

## AUTHENTICATION OF KEY BIOLOGICAL RESOURCES

### Bacterial strains

Storage: All bacterial cell strains are maintained in a master laboratory strain collection as frozen stocks with cryo-preserved in a -80°C freezer for long-term storage. Cryotubes are clearly labeled and a master spreadsheet with information about genotypes, phenotypes, strain generation, and strain validation is maintained and kept up-to-date. Bacterial strains used in experiments are freshly propagated from frozen stocks. Maintaining bacterial strains through any other methods is prohibited to prevent genetic drift. Lab members make their own frozen stocks for frequently used strains.

Strain validation: *F. tularensis* subsp. *holarctica* LVS (the attenuated live vaccine strain which can be used at BSL2) was obtained from Dr. Simon Dove and has been validated by whole-genome re-sequencing. Any strains obtained from other laboratories will be validated by whole-genome resequencing. All modifications are confirmed by PCR and subsequent Sanger sequencing at the Rhode Island INBRE Molecular Informatics Core. We will additionally validate newly-generated mutant strains with significant growth defects by whole-genome resequencing (e.g., using services provided by Seqcenter or SeqCoast Genomics).

### Plasmids

Storage: Original DNA stocks of each plasmid generated are stored in a master laboratory plasmid collection at -20°C. Stocks are clearly labeled and a master spreadsheet with plasmid data (how it was generated, antibiotic resistance genes, location of validation data) is maintained and kept up-to-date. Lab members have their own stocks of commonly used plasmids.

Plasmid validation: Standard plasmid backbones are re-sequenced and validated using long-read sequencing technology (e.g., using services provided by Plasmidsaurus). All modifications are confirmed by PCR and subsequent Sanger sequencing at the Rhode Island INBRE Molecular Informatics Core. If plasmids appear to be toxic to the *E. coli* host strain used for cloning and propagation, we will validate isolated plasmids using long-read sequencing technology (e.g., using services provided by Plasmidsaurus).

### Antibodies

All antibodies will be purchased from reputable commercial sources or obtained from public sources (e.g., BEI Resources) and these sources will be clearly listed in communications from the laboratory such as publications.

### Chemicals

All chemicals will be purchased from reputable commercial sources and these sources will be clearly listed in communications from the laboratory such as publications.