

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

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1 Introduction

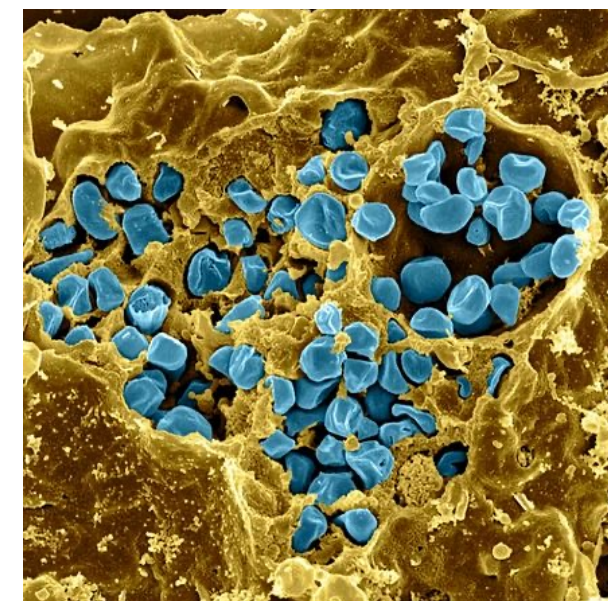


Figure 1: *F. tularensis* in macrophage.³

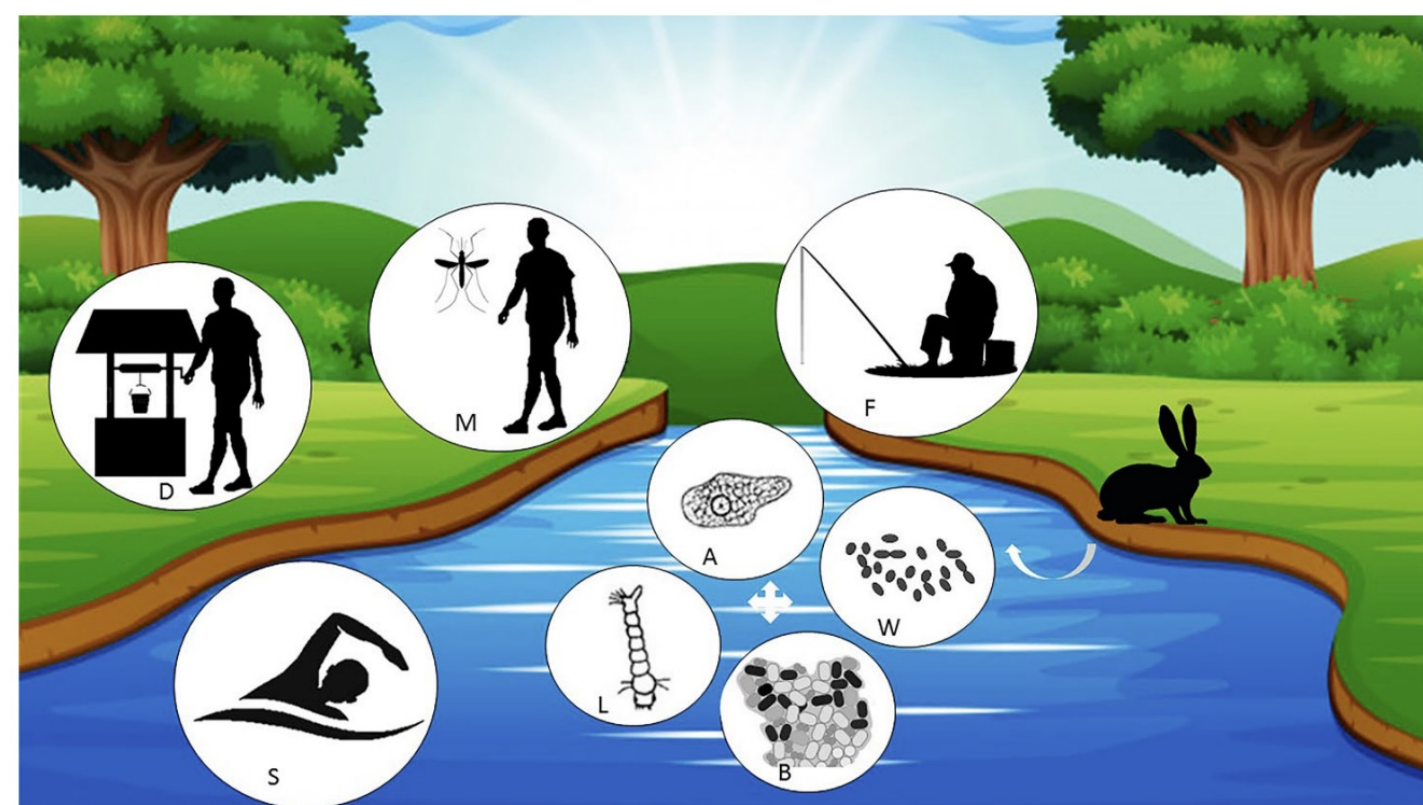


Figure 2: *F. tularensis* modes of aquatic transmission.⁶

- *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

