Aims

1. Perform genomic DNA sequencing of six *Francisella tularensis* subsp. *tularensis* Schu S4 isolates that are currently used in the US-government funded research, vaccine and drug development. Perform six genomes sequencing at 100 % coverage.

2. Define regions of difference (RDs or SNPs) by comparing genomes of six isolates to a published *F. tularensis* Schu S4 genomic sequence (GenBank AJ749949.2)

3. Submit complete genomic sequences of six Schu S4 isolates to GenBank.

Background and Rationale

Introduction

*Francisella tularensis* is a Gram-negative bacterium and a causative agent of zoonotic disease tularemia. In nature, *F. tularensis* is found in rabbits, squirrels, and a wide variety of mammals. It occasionally infects humans. Routes of entry into humans include bites by infected blood-feeding ticks, flies and mosquitoes, direct contact with infected animals especially when breaks in the skin are present, ingestion of undercooked meat from infected animals, and inhalation of contaminated water, dust and hay. Human cases of tularemia have been classified in six classic forms: ulceroglandular when skin ulceration and inflamed lymph nodes are present, glandular when inflamed lymph nodes exist without obvious skin ulceration, oculoglandular when eye involvement is present, pharyngeal, typhoidal when no other route is obvious, and pneumonic which includes the outcomes of infection by inhalation (Penn, 2009).

*F. tularensis* is highly infectious. Most virulent strains can cause disease in humans after inhalation of as few as 10 colony forming units (cfu) when presented in a form of respirable aerosol (Saslaw et al., 1961). Infections with highly virulent strains are lethal in up to 60% of individuals infected by inhalation if not treated with antibiotics (Hornick, 1998). *F. tularensis* has been weaponized for potential use as biothreat agent by several countries (Dennis et al., 2001). For this reason, *F. tularensis* has been designated Category A select agent by the Centers for Disease control (CDC) and NIH¹.

The *F. tularensis* species contains four subspecies: subsp. *tularensis*, (Type A) which is predominantly found in North America; subsp. *holarctica*, (Type B) which is found in Europe and Japan; subsp. *novicida* which is found in Thailand, Australia and the United States; and subsp. *mediaasiatica*, which is restricted to Central Asia. Type A is the most virulent of the subspecies, Type B is of intermediate virulence, and other subspecies have relatively low virulence (Penn, 2009; Thomas and Schaffner, 2010).

*F. tularensis* subsp. *tularensis* strain Schu S4 is a highly virulent smooth (S) colony morphology variant (#4) of a Type A strain Schu that was originally isolated from a skin ulcer of a person with tularemia in 1941 (Hesselbrock and Foshay, 1945; Eigelsbach et al., 1951). Schu S4 strain has been used in research of *F. tularensis* pathogenesis and in development of tularemia animal models (Dennis et al., 2001; Lyons and Wu, 2007) some of which have been used to test efficacy of tularemia vaccines and therapeutics.

¹http://www.niaid.nih.gov/topics/BiodefenseRelated/Biodefense/Pages/CatA.aspx
Furthermore, Schu S4 has been used as a parental strain for development of live attenuated tularemia vaccines.

**Rationale and Justification for Sequencing of *F. tularensis* Schu S4 Isolates**

Rationale and justification for sequencing of *F. tularensis* Schu S4 isolates in support of government-funded research and drug development efforts using different Schu S4 isolates are provided below.

- The US government-funded research and drug/vaccine development community is using Schu S4 isolates from different sources.
- NIAID, via BEI, currently distributes Schu S4 isolates which originated from two different sources.
- NIAID and BARDA, agencies of the US Department of Health and Human Services (HHS) are currently working on qualification of primate model of inhalational tularemia with the FDA\(^2\). The qualified model will be used to demonstrate efficacy of broad spectrum drugs and therapeutics. Schu S4 is currently used as a challenge strain in these studies. For this reason, Schu S4 strain characteristics, including its ability to cause disease resulting in the predictable clinical signs and outcome, are a critical component of the qualified tularemia animal model.
- Genome sequencing is the most cost-effective and ethical method for comparison of highly virulent *F. tularensis* isolates. Mice are most commonly used in tularemia research but they are extremely susceptible to all *Francisella* subspecies when infected by aerosol route. Studies in mice cannot show a difference in virulence among Schu S4 isolates. It is not ethical or feasible to perform primate studies to compare virulence of multiple Schu S4 isolates. The number of animals that would need to be used to ascertain the confidence in the results of virulence studies and the high cost of primate research further support use of less costly genetic analysis tools for comparison of Schu S4 isolates.

**Proposed Data Use and Sharing Plan**

- Submit genomic sequences of six Schu S4 isolates to GenBank alongside a brief description of their source.
- Store all genetically unique Schu S4 isolates in BEI repository and make them available to research community.
- Submit genomic sequences of the Schu S4 isolates currently used in NIAID primate tularemia model development studies to the FDA in support of primate tularemia model qualification.
- Encourage research community collaboration on annotation and comparison of Schu S4 isolates genomes, and results publication in a scientific journal.
- Harmonize use of Schu S4 isolates in drug and vaccines research and development across US government agencies.
- Discuss potential benefits and risk of using Schu S4 isolates that differ in their genomic sequence with tularemia research community.

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F. tularensis Schu S4 isolates for genome sequencing

List of Schu S4 isolates submitted for sequencing is provided in Table 1.

**Table 1. Schu S4 Isolates for Genome Sequencing and Their Source**

<table>
<thead>
<tr>
<th>Schu S4 Isolate Source and Lot Number</th>
<th>Comment</th>
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<tr>
<td>BEI, NR-28534</td>
<td>Master cell bank, manufactured in 2005 at Midwest Research Institute (MRI) for Dynport Vaccine Company (DVC). Isolate originated at USAMRIID and was stored at Salk Institute prior to manufacture at MRI.</td>
</tr>
<tr>
<td>BEI, NR-10492</td>
<td>Submaster cell bank, manufactured in 2005 at MRI for DVC. Related to BEI, NR-28534.</td>
</tr>
<tr>
<td>BEI, NR-643</td>
<td>Submaster cell bank of a master cell bank deposited at BEI in 2004 by CDC. NR-643 was manufactured and vialled at BEI in 2005.</td>
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<tr>
<td>Ohio State University (OSU), Columbus, OH</td>
<td>Originally from Centers for Disease Control (CDC). May be related to BEI NR-643.</td>
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<tr>
<td>University of Umeå, Umeå, Sweden, FSC237</td>
<td>This isolate genome has been sequenced and published (GenBank Accession number AJ749949.2, Larsson P et al. Nat Genet. 2005;37(2):153-159).</td>
</tr>
<tr>
<td>Albany Medical College, Albany, NY</td>
<td>FT4 (strain #5) obtained from USAMRIID which was derived from the Makesson Strain after several passages through mice.</td>
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**References**


Genome Sequencing of *Francisella tularensis* subsp. *tularensis* Schu S4 Isolates
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