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Testing Green Fluorescent Protein at Different Temperatures and its Relation to ROS Production

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Testing Green Fluorescent Protein at Different Temperatures and its Relation to ROS Production Lauren Cox1 Patrick L. Hindmarsh2 1School of Biological Sciences, Louisiana Tech University 2Associate Professor, School of Biological Sciences, Louisiana Tech University Microorganisms or multicellular organisms respond to stress in different ways depending on the type of stress, such as: temperature, infection, environmental factors, and cancer. These different stressors can all contribute to production of reactive oxygen species (ROS). Temperature is a critical stressor that microorganisms are constantly exposed to and must respond. Our lab has developed a ROS sensitive yeast enhanced green fluorescent protein (royGFP). Two substitutions in GFP result in royGFP; introduction of cystines at positions 147 and 204, (S147C and Q204C). These mutations make it possible to measure the production of ROS by the change in fluorescent excitation. In the presence of ROS agents we have observed changes in excitation confirming the functionality of our royGFP construct. In the presence of ROS a disulfide bond is formed between the introduced cystines and this distorts the structure of GFP changing the excitation wavelength from 485 nm in the absence of ROS to 400 nm in its presence. For my experiments I have used a fluorescent plate reader that can maintain different temperatures E. coli cells expressing royGFP and our wild type yEGFP. The temperatures range from physiological temperatures for E. coli (37 to 42oC) and beyond at 45oC.