



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

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1 - Introduction

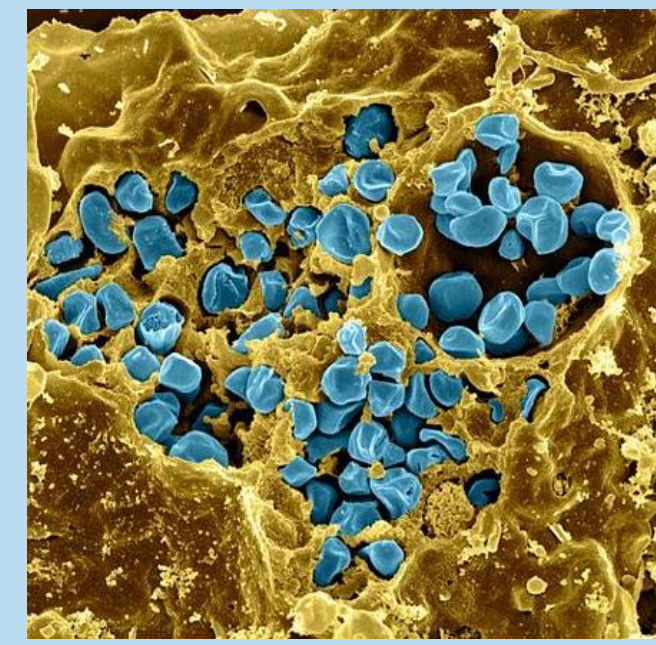
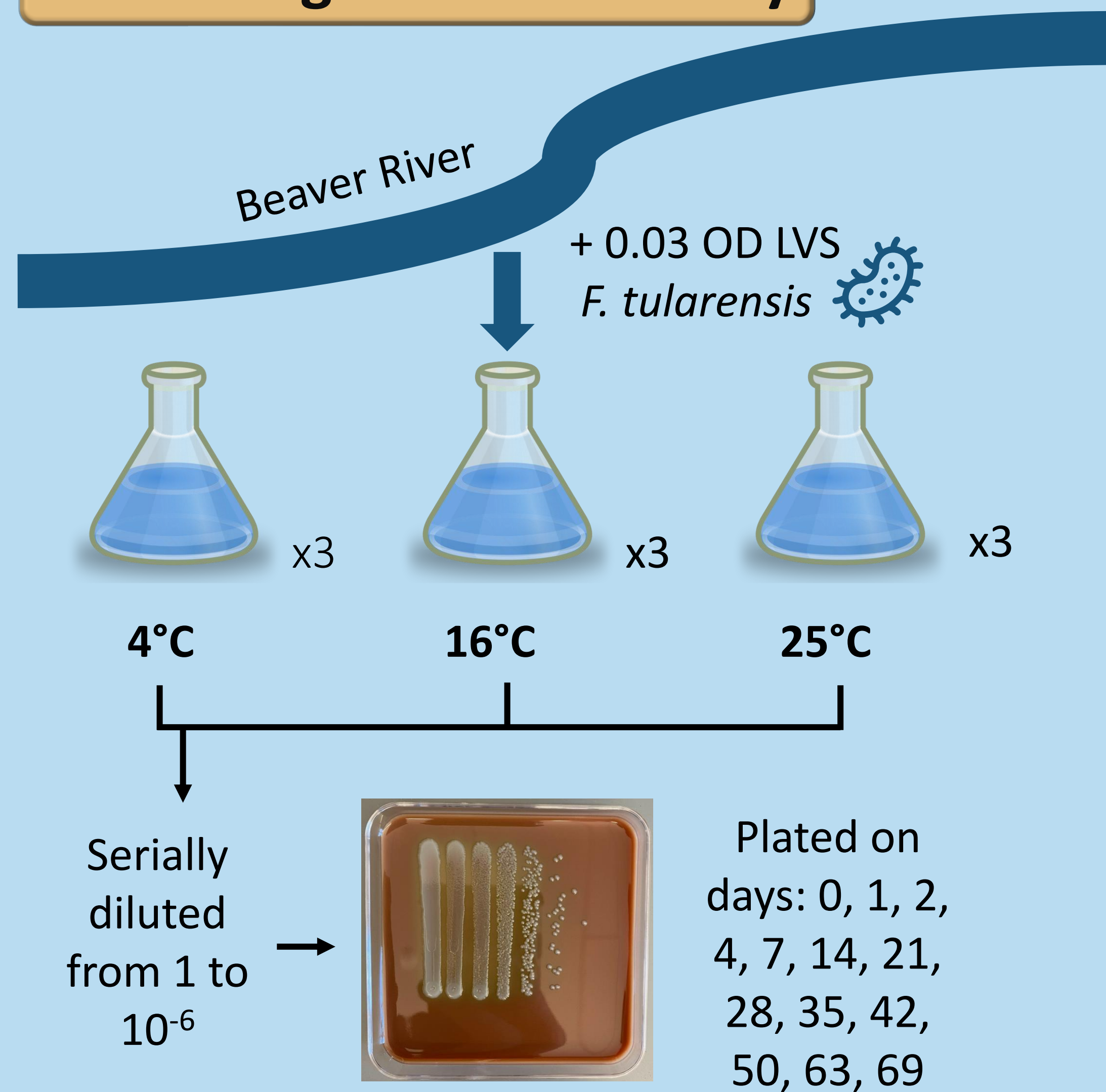


