Assessing *in vitro* control of gene expression in *Francisella tularensis* by ribosomal protein bS21

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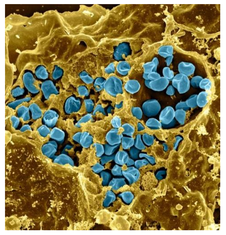
Introduction

The bacterial pathogen *Francisella tularensis* and ribosomal protein bS21



Francisella tularensis
- Gram-negative
- Causes tularemia
- Potential bioweapon
- Type VI secretion system is critical for virulence

Figure 1. *F. tularensis* cells (blue) inside macrophage (yellow). Fischer, PNAS cover, 2006.

Small subunit ribosomal protein bS21
- Three homologs encoded by *F. tularensis*
- Possibly regulate translation initiation
- bS21-2 positively controls translation of type VI secretion system proteins
- bS21-2 necessary for intramacrophage growth

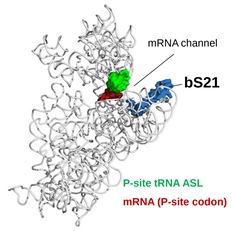


Figure 2. The location of bS21 in the 30S subunit in *Escherichia coli*. PDB 4V50; figure made by Dr. Gregory.

Assessing regulation of translation by bS21-2

***In vivo* assay**

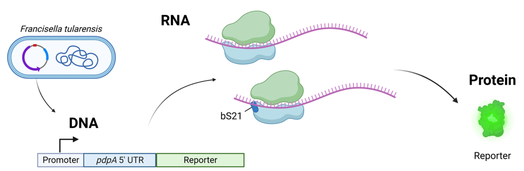
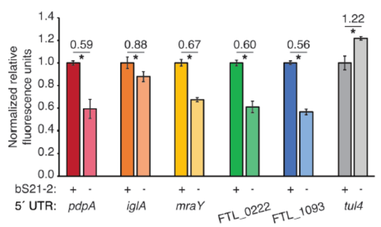


Figure 3. Diagram of plasmid-encoded translational reporter and processes leading to reporter production.

Leader sequences lead to preferential translation by ribosomes with bS21-2

Figure 4. 5′ UTRs are sufficient to cause bS21-2 mediated changes in translation. 5′ UTRs of indicated genes are fused in frame with GFP (as in Fig. 3) and introduced into cells with or without bS21-2. *P < 0.05. ns = not significant. Trautmann et al., 2023.



***In vitro* assay**

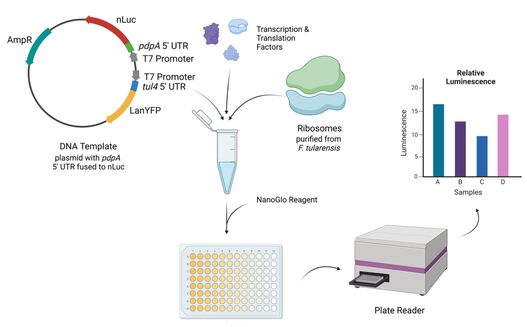
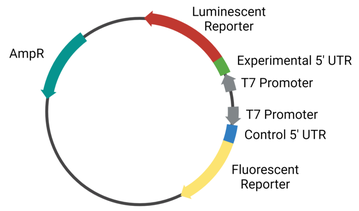


Figure 5. Diagram depicting process of *in vitro* assay. Assay requires NEB PURExpress Δ Ribosome Kit, Nano-Glo® Luciferase Assay System, purified ribosomes, and a reporter template.

Specific Components

Dual-Reporter Plasmids



A dual-reporter system can look at translation efficiency of two distinct proteins and measure them using either luminescence or fluorescence.
Components:
1. Experimental 5’ untranslated region (5’ UTR) fused to luminescent reporter (nLuc)
2. Control 5’ UTR fused to fluorescent reporter (LanYFP, iLov, or eGFP)

Figure 6. Diagram depicting a dual-reporter plasmid.

