



Determining the genetic requirements for *Francisella tularensis* survival in freshwater

Aisling Macaraeg¹, Hannah Trautmann¹, Sierra Schmidt¹, and Kathryn Ramsey^{1,2}

¹Department of Cell and Molecular Biology, University of Rhode Island

²Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island



1 Introduction

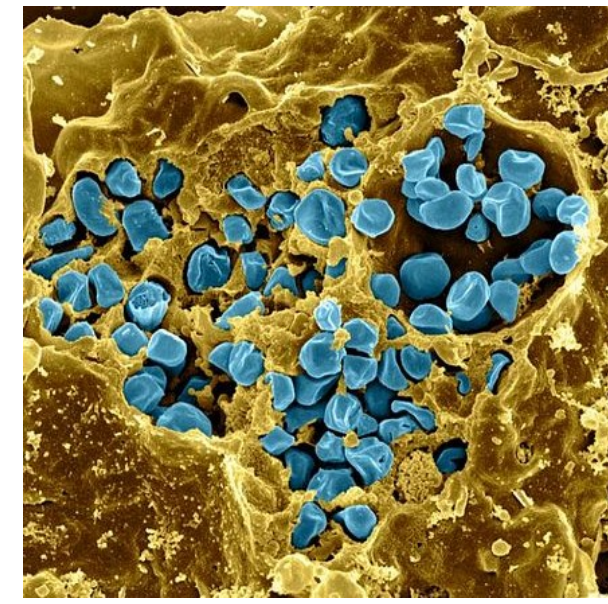


Figure 1: *F. tularensis* in macrophage.³

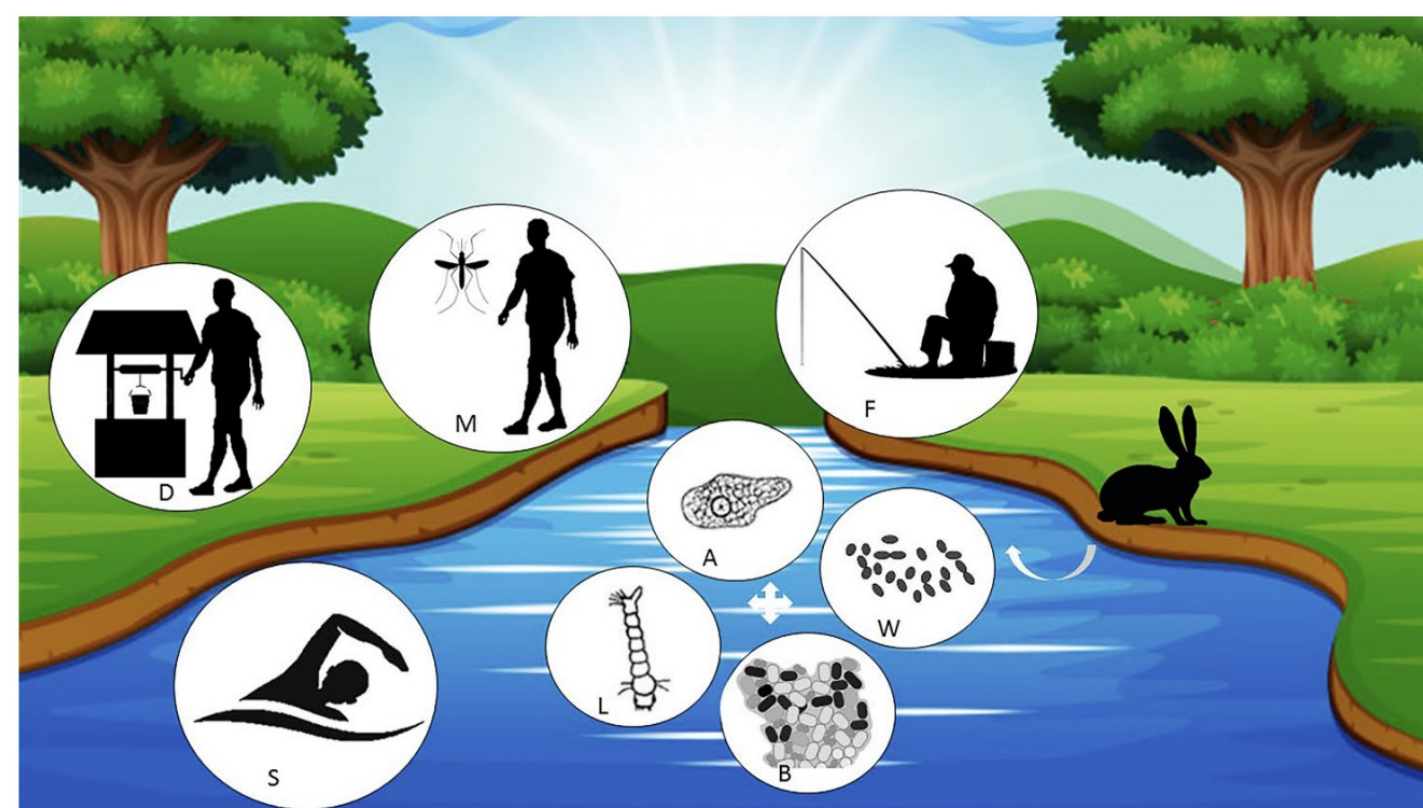


Figure 2: *F. tularensis* modes of aquatic transmission.⁶

- *Francisella tularensis*
 - Gram-negative, pathogenic bacterium
 - Causes the disease tularemia
- *F. tularensis* can **survive in freshwater** for long periods of time and subsequently **infect animals and humans**⁴
- A gene has been found that is important during the transition between the host and aquatic environment⁷

Main Question:

What are the genetic requirements for survival of *F. tularensis* in freshwater?