Figure 1: *F. tularensis* in macrophage.¹

- *Francisella tularensis* is a Gram-negative pathogenic bacterium
- Causative agent of a potentially deadly disease tularemia

- *F. tularensis* can survive in freshwater for long periods of time and subsequently infect animals and humans³
- Lower temperatures (above freezing) have been shown to allow for the longest survival of *F. tularensis* in sterile freshwater⁴
- A genes has been found that is important for survival of *F. tularensis* during the transition between the host and aquatic environment⁵

2 – Plating for Cell Viability



3 - Results

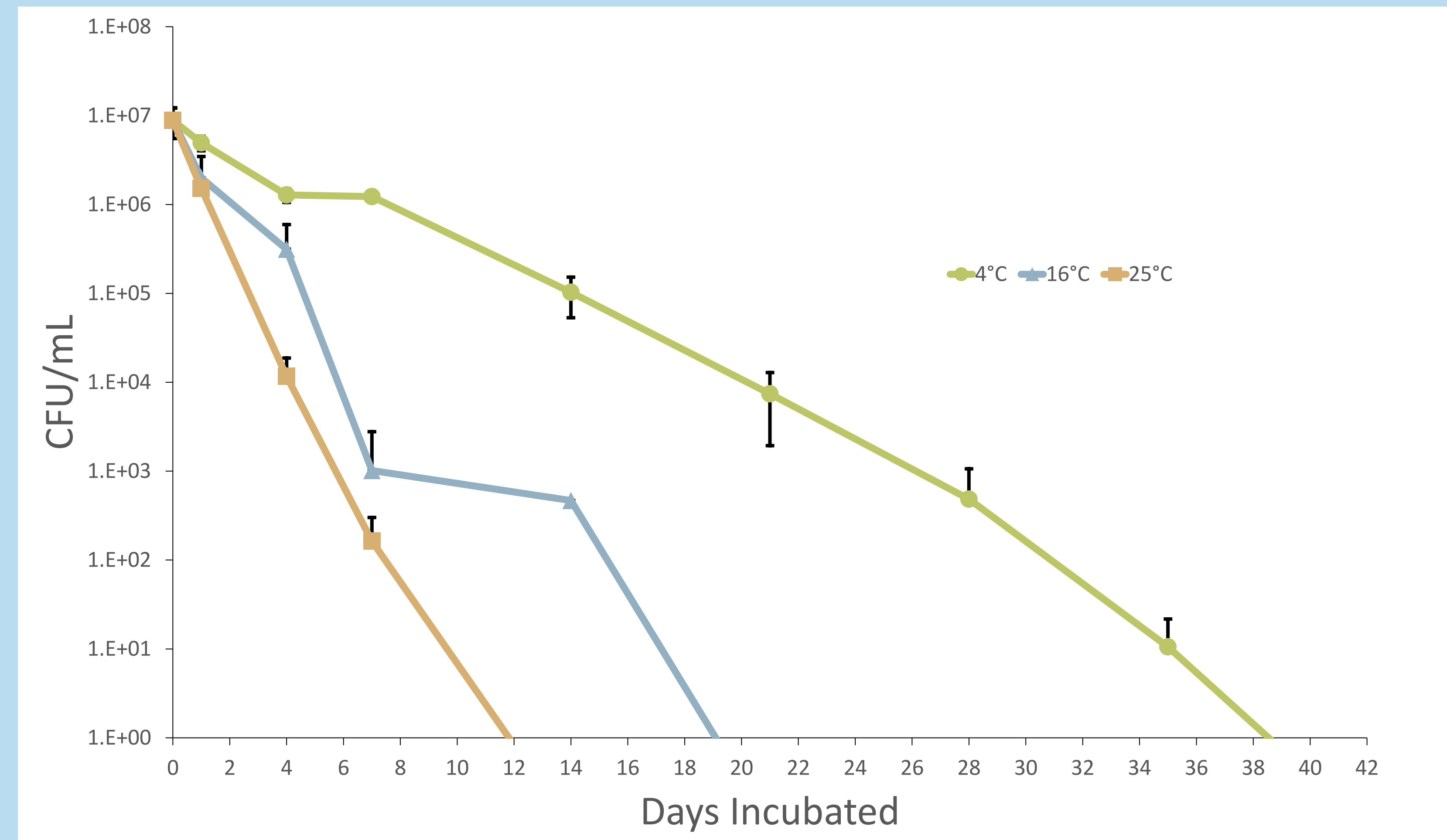


Figure 2: Survival of *F. tularensis* in river water at various temperatures: Replicate 1.

Average colony forming units (CFU) per mL recovered at indicated time points. The longest surviving viable cells were found at 4 °C.

