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Recommended Citation
Heinz, Drake and Hindmarsh, Patrick L., "Optimization of GFP Biosensor" (2019). *ANS Research Symposium*. 37.
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Optimization of GFP Biosensor

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Advances in medical therapies has advanced significantly in the last 25 years with the exception of those targeting microorganisms. The developments in antibiotics in the first half of the 20th century was very robust, but selective pressures have enriched bacterial populations for resistance. In order for the scientific community to maintain the ability to keep pathogens in control depends on the development of new and novel antibiotics. For my project I am developing a reactive oxygen species (ROS) sensitive green fluorescent protein (GFP) that can be expressed in E. coli and Candida albicans. My system will be able to identify novel antibiotic and antifungal compounds. The question I would like to answer is whether I can develop a ROS-sensitive GFP that has increased specificity to ROS by eliminating cysteines unrelated to our experimental design. This biosensor utilizes changes to two proximal amino acids to cysteines (S147C and Q204C) made using Quik-change mutagenesis. Mass spectroscopy and sequencing will check if the mutation occurred correctly. In the presence of ROS, these two cysteines form a disulfide bridge distorting the excitation of GFP. We can detect this using the clear bottom plates and the plate reader to see if there is a gain or loss of fluorescence. The percent change in fluorescence can show the presence of ROS. In addition to these mutations, there are two natural cysteines at positions 48 and 70 in GFP that under an oxidizing environment will form disulfide bridges with other proteins, complicating the analysis of our biosensor. Several groups have altered these cysteines to different amino acids in order to eliminate the oligomerization of GFP. Unfortunately, some of the amino acid substitutions have shown negative affects on the fluorescence (1). The goal of this project is to replace these two natural cysteines while maintaining the fluorescence of GFP. Alanine and serine substitutions have been shown to decrease fluorescence, while hydrophobic substitutions, valine and isoleucine, have been suggested to maintain fluorescence (2). In addition to valine and isoleucine, we will introduce leucine, methionine and phenylalanine. I will use combinations of these amino acids to mutate positions 48 and 70 and test these using a fluorescence plate reader to identify mutants that maintain wild type fluorescence followed by testing under oxidizing conditions using western blot analysis to detect oligomerization. With a functional ROS-sensitive GFP biosensor, novel antibiotics can be identified that can be used properly without increasing ROS. Furthermore, this biosensor can be widely used at discovering other molecules in the cells that also increase ROS.