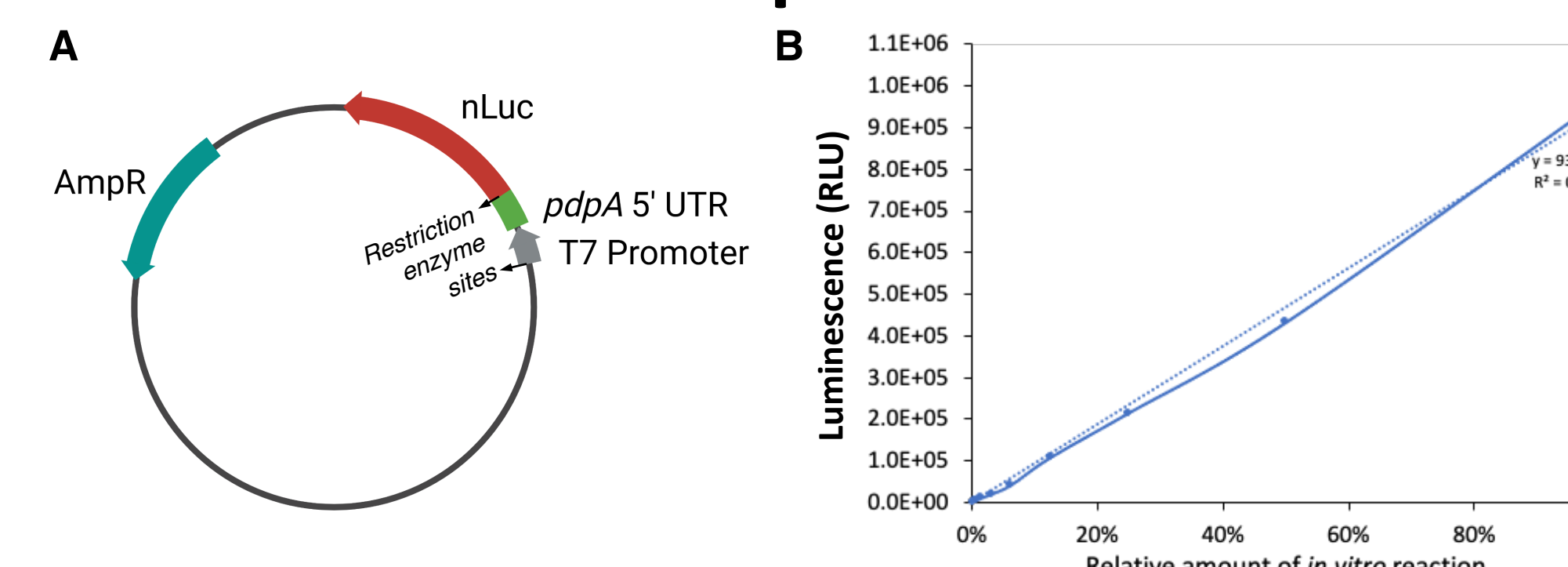


Figure 6. Generating and validating NanoLuc reporters for in vitro translation assays. A. Schematic of pKR212, a plasmid containing DNA specifying an mRNA with the *pdpA* 5' UTR fused to the gene for the NanoLuciferase (nLuc). Inclusion of DNA specifying restriction enzyme sites flanking the 5' UTR allows for easy exchange of leader sequences using standard cloning techniques. B. Standard curve demonstrating sensitivity and linear range of in vitro translation assays using NEB-provided ribosomes, the pKR208 reporter template, which specifies a *tul4* 5' UTR – NanoLuc reporter fusion, and the Promega Nano-Glo™ Luciferase Assay System.