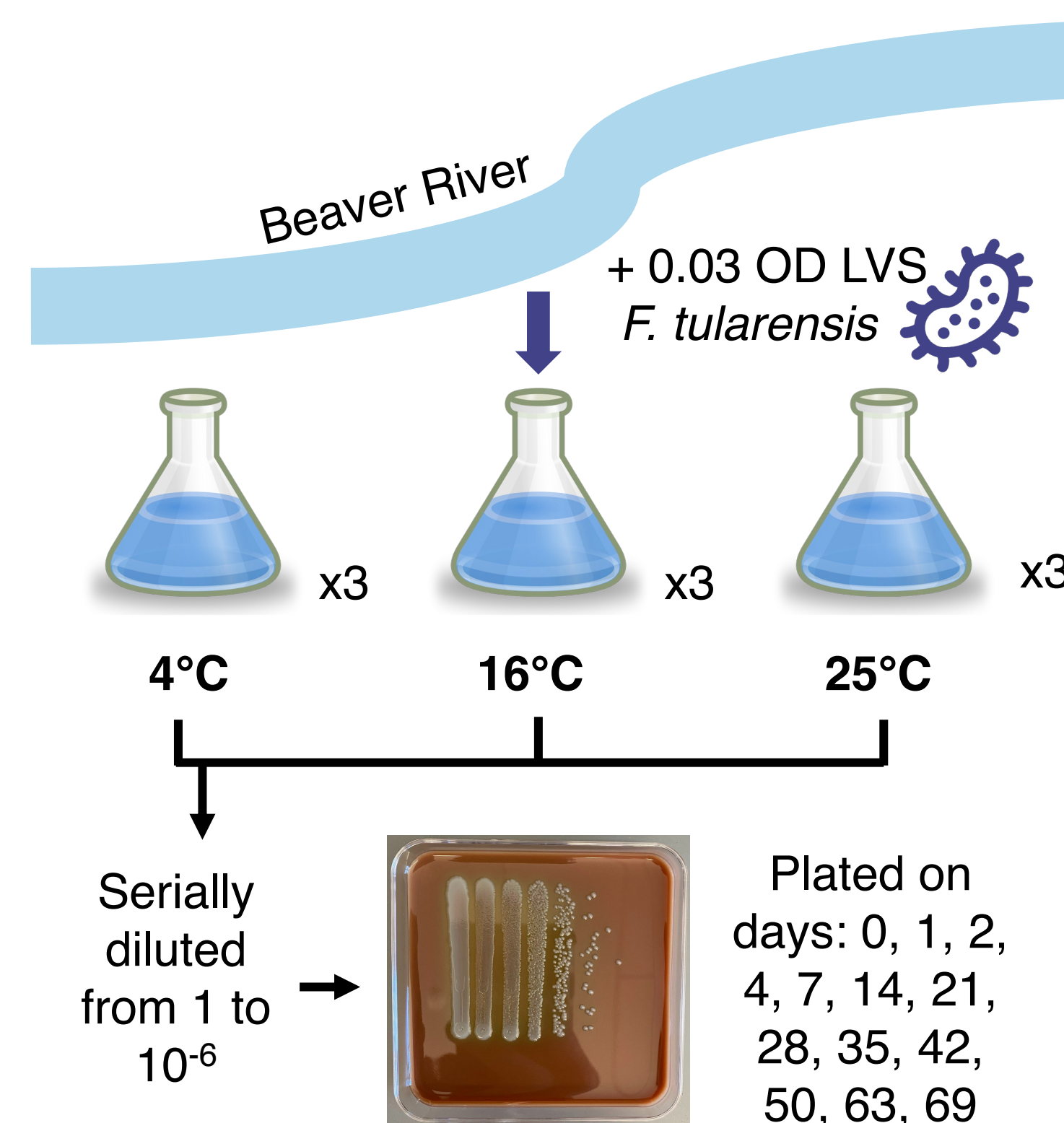


Figure 3. Workflow for Cell Viability. Freshwater was collected from the Beaver River, Rhode Island. The water was filter sterilized and inoculated with *F. tularensis* LVS. The initial inoculum was distributed to nine flasks, three of each were placed at three different temperatures: 4°C, 16 °C, and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