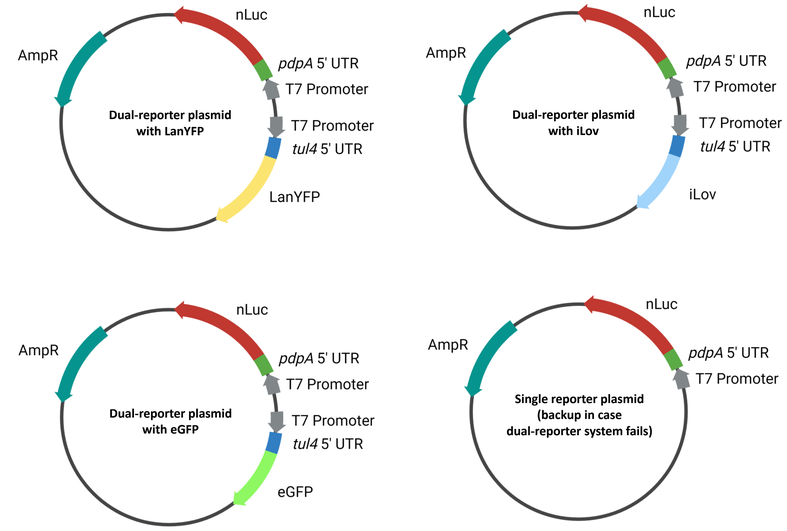


Figure 7. Reporter constructs we have generated to test in *in vitro* assay.

Ribosome Purifications

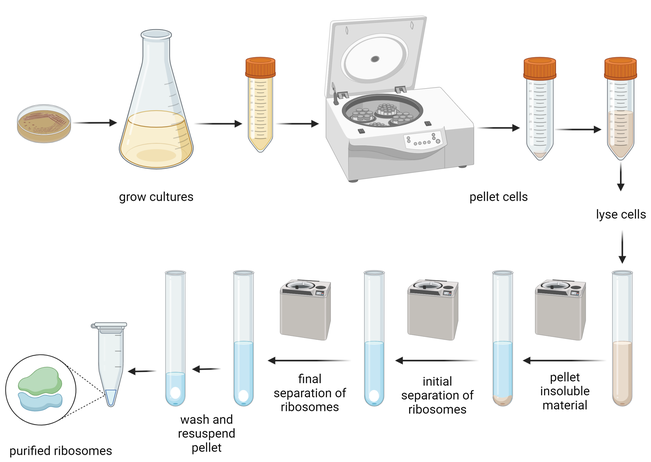


Figure 8. Workflow for ribosome purifications.

Results

Ribosome Purifications
- Purified high concentrations of *E. coli* ribosomes.
- Purified *F. tularensis* ribosomes, concentrations not sufficient for *in vitro* assay.

Dual-Reporter Plasmid Cloning
- We have cloned easily-modifiable versions of the plasmids that we can use to test the assay.
- We can detect all reporters (nLuc, LanYFP, iLov, eGFP).

***In Vitro* Assay Results**
- Our purified *E. coli* ribosomes have reproducibly similar activity to kit-provided ribosomes.
- Previously purified *F. tularensis* ribosomes demonstrated less activity.
- Recently purified *F. tularensis* ribosomes have yet to be tested.

Figure 9. *E. coli* ribosome pellet after purification.

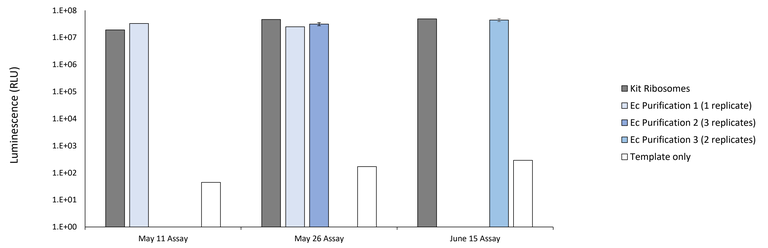


Figure 10. Relative luminescence values for nLuc after translation by purified *E. coli* ribosomes.

In Progress

1. Purify active *Francisella tularensis* ribosomes.
   - Optimize growth conditions for cells and purification steps.
2. Test translation efficiency of specific 5’ UTRs using the *in vitro* assay.

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