2 Freshwater Survival Assay

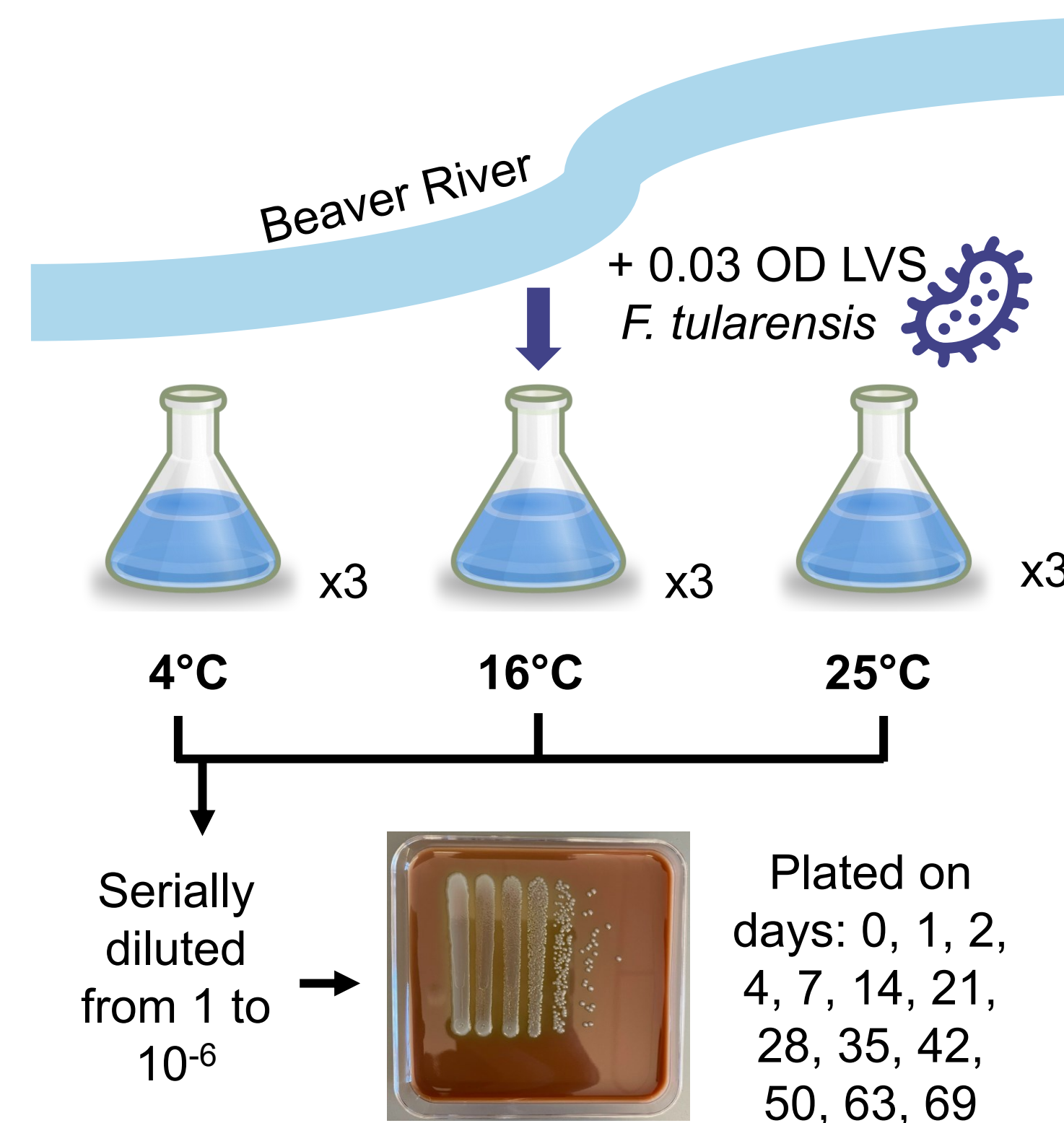


Figure 3. Workflow for Cell Viability. Freshwater was collected from the Beaver River, Rhode Island. The water was filter sterilized and inoculated with *F. tularensis* LVS. The initial inoculum was distributed to nine flasks, three of each were placed at three different temperatures: 4°C, 16 °C, and 25 °C. Samples were serially diluted and plated over a period of 10 weeks.

