

# Genetically Transformed *Legionella* for Application in Engineering Process Validation in the Built Environment

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STATUS: New X Continuation

## Background

- *Legionella* is the single largest cause of waterborne diseases outbreaks in the U.S. Its survival and transport in engineered water systems is not well understood. The challenges in the detection of *Legionella* is an impediment in it's thorough study in the built environment. In particular, suppression of slow growing *Legionella* colonies by fast growing commensal bacterial population is the major challenge. In addition, distinguishing *Legionella* colonies from some other environmental bacteria can be difficult. There is a need for innovation in studying the fate and transport of *Legionella* in engineering process validation in water distribution systems. Previous efforts have resulted in a mutant that is not realistically stable in environment.

## Objectives

- To engineer an easy to detect *Legionella* mutant that is stable in water. The proposed mutant can be used to study the fate, transport and survival of *Legionella* in engineered water systems.

## Approach

- Transforming *Legionella* by inserting the target gene in *Legionella* chromosomal DNA. The desired gene construct will consist of molecular system for inducing gene expression based on cumate. The proposed work will be performed using a commercially available molecular tools.

## Key Deliverables

- A genetically modified *Legionella* that is stable and easy to detect in presence other heterotrophic bacterial population. The proposed mutant *Legionella* will have applications in the validation of engineering processes such disinfection and transport studies.

## Key Findings

- Genetic transformation of *Legionella* was successfully achieved.
- The transformed *Legionella* has a higher growth rate than wild type.
- Genetic modification resulted in reducing growth time from 5 to 2 days.
- The mutant has less stringent nutrient requirement & can grow in TSB
  - L-cysteine is not required
  - Iron salt is not required
  - Antioxidant is not required
  - Less reliant on antibiotics
- The complex phenotypes in transformed *Legionella* are believed to be because of CymR gene used as a repressor in cumate-inducible systems.
- Mutant is not stable in environment.

## Budget Requested

\$10,000

Project Duration - January – Sept 2019