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Results from screening the *F. novicida* transposon two-allele mutant libraryX.-H. Lai¹, L. Gallagher², C. Manoil², F. Heffron¹¹Oregon Health Science University, Portland, United States, ²University of Washington, Seattle, United States

The availability of the *F. novicida* Tn 2-allele mutant library provides opportunity to study genes of interest in parallel at a genomic level for the first time. We aimed to study the cytopathogenicity of each mutant by screening this library in several steps. Firstly, J774 macrophage-like cells were seeded in 24-well plates and infected with OD₆₀₀-unnormalized overnight cultures. Changes of J774 cell morphology were followed since infection. Mutants were put into different categories such as faster killing, slower killing and normal killing groups per their killing kinetics. To verify some of those observations, individual mutant culture was OD₆₀₀ normalized and used to infect J774 cells for morphology observation, CFU counting, and LDH measurement in 6-, 24-, 96-well plates respectively. To test in vivo significance, mutants were selected to infect Balb/C mice and animal survival was monitored.

In summary, some mutants have differential in vitro growth defect, and some mutants are faster or slower in killing J774 cells than U112. In vitro results of host cell cytopathogenicity and bacterial intracellular growth ability are the two major criteria to judge the potential of a mutant for attenuation. Utilizing host cell morphology change as an initial screening standard has several advantages over the use of intracellular bacterial growth in that it makes possible examination of one experimental sample at multiple time points, thereby reducing the chance of overlooking mutants that could be normal in intracellular growth. The strategy could also be useful for screening libraries of human virulent *F. tularensis* strains.