Developing an assay to compare translation by *Francisella tularensis* ribosomes with different bS21 homologs

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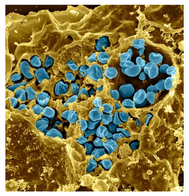
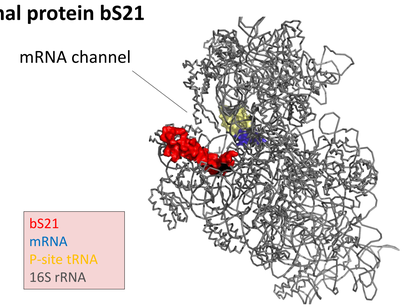
Introduction

***Francisella tularensis* is a bacterial pathogen that encodes three homologs of ribosomal protein bS21**



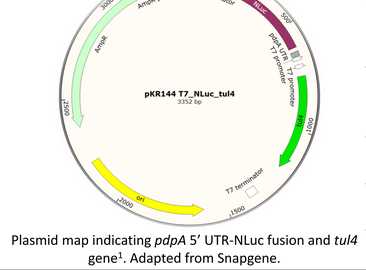
F. tularensis is a Gram negative facultative intracellular bacterium

- Causes tularemia
- Highly infectious
- Considered potential bioweapon

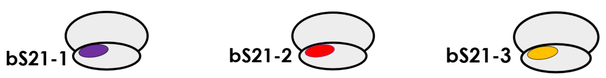


bS21 in *E. coli* is in close proximity to the mRNA exit channel in the 30S subunit[4]. Figure made by Hannah Trautmann. PDB entry: 4V50

***F. tularensis* has heterogenous populations of ribosomes**



- Three bS21 homologs in *F. tularensis*
- Role in translation initiation
- *rpsU2* encodes bS21-2
- bS21-2 important for virulence



The *pdpA* 5' UTR leads to reduced translation in cells without bS21-2

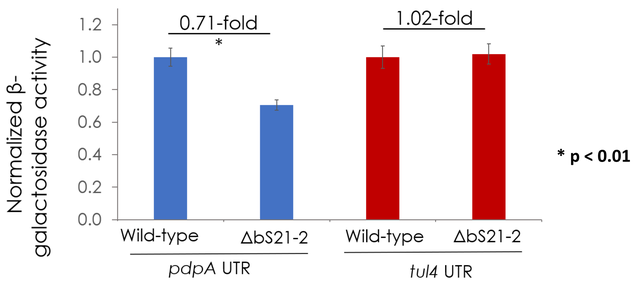


Figure 1: Cells lacking bS21-2 translate the *pdpA* 5' UTR less efficiently than wild-type cells. β-galactosidase activity was assessed in reporter fusions with the *tul4* promoter and either the *pdpA* or *tul4* UTR. No significant difference was detected between wild-type and ΔbS21-2 in the *tul4* UTR reporter. Activity was normalized to wild-type cells for each UTR reporter.

Developing an *in vitro* assay

Plasmid map indicating *pdpA* 5' UTR-NLuc fusion and *tul4* gene[1]. Adapted from Snapgene.

- Amplified *pdpA* 5' UTR-NLuc fusion using PCR
- Digested then ligated into plasmid with T7 promoter
- Transformed into *E. coli*
- Purified plasmid and confirmed sequence
- Phenol-chloroform purified plasmid

In vitro assay method

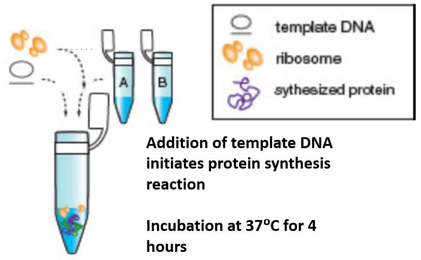
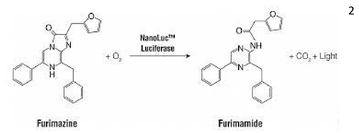


Diagram depicting the protocol for using the PURExpress Δribosome kit to produce NLuc and LpnA. Adapted from NEB PURExpress product diagram[3].

- Detection
  - *pdpA* 5' UTR-*NLuc*: luminometer
  - *tul4* 5' UTR-*tul4*: immunoblot for LpnA
- More sensitive and faster
- Observe interaction between purified ribosomes and transcript to examine relationship between 5' UTR and translation



Results

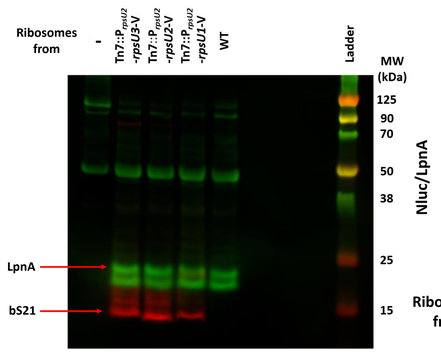


Figure 2: Immunoblot detecting production of LpnA from replicate 1 of *in vitro* assay. Amounts of bS21-V homolog present in purified ribosomes are also detected.

