

## THE OFFICE OF UNDERGRADUATE RESEARCH AND INNOVATION

### Project Summary Report

Please submit report by May 15, 2022 to:

The Office of Undergraduate Research (URI)2 Email: uri2@etal.uri.edu

**Date:** May 2<sup>nd</sup>, 2022

**Project Title:** Assessing promoter sequences important for regulation of the *rpsU2* gene in a pathogenic bacterium

**Primary Student Name:** Aisling Macaraeg and Dan Floyd

**Project start date:** December 1<sup>st</sup>, 2021

**Project end date:** May 2<sup>nd</sup>, 2022

**Faculty Mentor Name:** Dr. Kathryn Ramsey

**Department:** CMB/BPS

#### Abstract:

Provide a one paragraph abstract so that readers who are not specialists in the subject will understand the purpose, results, and impact of your project.

*Francisella tularensis* is a pathogenic bacterium that is the causative agent of the disease tularemia, which can be fatal in humans and other animals. To work with this bacterium in a research laboratory setting, we use the live vaccine strain (LVS), which is attenuated and does not cause disease in humans. The genome of *F. tularensis* encodes three homologs (versions) of the small ribosomal protein, bS21. One of these homologs, bS21-2, is of interest to the Ramsey lab because it is important for virulence, the bacteria's ability to cause disease. However, we do not know what factors lead to production of bS21-2. This project aimed to uncover more information about regulation of bS21-2, building on preliminary data from the Ramsey lab which suggested that the bS21-2 protein may repress its own production by reducing the amount of messenger RNA produced from the gene that encodes bS21-2, *rpsU2*. Specifically, this project assessed whether the activity of the promoter or the 5' untranslated region (UTR) sequence of the *rpsU2* gene is responding to the presence of the bS21-2 protein. The promoter and the 5' UTR are two different regulatory sequences that precede the gene of interest, *rpsU2*. We investigated this question through construction of reporter gene fusions of these upstream regions to the gene encoding  $\beta$ -galactosidase, *lacZ*, and completion of  $\beta$ -galactosidase assays in strains with and without bS21-2. We found no meaningful difference in  $\beta$ -galactosidase activity between our two strains, which did not validate the previous findings. Due to this discrepancy, we compared the *lacZ* mRNA abundance from our reporter fusion cells that either contain or lack bS21-2 using real-time PCR, as this was the method used in the preliminary research. The data showed 7-fold more *lacZ* mRNA in the strain without bS21-2, suggesting that the presence of bS21-2 leads to reduced amounts of mRNA. Because there is a difference of mRNA but no difference in protein activity, we hypothesize that there is an unknown mechanism of regulation happening post-transcriptionally; further research is necessary to validate these results and identify this unknown mechanism.

## Outcomes:

### 1. What did you discover? Discuss your project's significant findings and accomplishments.

We discovered that *rpsU2* is regulated in a complex manner that involves its own gene product, bS21-2. Initially, our goal was to determine which upstream region (the promoter or 5' UTR) of the *rpsU2* gene could be responding to bS21-2, to expand upon initial research that found there was 25-fold more *rpsU2* mRNA produced in cells lacking bS21-2. However, using an approach that fused upstream regulatory regions to the  $\beta$ -galactosidase reporter gene, we only about a 30% increase in protein activity in the cells lacking bS21-2. Given the relatively small difference in activity in cells with or without bS21-2, we were unable to determine the regulatory region that could be responding to the protein. We subsequently considered that protein activity may not be an accurate measure of transcript abundance in this model, and instead isolated RNA to assess *rpsU2* transcripts more directly. We then performed real-time PCR to compare the strains that we constructed to the samples that were used in previous study, which did not contain the *lacZ* fusion. We were expecting that, in cells with both components of the *rpsu2* regulatory region (promoter and 5' UTR) fused to *lacZ*, the amount of *lacZ* mRNA would mimic the amount of *rpsU2* mRNA in wild-type cells. In wild-type cells, it was found that there is a 25-fold increase in *rpsU2* mRNA between cells with bS21-2 and cells without bS21-2. When we completed the RT-PCR, the results differed from our expectations, as we found only about a 7-fold increase. There are several possible explanations for this discrepancy. For the strains that we constructed, the *rpsU2* regulatory sequences were copied and engineered with the gene fusion of *lacZ* in another part of the chromosome. It can be difficult to find the bounds of upstream regulatory sequences, and this engineered strain could be missing some key components. The original data we are validating have the sequences in the native context, and therefore have all the necessary components. Another reason could be that, because of the replicated *rpsU2* regulatory regions in the engineered strains, there are two *rpsU2* promoter regions on the chromosome where the protein bS21-2 could be acting, while in the native context there is only one. This could be a reasonable explanation as the transcript abundance found in the native context was 16-fold, and in the engineered strains was 7-fold. The difference is about half, consistent with the hypothesis that bS21-2 is split between two loci. We accomplished some major milestones in lab this semester, such as making plasmids, completing  $\beta$ -galactosidase assays, isolating RNA, and real-time PCR. We found limitations in the use of reporter fusion strains which will help improve the design of future experiments for the Ramsey lab.