3 Transposon Insertion Sequencing

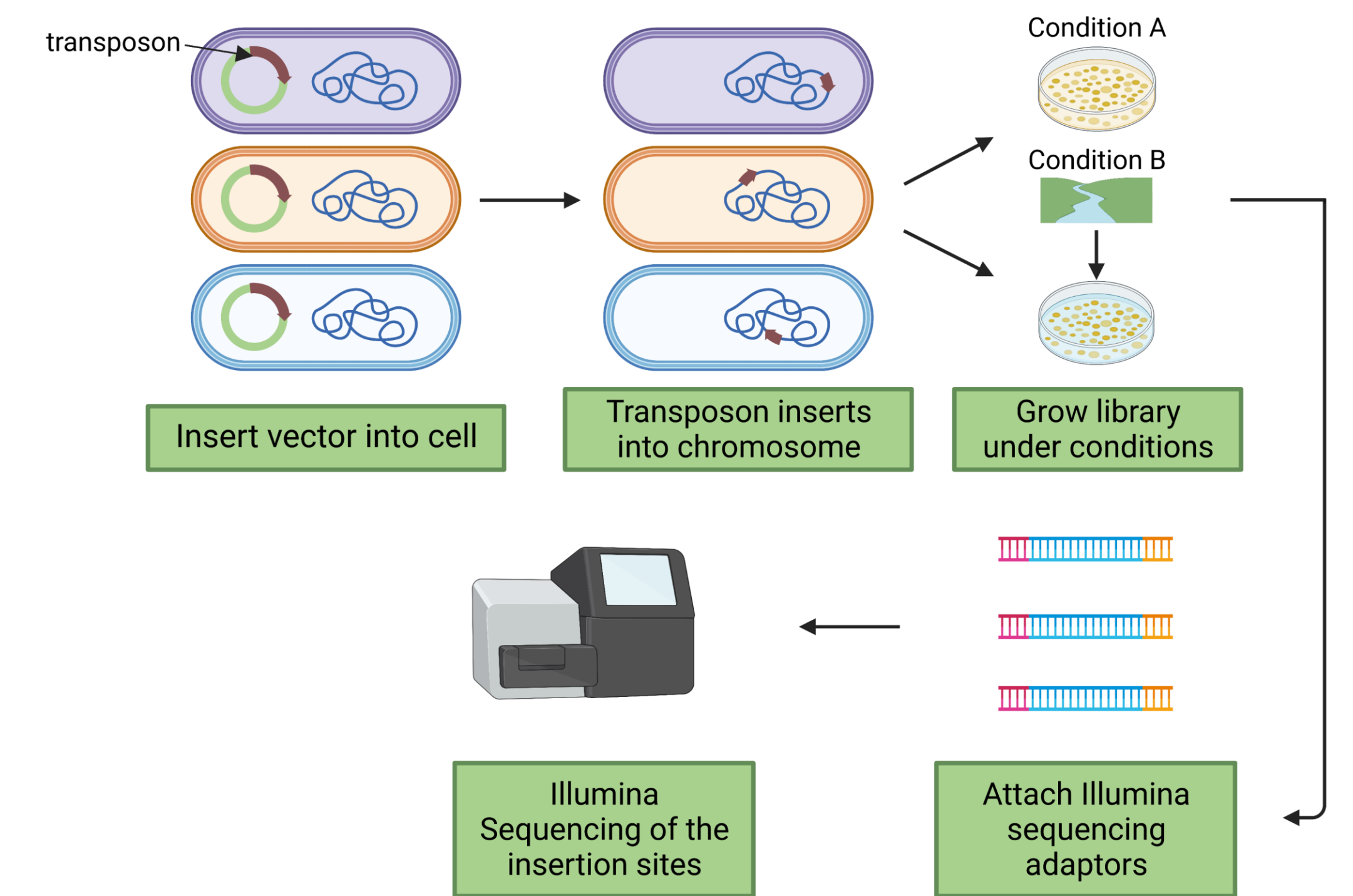


Figure 4: Workflow of transposon insertion sequencing protocol¹

This is modified from Chao *et al.* 2016.

4 New Transposon Delivery Plasmid



Initial transformation efficiency results: 1.88×10^4

Transposon: Fragment of DNA that “jumps” into the chromosome

Why make a new plasmid?

- Modified the ends of the transposon to be compatible with the 2011 update to the Illumina adapters⁵
- The new plasmid incorporated this modifications (depicted in brown)