Purified *F. tularensis* ribosomes have more variability in activity and density

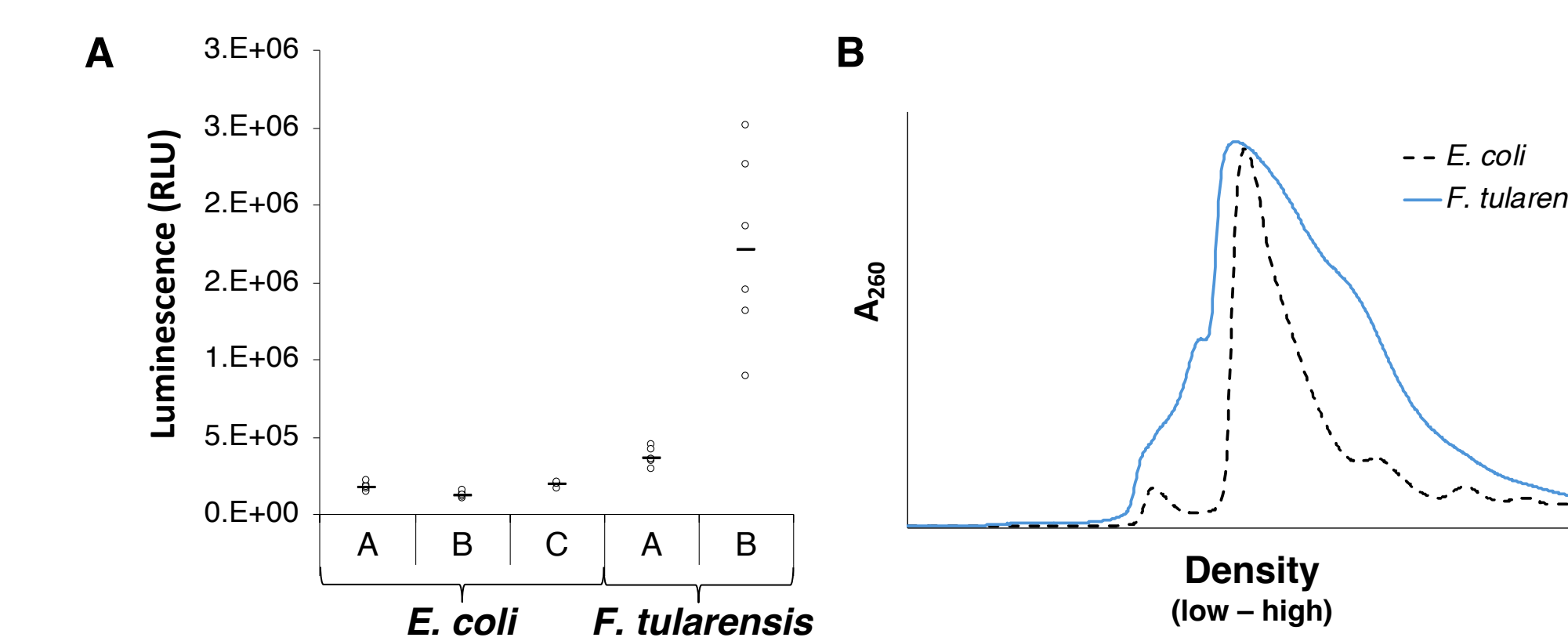
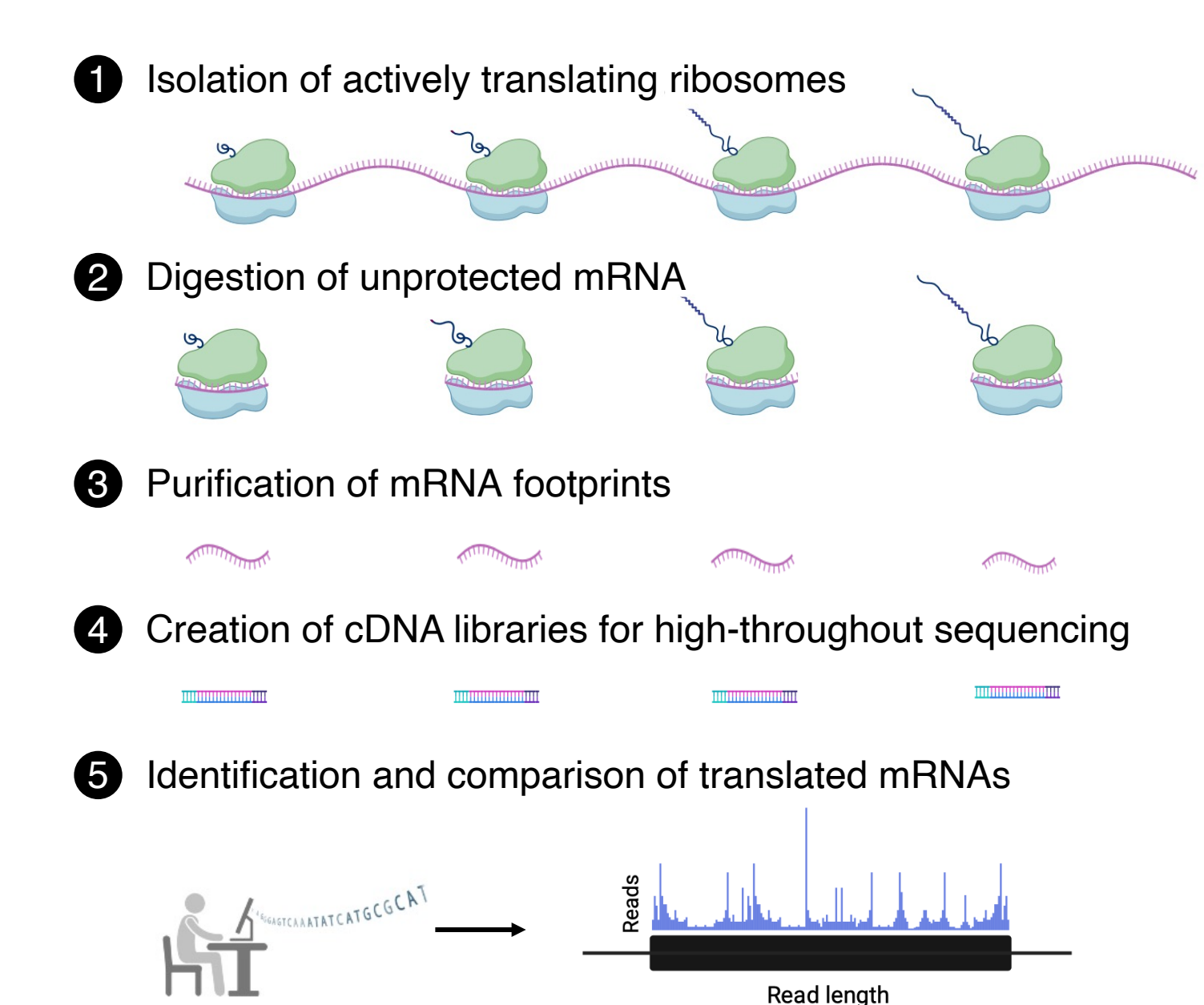


