Cloning a plasmid to assess *in vitro* control of gene expression by a ribosomal protein homolog

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Introduction

The bacterial pathogen *Francisella tularensis*, bS21-2, and translation initiation

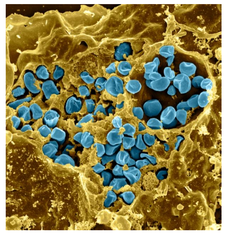


Figure 1. *F. tularensis* cells (blue) inside macrophage (yellow). Fischer, PNAS cover, 2006

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

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

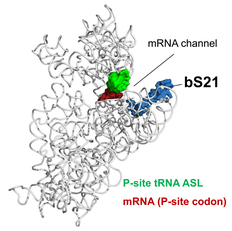


Figure 2. The location of bS21 in the 30S subunit in *Escherichia coli*. PDB 4V50; figure made by Dr. Gregory

Figure 3. Diagram depicting ribosome undergoing initiation

Translation initiation:
• During initiation, the ribosome binds at the start codon to the mRNA
• To help the ribosome bind at the proper location, the anti Shine-Dalgarno (SD) sequence on the ribosome binds to the Shine-Dalgarno on the mRNA
• bS21 is in the ribosome next to the SD sequence on the mRNA, and its proximity raises the possibility of regulation

Assessing the role of leader sequences in regulation by bS21-2

Translational reporters used to assess contribution of bS21-2

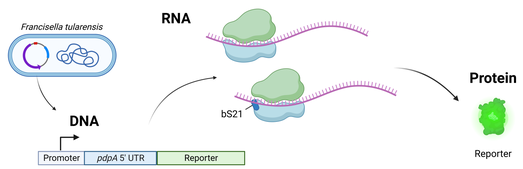


Figure 4. Figure depicting a cloned plasmid introduced to a cell undergoing transcription and translation

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

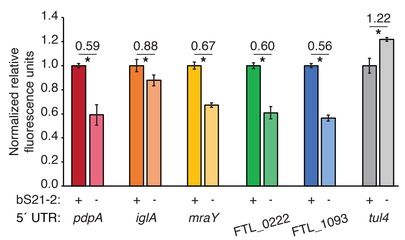
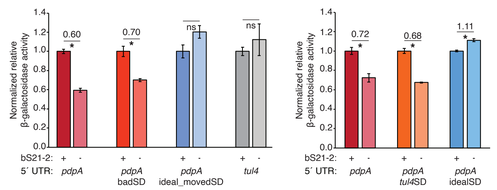


Figure 5. 5´ UTRs are sufficient to cause bS21-2 mediated changes in translation. Trautmann et al., 2023

An imperfect Shine-Dalgarno sequence is necessary for responsiveness to bS21-2



Shine-Dalgarno

pdpA	aguuauguucuaauua**aguagg**aca aug auagcaguaaaagau
pdpA badSD	aguuauguucuaCCguCCAUCAaca aug auagcaguaaaagau
pdpA ideal_movedSD	aguuauguA**AGGAGG**UCCAUCAaca aug auagcaguaaaagau
*pdpA tul4*SD	aguuauguucua**AGGAGU**AUCaca aug auagcaguaaaagau
pdpA idealSD	aguuauguucua**AGGAGG**UCAaca aug auagcaguaaaagau
tul4	gaguauaugugaauauuuaaaaaa**aggagu**aucuau aug aaaaaaauaauuaag

Figure 6. Changes in levels of translation disappear when the Shine-Dalgarno sequence of *pdpA* is modified to an ideal Shine-Dalgarno. Trautmann et al., 2023

Study Goals

Overall Goal: Can we recapitulate bS21-2-mediated regulation *in vitro*?

In vitro assay:

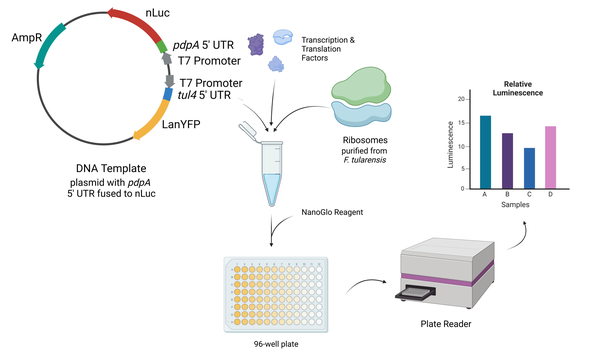


Figure 7. Diagram depicting process of *in vitro* assay

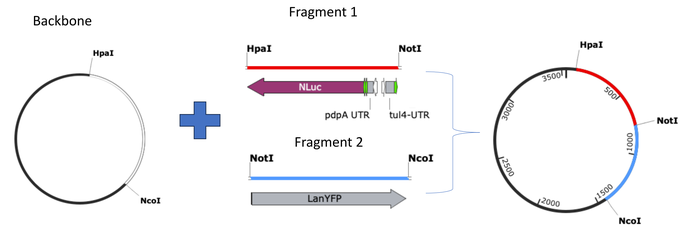
Summer Goals:

1. Complete cloning a dual-reporter plasmid for *in vitro* translation assays
2. Clone a plasmid that will allow us to easily modify and switch out the 5' UTR sequences for genes we want to study, including *pdpA*
3. Clone a plasmid with the *pdpA 5'* UTR containing an ideal Shine-Dalgarno sequence to see if we can recapitulate the loss of regulation by bS21-2 *in vitro*

Process of DNA Cloning

Step 1: Amplify DNA
Step 2: Cut DNA so ends match
Step 3: Purify the DNA
Step 4: Ligate DNA together
Step 5: Transform plasmid into *E.coli* cells
Step 6: Grow bacteria with plasmid
Step 7: Isolate plasmid
Step 8: Confirm plasmid by sequencing

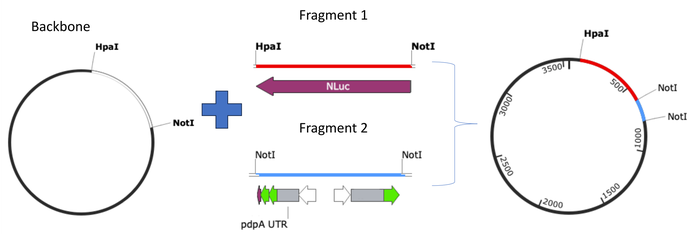
Cloning a dual-reporter plasmid for *in vitro* translation assays:



- Control UTR fused to LanYFP (fluorescent)
- Wildtype (unmodified) *pdpA* 5' UTR fused to nLuc (luminescent)

Figure 8. Diagram depicting creation of dual-reporter plasmid

Cloning a dual-reporter plasmid that can be easily modified to test different leader sequences:



- Control UTR fused to LanYFP (fluorescent)
- Wildtype (unmodified) *pdpA* 5' UTR fused to nLuc (luminescent)
- Can easily cut out UTRs and switch them

Figure 9. Diagram depicting final dual-reporter plasmid

In Progress

1. In process of using this plasmid as a template to modify the Shine-Dalgarno sequence in the *pdpA* 5'UTR
2. Test translation efficiency using an *in vitro* assay

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