6 Survival of *F. tularensis* in Freshwater at 4°C

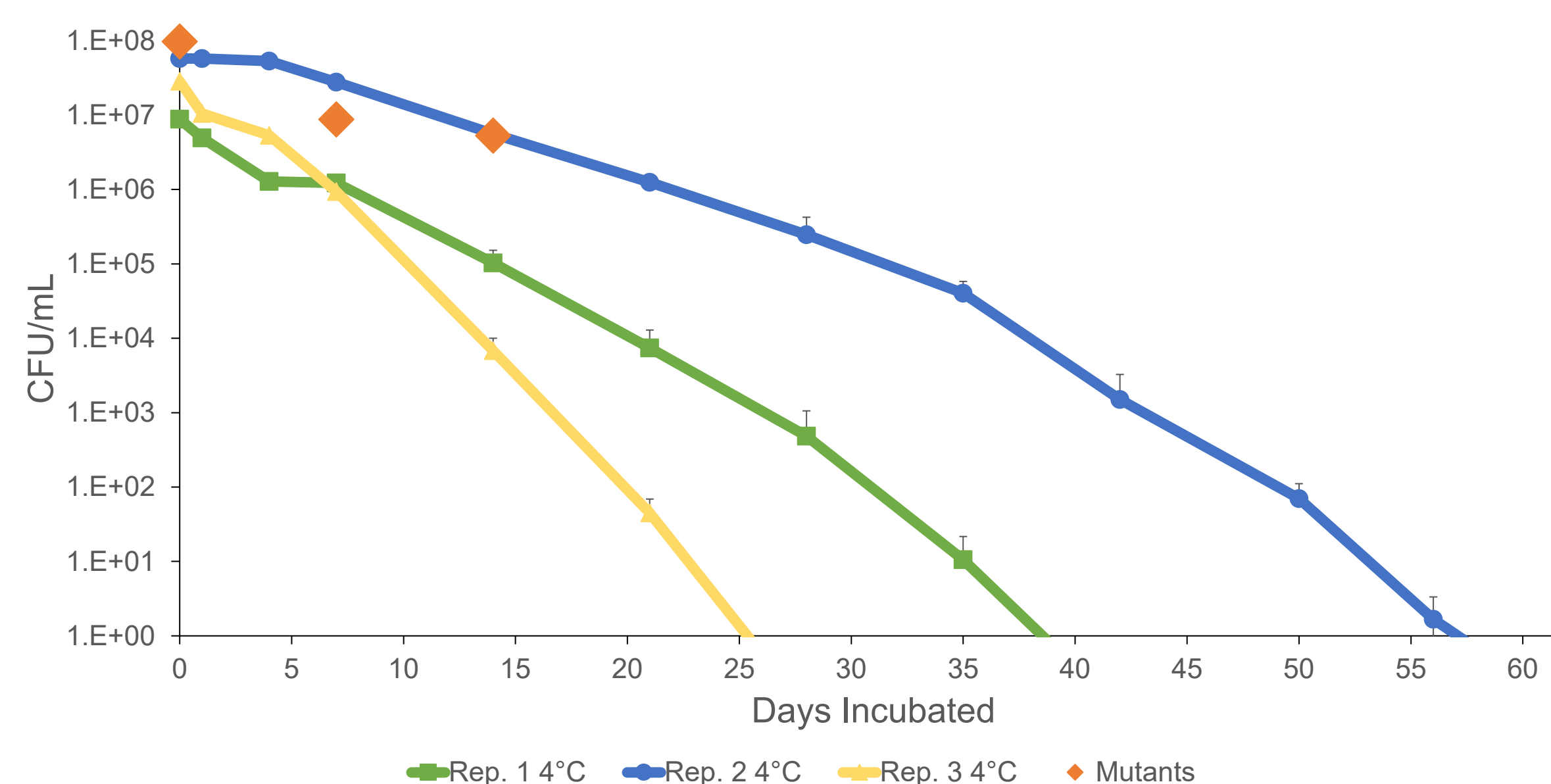


Figure 6: Comparison of *F. tularensis* survival in river water at 4°C across experiments.

Average CFU per mL recovered at indicated time points for three replicate experiments, with cells incubated at 4°C. The longest survival was 56 days in replicate 2. In orange diamonds, the average CFU per mL after