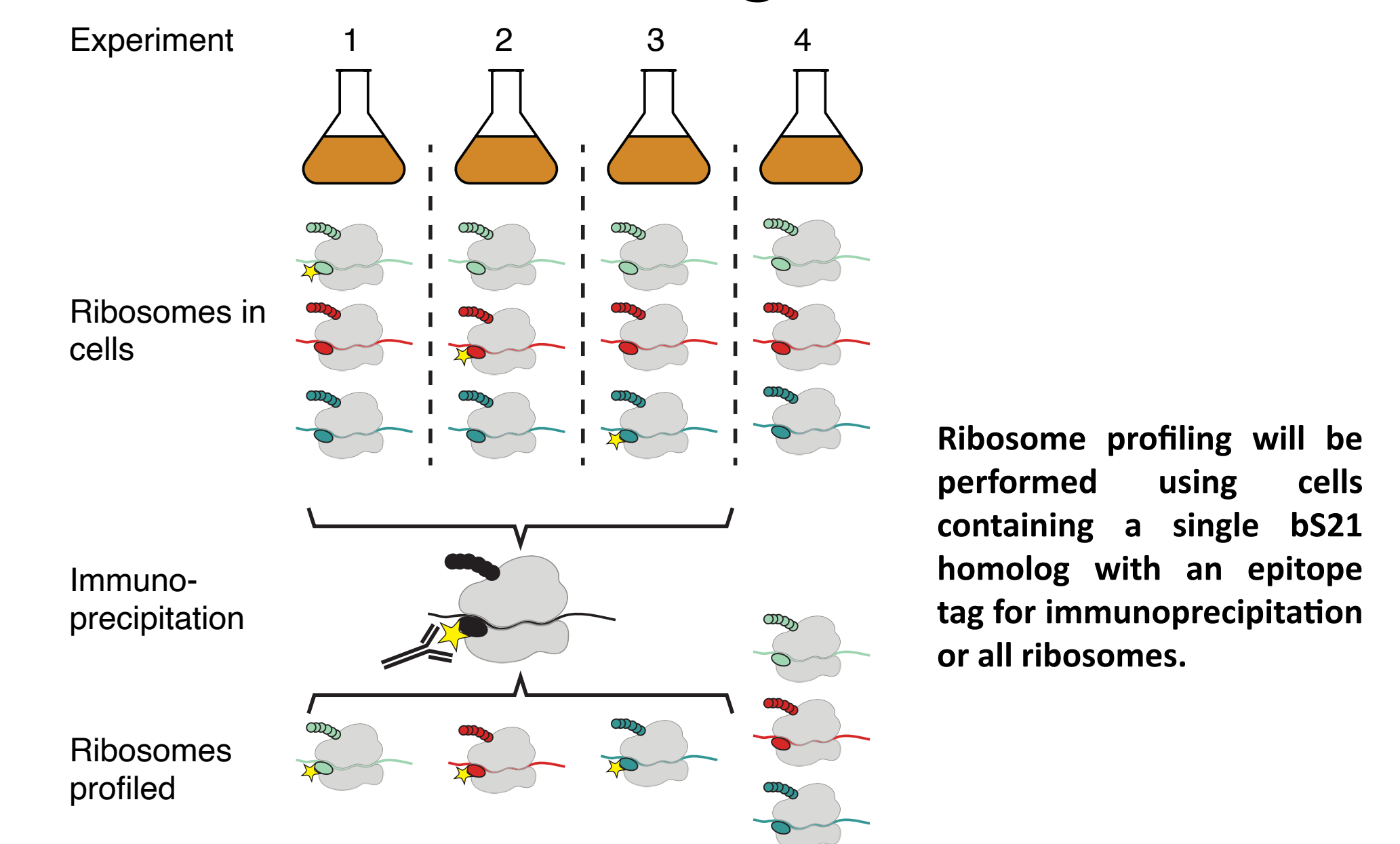
Figure 7. Comparison of ribosomes purified from *E. coli* and *F. tularensis* LVS. All ribosomes were purified using a sucrose cushion. A. Chart comparing activity of *E. coli* and *F. tularensis* ribosomes in in vitro translation assay. Letters on x-axis represent independent ribosome purifications, circles represent technical replicates, horizontal lines represent averages of technical replicates. Assays used the pKR214 reporter template, which specifies a *tul4* 5' UTR – NanoLuc reporter fusion. B. Sucrose gradient sedimentation profiles of purified ribosomes from indicated species.