3 Transposon Insertion Sequencing

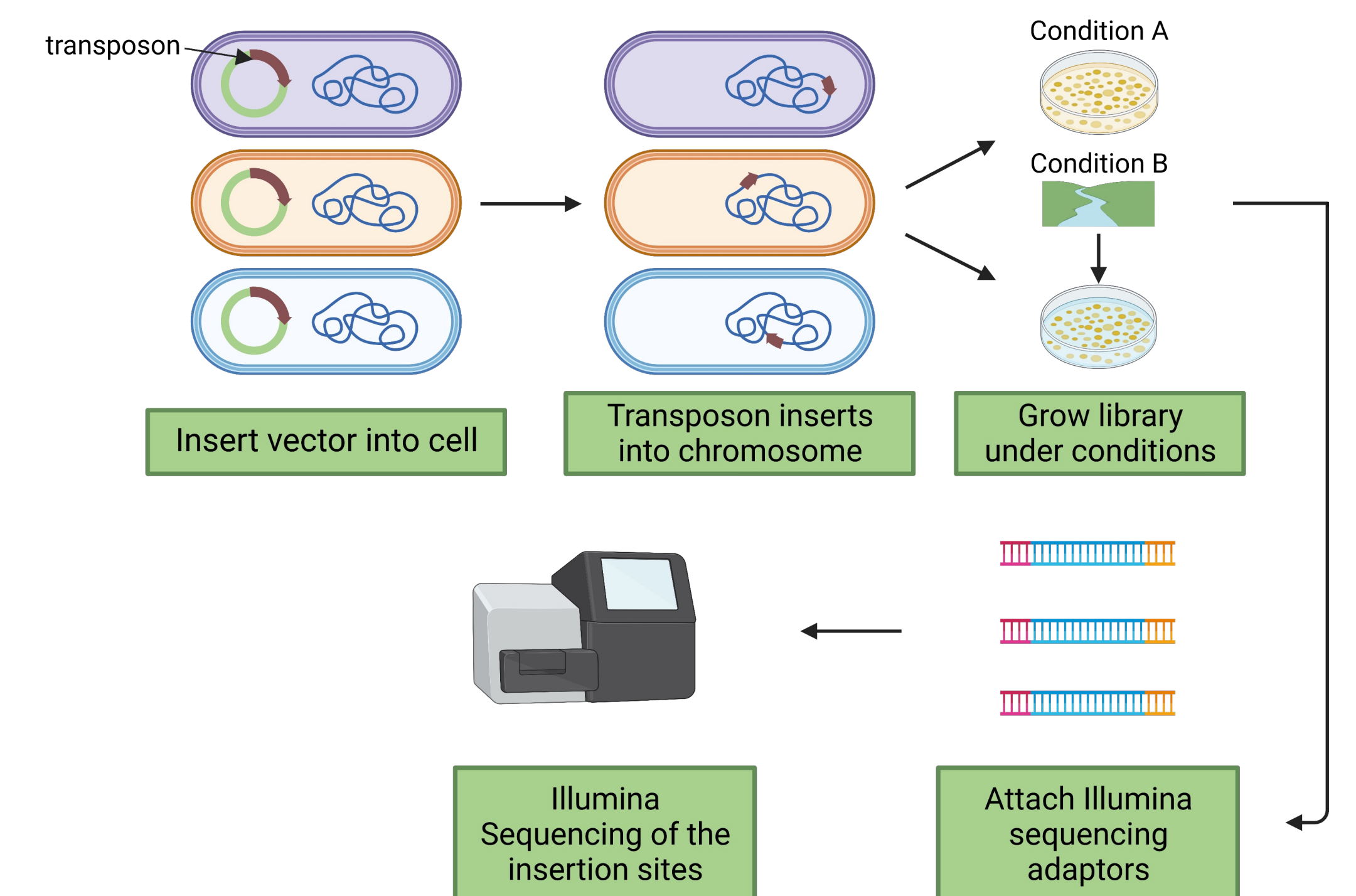


Figure 4: Workflow of transposon insertion sequencing protocol (modified from ref 1).

