KRISTEN HAGGERTY, STUART CANTLAY, and JOSEPH HORZEMPA, Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV 26074. *Francisella tularensis* expressing TC3D Leader Peptide and Promotor from tdtomato Enhances Fluorescence

Francisella tularensis is a gram-negative, intracellular pathogen capable of causing infection at a low dose of <10 CFU. For this reason, this bacterium has been classified as a Category A Bioterrorism Agent by the Center for Disease Control (CDC). Fluorescence microscopy is a valuable tool that investigators can utilize to study the many interactions between *F. tularensis* and its host cells. However, in our hands, *F. tularensis* is only weakly fluorescent when expressing certain fluorescent proteins such as emGFP and BFP using previously described molecular tools. On the other hand, our laboratory previously described robust expression of tdTomato while under the control of the *Francisella* glucose-repressible promoter (FGRp; this plasmid construct was referred to as pTC3D). However, *F. tularensis* bacteria were unable to produce robust expression of tdTomato or other fluorescent proteins, such as emGFP, while these genes were under the control of the *groE* promoter (which is much more robust). Therefore, we analyzed the DNA upstream of *tdtomato* in pTC3D. Here, we identified an in-frame N-terminal leader peptide that is likely translated with tdTomato potentially producing a stable fusion. The previously identified FGRp sequence alone could not produce robust expression of fluorescent proteins. Moreover, a frame-shift disrupting the coding sequence of the leader peptide resulted in poor fluorescent protein expression. Inclusion of the leader peptide in-frame controlling expression of Emerald GFP, *emGFP*, under the control of the FGRp, however, resulted in robust green fluorescence suggesting that this domain can be used to stabilize fluorescent proteins in *F. tularensis.* Application of this new biotechnology will expand our ability to investigate *F. tularensis* through fluorescence microscopy.