### 2. Comment on additional outcomes not directly associated with the project's objectives (e.g., partnerships formed, additional funding support gained, presentations)

We both had the opportunity to practice our science communication skills when updating our professor, lab mates, and members of another lab in the department about the project's process during joint lab meetings. This was important as undergraduates working on a niche molecular biology project to be able to communicate the science behind the project. Additionally, Aisling had the opportunity to present the project to the class CMB 482 as a guest lecturer, which was beneficial both for her communication skills and for exposing other undergraduates to the unexpected nature of research.

**Lessons Learned:**

1. Reflecting on strategies and activities, what worked and what did not work?

Our original strategy of using reporter fusions and protein activity as a proxy for measuring transcript abundance was unsuccessful so we moved onto the strategy of using real-time quantitative reverse transcription PCR (RT-qPCR). From the RT-qPCR, we generated data different from our beta-galactosidase assays and were able to draw some conclusions from that data. In the future, continuing to use the RT-qPCR approach and including more upstream DNA to pinpoint which component of the upstream regions are necessary is most likely the next step. It also became difficult for both partners to work on the project in equal amounts of time and maintain communication about the project over the course of the semester. Other projects reduced the amount of time Dan was able to work on this project and so it was difficult for both partners to be on the same page regarding the progress made on this project.

2. What would you do differently next time?

After discussing this question, we decided having more checkpoints along the way to provide more analysis and insight on the progress made during this project would be the best improvement for future endeavors. Additionally, if two partners were to work on a similar project in the future, planning days to work on the project far in advance may result in better communication and more efficient work done.

**Lessons Applied:**

1. How will the lessons learned from this project affect your future work? Do you anticipate any long-term outcome from your project?

Dan is graduating this May and will be moving on to a laboratory technician position at the Massachusetts General Hospital Center for Infectious Disease. The work he will be doing there will involve similar and relevant skills he acquired and refined through the work done on this project, such as RNA isolation, DNA extraction, PCR, and gel electrophoresis. Along with lab techniques, he also improved his communication and organizational skills.

Aisling will be continuing to work on this project in the next year as she continues to conduct research in the Ramsey lab. Relevant skills such as real-time PCR,  $\beta$ -galactosidase assays, and lab safety are all tools that she feels confident using independently in lab and hopes to contribute to other spaces in her future. Additionally, Aisling will be participating in the RI-INBRE SURF program this summer and will be putting her organizational skills, time management, and science communication skills to use.

Will you be presenting your project at a conference or showcase, and if yes, where, and when?

No, we will not be presenting our project at a conference or showcase.

Additional Comments (regarding program, experience, etc.):

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Date	Vendor	Cost	Items
3/7/2022	Wilkem Scientific	\$ 319.23	Laboratory consumables for beta-galactosidase assays
3/11/2022	ThermoFisher	\$ 372.86	Specalized enzyme for gene expression analysis (reverse transcriptase)
4/25/2022	RIGSC	\$ 149.36	Equipment fees for quantiative PCR
	TOTAL	\$ 841.45	

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