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**Identification of *Francisella* loci that impact expression of *ripA***T. Kijek<sup>1</sup>, J. Fuller<sup>1</sup>, B. Mortensen<sup>1</sup>, S. Taft-Benz<sup>1</sup>, T. Kawula<sup>1</sup><sup>1</sup>University of North Carolina, Microbiology and Immunology, Chapel Hill, United States

**Aim:** The goal of this study was to identify loci in *F. tularensis* that impact expression of RipA, a known virulence determinant in LVS.

**Methods:** We created a *ripA-lacZ* reporter and integrated it onto the chromosome of *Ft* LVS. This reporter strain, JF127, was subjected to transposon mutagenesis. Transformed bacteria were selected on media containing X-gal for blue/white screening. Mutants exhibiting an altered colony phenotype compared to JF127 were subjected to further study. Expression of *ripA* in these mutants was compared to the parent strain using a standard  $\beta$ -galactosidase assay. Transposon insertion sites were identified by amplifying adjacent genomic sequence using semi-degenerate PCR. Mutants that exhibited a decrease in *ripA* expression were evaluated for their ability to replicate within J774.1 and TC-1 cell lines.

**Results:** 77 of roughly 7000 total mutants were selected for further analysis based on their blue/white phenotype. Of these, 22 harbored transposon insertions in *lacZ*. Transposon insertions into FTL0329, FTL1306, FTL1929, FTL0895 (*hupB*) and FTL1810 (*nusA*) each resulted in a  $\geq 2$ -fold decrease in *ripA* expression. Transposon insertions into FTL0439 (hypothetical), FTL0957 (*blaA*), and FTL0707 all resulted in a  $\geq 2$ -fold increase in expression of *ripA*. Interestingly, we obtained 3 total insertions in FTL0439 and 2 insertions in FTL0073. Deletion of FTL0073 in *Ft* LVS resulted in a mutant strain with reduced intracellular growth in both J774.1 and TC-1 cells. Additionally, this mutant also demonstrated reduced virulence in a mouse model of inhalation tularemia.

**Conclusions:** The use of a *ripA-lacZ* reporter allowed us to rapidly screen a transposon library for loci that impacted expression of *ripA*.  $\beta$ -galactosidase activity of mutants vs the parent strain were in agreement the observed colony phenotype. Additionally, one of the mutants, FTL0073, was found to be defective for growth in both *in vitro* and *in vivo* models of *F. tularensis* infection.