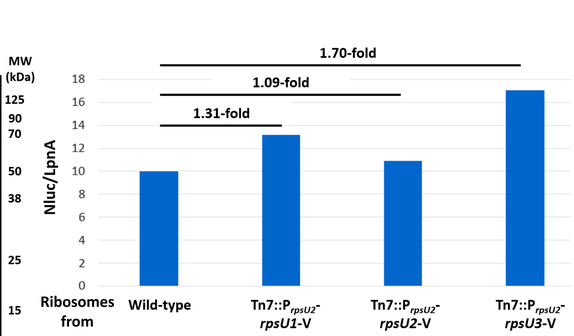


Figure 3: Relative translation of transcripts with *pdpA* versus *tul4* 5' UTRs from replicate 1 of *in vitro* assay. Ratio of luminescence produced from NLuc to amount of LpnA quantified from immunoblot for each sample.

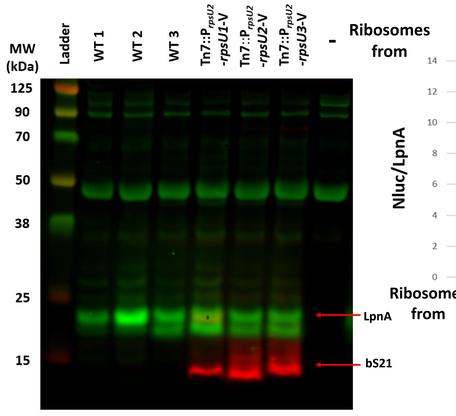


Figure 4: Immunoblot detecting production of LpnA from replicate 2 of *in vitro* assay. Amounts of bS21-V homolog present in purified ribosomes are also detected.

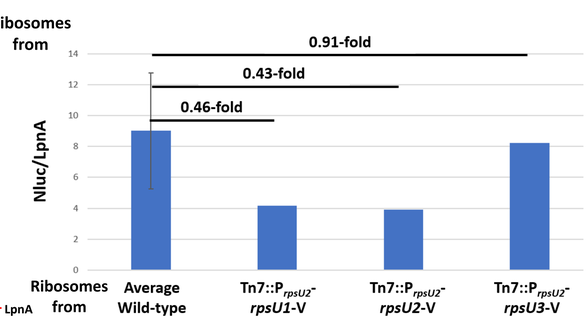


Figure 5: Relative translation of transcripts with *pdpA* versus *tul4* 5' UTRs from replicate 2 of *in vitro* assay. Ratio of luminescence produced from NLuc to amount of LpnA quantified from immunoblot for each sample. Ratios from wild-type ribosomes were averaged and error bar represents standard deviation.

Conclusions

- Successfully created pKR144 plasmid for *in vitro* transcription/translation assay
- Able to clone in *E. coli* (not toxic, compared to previous reporter constructs)
- Able to detect translation by *F. tularensis* ribosomes (Figure 6)
- All bS21 homologs allow translation of *pdpA* 5' UTR
- Reductions in PdpA in cells lacking bS21-2 may be due to less bS21 overall

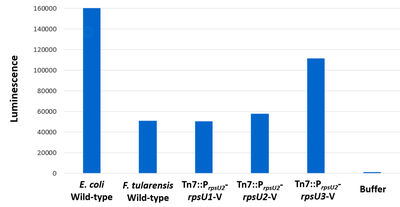


Figure 6: Luminescence produced by *E. coli* compared with *F. tularensis* ribosomes. Max value of x-axis was minimized to 1.6 x 10^5. Actual *E. coli* value: 1.4 x 10^7

Ribosomes with bS21-3 seem to translate transcripts with the *pdpA* 5' UTR most efficiently

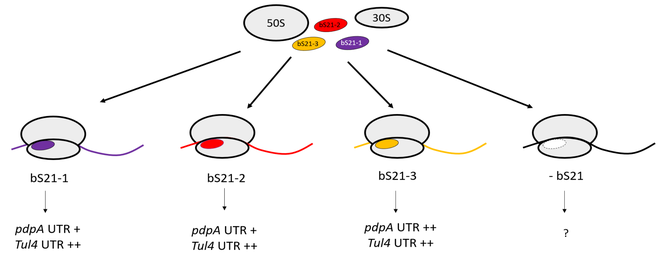


Diagram depicting a potential model for how bS21 homologs may differentially regulate translation of the type VII secretion system protein pdpA's 5' UTR.

Next Steps

- Test ribosomes in biological triplicate to assess variability
- Test ribosomes with and without bS21
- Use ribosomes isolated from cells with inducible bS21 to ensure consistent abundances
- Test other 5' UTRs fused to NLuc to examine impacts on relative translation

Acknowledgements

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References

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3. PURExpress *In Vitro* Protein Synthesis, *New England BioLabs* (2021).
4. Van Duin, J., & Wijnands, R. (1981). The function of ribosomal protein S21 in protein synthesis. *European Journal of Biochemistry*, 118(3).

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