4 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

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

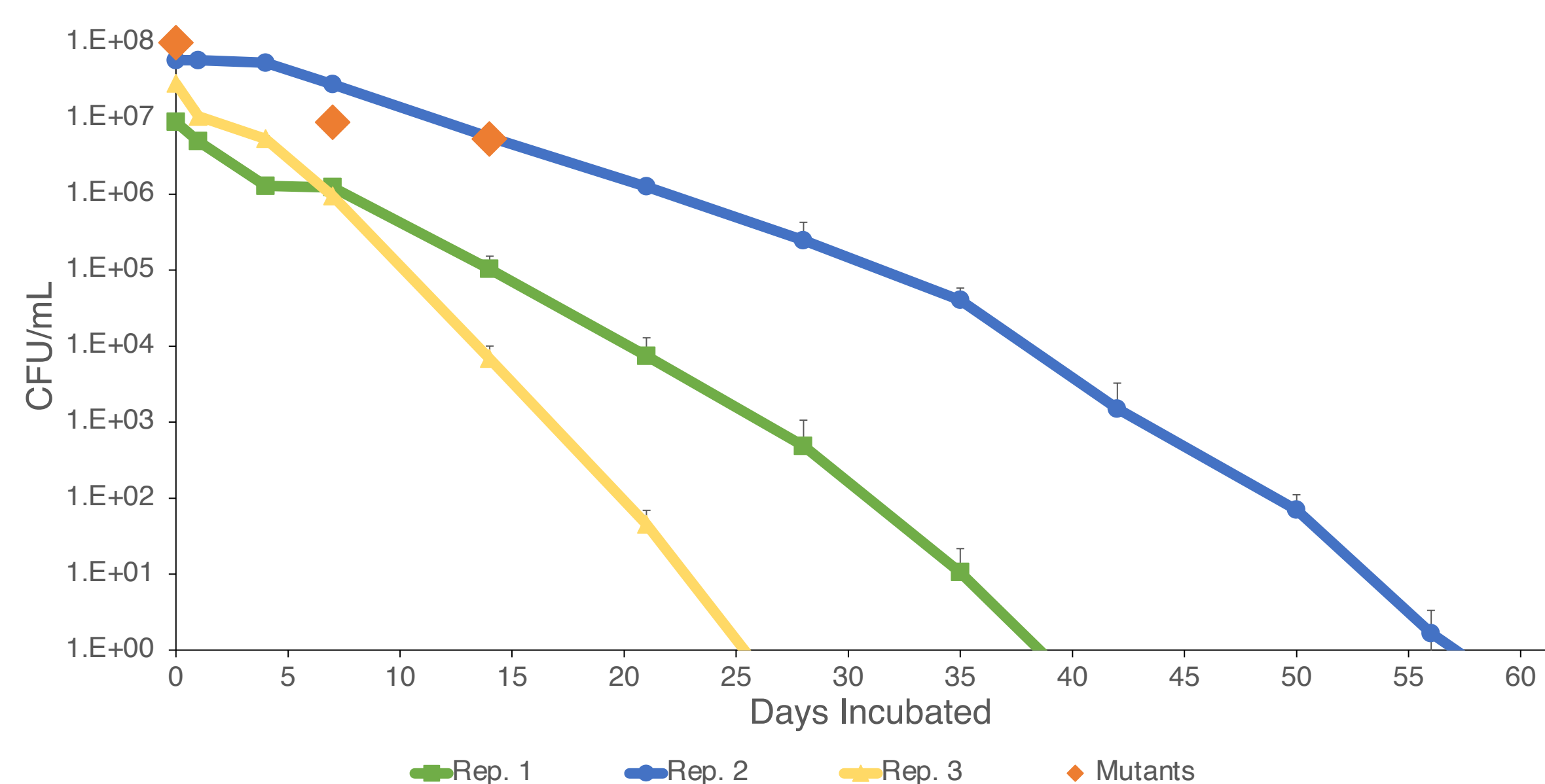
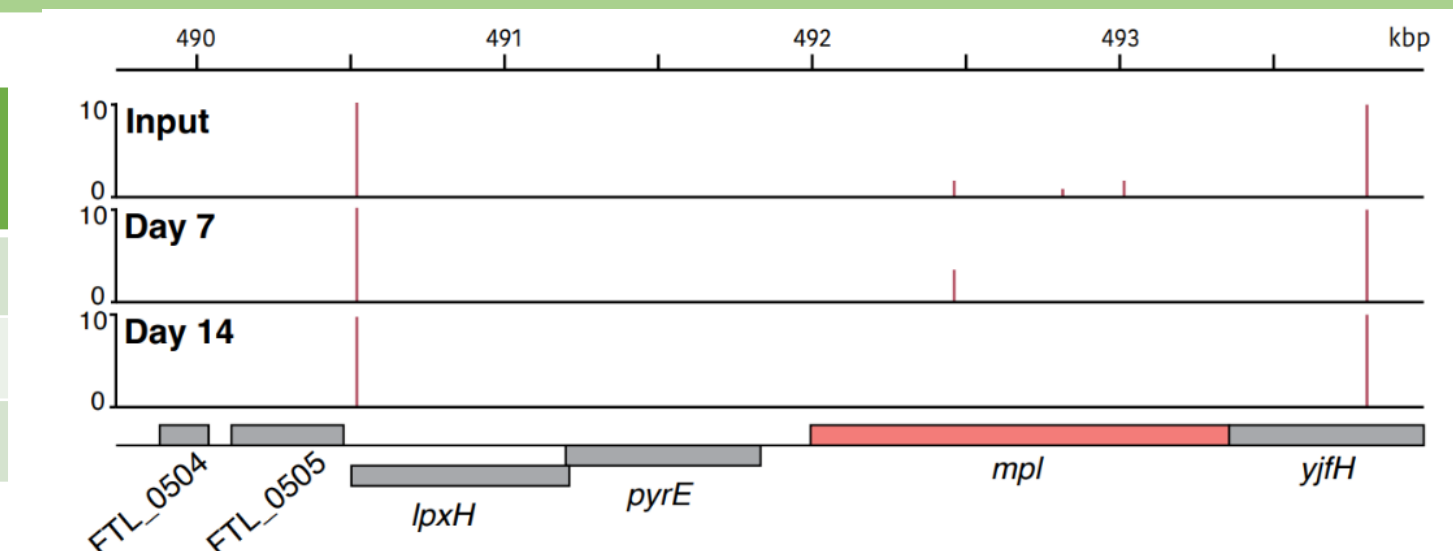


