The causative agent of tuberculosis (TB), (Mtb), infects a third of the world's population. After entering the lungs, Mtb is phagocytosed by alveolar macrophages. Mtb can persist inside the phagosomes of these macrophages despite being a hostile environment due to the presence of reactive oxygen and nitrogen species (ROS and RNS) produced by NADPH oxidase and inducible nitric oxide synthase, respectively. ROS and RNS can react with bacterial lipids, carbohydrates, proteins and nucleic acids causing free radical stress. Mtb may also be exposed to antibiotic stress such as rifampicin, a first-line drug used for TB treatment. Despite these various stressors, Mtb can persist for long-periods of time within the patient. The aim of this summer project was to determine the genetic basis of how Mtb can persist for long periods of time inside the patient. This was done by measuring the survivability of a transposon mutant library (Table 1) of

Preliminary experiments were conducted before subjecting the transposon mutants to the various stressors to determine the concentrations that should be used for treatment. This involved exposing WT Msm to various concentrations of stressors. These concentrations were based of similar research using these stressors in mycobacteria. The ideal concentration would be one that resulted in killing without being fully bactericidal to allow for differentiation of susceptibilities between the transposon mutants. Survivability was measured by calculating the number of colony-forming units (CFUs) per ml of culture after exposure.

The concentrations of $H_2O_2$ tested were 0.2, 2, and 20mM whereas DETA/NO concentrations tested were 0.05, 0.5, and 5mM. Aliquots of WT liquid culture at OD 0.3 grown in the same liquid medium described above were exposed to the various concentrations of hydrogen peroxide and DETA/NO listed above for 3h at 37C. To count any CFUs, Miles and Misra spotting was performed which involved performing 10-fold serial dilutions of the aliquots after the 3h exposure and pipetting 20µl of each in triplicate on LEMCO agar. CFUs were counted, allowing the concentration of bacteria (CFU/ml) to be calculated. The highest concentrations of both H