Ribosome Profiling

Overview of ribosome profiling



Profiling ribosomes with specific bS21 homologs



Ribosome profiling will be performed using cells containing a single bS21 homolog with an epitope tag for immunoprecipitation or all ribosomes.

Purification of 70S ribosomes by immunoprecipitating a bS21 homolog

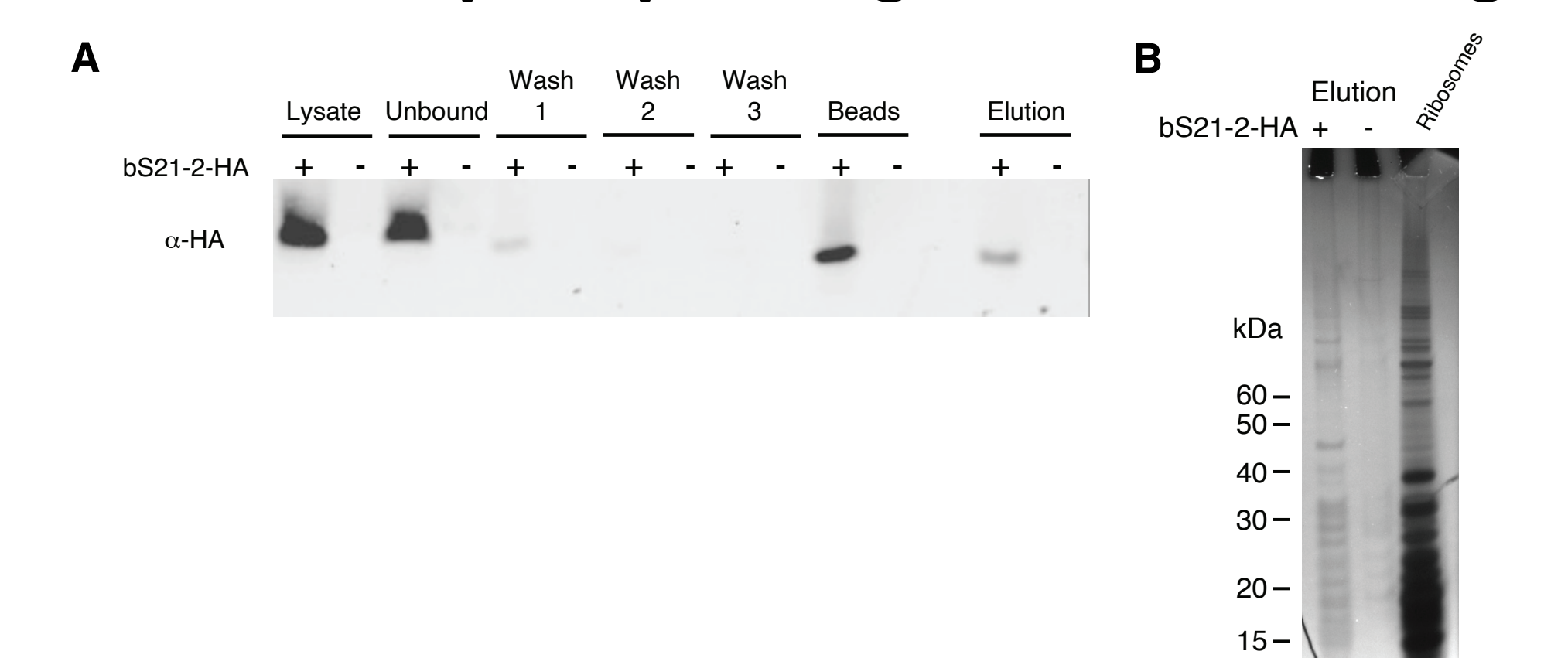


Figure 8. Assessing purification of 70S ribosomes by immunoprecipitating bS21-2-HA. *F. tularensis* cells either contained a plasmid ectopically expressing bS21-2-HA ("+") or an empty vector ("-"). A. Immunoblot analysis of indicated samples taken during immunoprecipitation of bS21-2-HA or of final elution. B. Silver stained gel of final elution, comparing immunoprecipitated material to empty vector control and all *F. tularensis* ribosomes purified by sucrose cushion.