Figure 6: Comparison of *F. tularensis* survival in river water at 4°C across experiments.

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.

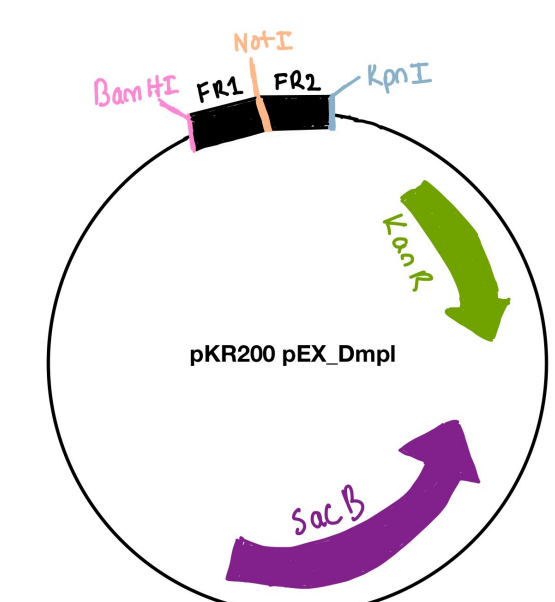
6 Transposon Insertion Sequencing Data

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



7 Ongoing Studies

- Clone a plasmid to modify the *F. tularensis* genome
- Create a strain of *F. tularensis* that lacks the *mpl* gene
- Determine the relative survival of different mutants in freshwater, potentially validating the importance of *mpl* to the survival of *F. tularensis* in a key environmental condition



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 Mpl, murein peptide (UDP-*N*-acetylmuramate:L-alanyl-γ-D-glutamyl-meso-diaminopimelate) ligase
 - Important for cell wall synthesis

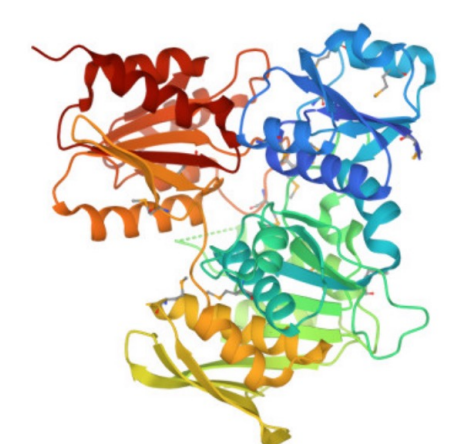


Figure 6: Crystal Structure of Mpl from *Psychrobacter arcticus*²

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