incubating transposon mutant library in freshwater. Indicated timepoints (day 0, 7, 14) are also when gDNA was extracted for transposon insertion sequencing.

5 Creation of a Transposon Mutant Library

F. tularensis genome: 1,895,944 bp

Number of genes: 2,020

Final transformation efficiency: 2.44×10^3

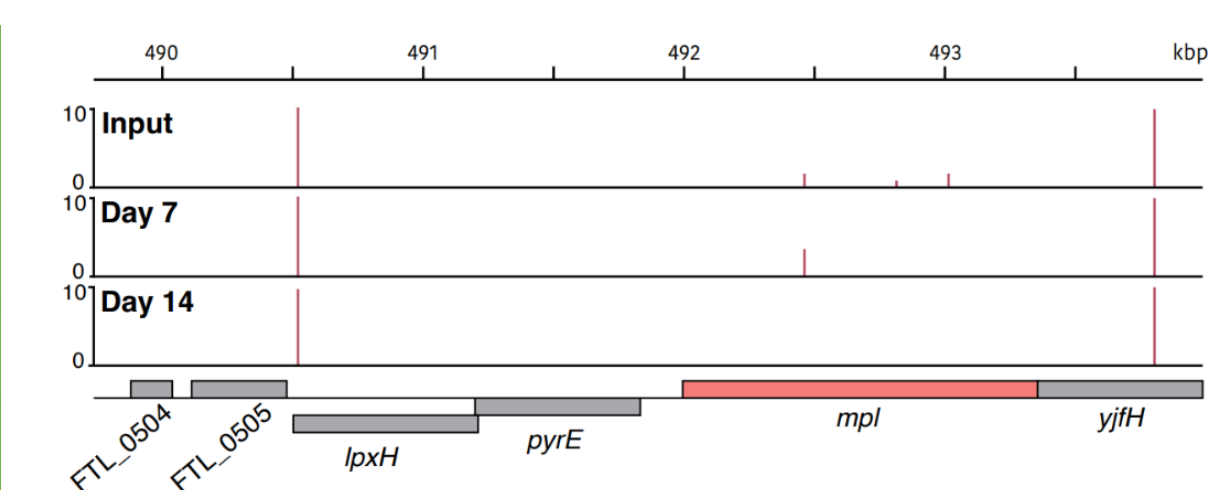
Estimated number of transposon mutants: ~5,572