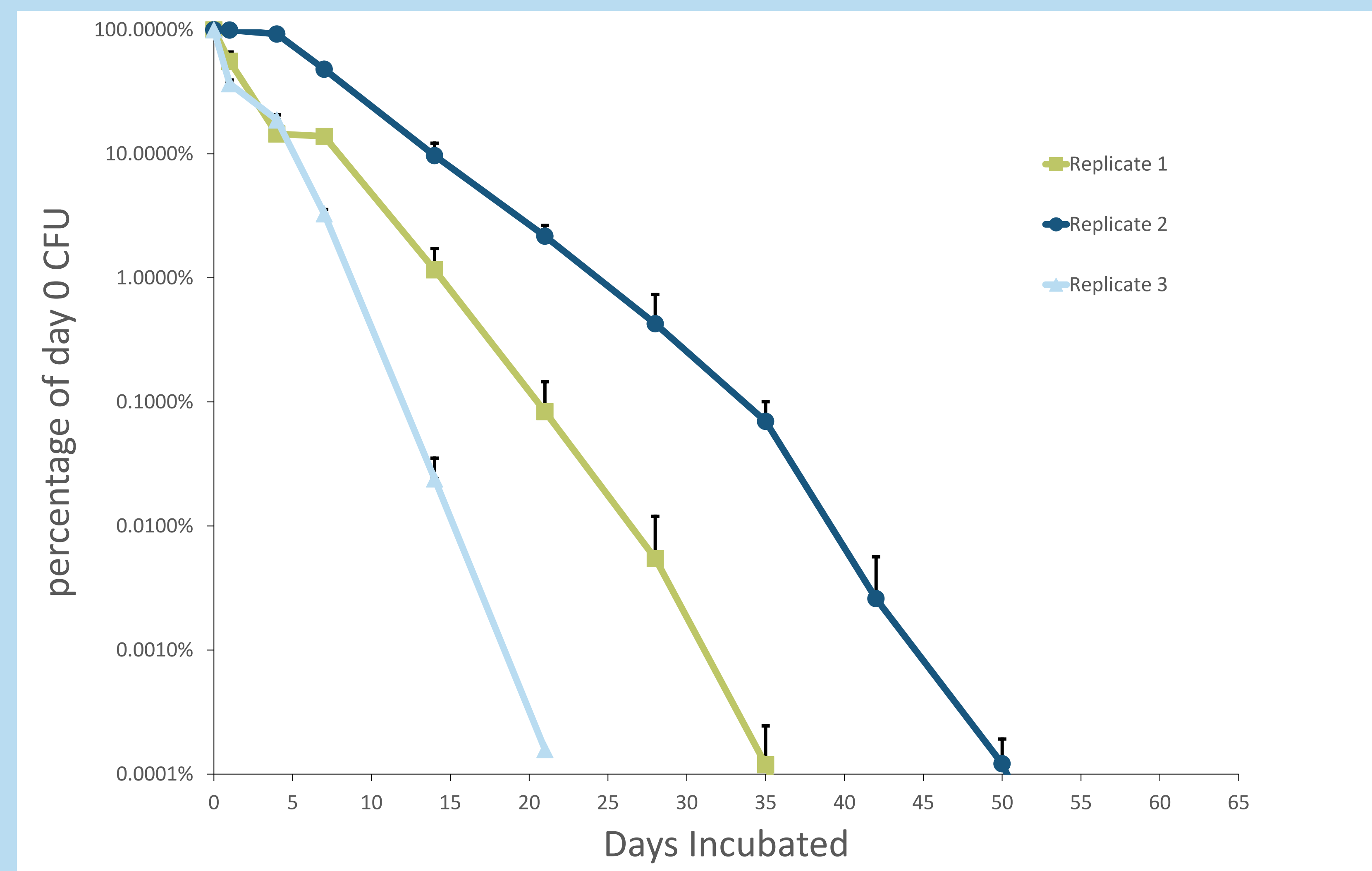


Figure 3: Comparison of *F. tularensis* survival in river water at 4°C across experiments.

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.

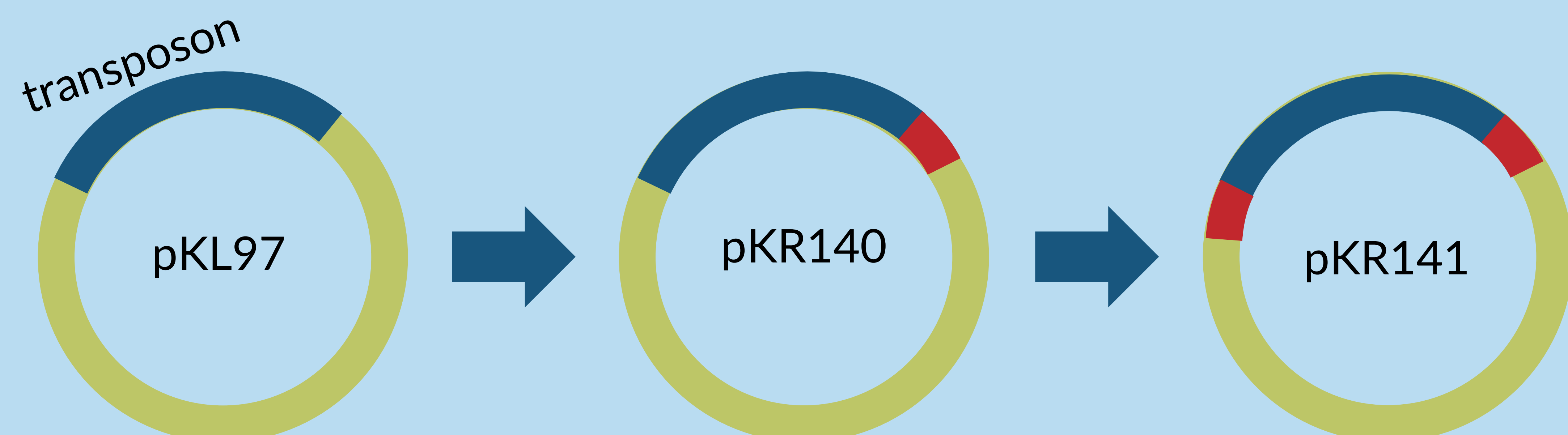
5 - Discussion

- **Importance:** *F. tularensis* can be transmitted when surviving in freshwater conditions to humans.
- **Cells remained viable from 21 - 56 days at 4 °C.**
- Starting inoculum may impact the length of viability.
- Final results for replicate 3 are to be determined.
- Successfully created a new plasmid for the IN-Seq method
- Expect to make mutant library with 150,400 mutants using 8 electroporations
- Tularemia is endemic in central and northern Norway.⁴ There, river water is around 7.7 °C – 20.3 °C from the months of August and September, and *F. tularensis* can be found in these aquatic reservoirs.⁶ The water is warmest during these two months, and the remainder of the year the temperature is under 7.7 °C. This evidence aligns well with the finding that the condition of 4°C provided the longest viable cells.

6 - Future Directions

- Creation of mutant library for Tn-Seq
- Using Tn-Seq to investigate the gene(s) responsible for this effect of the bacteria's ability to survive at 4°C.

4 - New Transposon Insertion Sequencing (Tn-Seq) Vector



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

Why make a new plasmid?

Around 2011, with the development of the method for Tn-Seq we will use (IN-Seq)⁵ the base pairs for the adapters changed, so the new plasmid updated these sequences. (shown in red)

Initial transformation efficiency results: 1.88 x 10⁴

Literature Cited

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