Figure 5: Example of transposon mutant library isolated on cysteine heart agar with hemoglobin plate

7 Transposon Insertion Sequencing Data

Library	Transposon Sequencing Reads	Number of Insertions/Mutants Detected
Input	377,164	5,733
Day 7	696,629	6,821
Day 14	502,258	6,168



8 Conclusion and References

- *F. tularensis* can remain viable between 21 and 56 days at 4°C in freshwater
- Assessed survival of transposon mutant library after incubation in freshwater kept at 4°C
- **FTL_0508, mpl**, is a candidate for a gene essential for *F. tularensis* survival in freshwater
 - Encodes for UDP-*N*-acetylmuramate:L-alanyl-γ-D-glutamyl-meso-diaminopimelate ligase
 - Important for cell wall synthesis

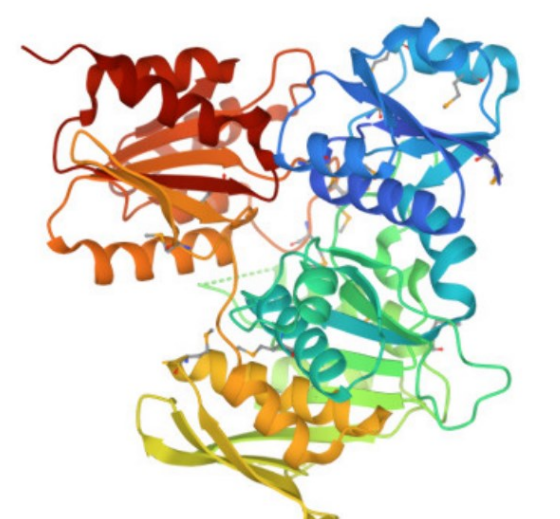


Figure 6: Crystal structure of UDP-*N*-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase from *Psychrobacter arcticus*²

Reference List

1. Chao, M.C., Abel, S., Davis, B.M., and Waldor, M.K. (2016). The design and analysis of transposon insertion sequencing experiments. *Nat Rev Microbiol* 14, 119-128.
2. Das, D., Hervé, M., Feuerhelm, J., Farr, C.L., Chiu, H., Elsiger, M., Knuth, M.W., Klock, H.E., Miller, M.D., Godzik, A., *et al.* (2011). Structure and function of the first full-length murein peptide ligase (Mpl) cell wall recycling protein. *PLoS One* 6, e17624.
3. Fischer. (2006). Cover image. 103(39)
4. Golovliov, I., Bäckman, S., Granberg, M., Salomonsson, E., Lundmark, E., Näslund, J., Busch, J.D., Birdsell, D., Sahl, J.W., Wagner, D.M., *et al.* (2021). Long-Term Survival of Virulent Tularemia Pathogens outside a Host in Conditions That Mimic Natural Aquatic Environments. *Appl Environ Microbiol* 87, 2713.
5. Goodman, A.L., Wu, M., and Gordon, J.I. (2011). Identifying microbial fitness determinants by insertion sequencing using genome-wide transposon mutant libraries. *Nat Protoc* 6, 1969-1980.
6. Hennebique, A., Boisset, S., and Maurin, M. (2019). Tularemia as a waterborne disease: a review. *Emerg Microbes Infect* 8, 1027-1042.
7. Williamson, D.R., Dewan, K.K., Patel, T., Wastella, C.M., Ning, G., and Kirimanjeswara, G.S. (2018). A Single Mechanosensitive Channel Protects *Francisella tularensis* subsp. *holarctica* from Hypoosmotic Shock and Promotes Survival in the Aquatic Environment. *Appl Environ Microbiol* 84, 2203.

Acknowledgments

We would like to thank the Ramsey lab as well as the Gregory Lab for productive joint lab discussions. We would also like to thank the Jenkins lab for the use of their incubator and the Nelson lab for the use of their benchtop. A big thank you to Janet, with all of her advice and help throughout the entirety of this project! Research reported in this presentation was supported by the Rhode Island Institutional Development Award (IDeA) Network of Biomedical Research Excellence from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103430.