

## 2007 Ohio Student Research Forum

Wright State University  
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## RESEARCH ABSTRACT FORM

**TITLE:** Generation of a host strain for improved gene targeting in the model genetic system *Aspergillus nidulans* containing a reversible disruption of Ku80

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*Aspergillus nidulans* is widely used as a model genetic system to study basic principles of biology. Previous research has shown that deletion of the Ku70 and Ku80 genes in *A. nidulans* greatly increases transformation efficiency for gene tagging or gene deletion studies (Nayak et al. 2005). However, for further studies these deletions must be replaced with the wild type genes. This project therefore focused on generating a disruption of Ku80 in a strain of *A. nidulans* that can be used for improved gene targeting experiments before reversing the disruption back to wild type Ku80. We created a disruption construct of Ku80 from the *A. nidulans* genome using PCR amplification, cloned it into a host plasmid, and transformed the plasmid into *A. nidulans* to disrupt Ku80. Our transformations were successful, and most likely contain the Ku80 disruption. To regenerate wild type Ku80, we have selected for recombination using 5-Fluoroorotic acid (FOA), which causes a lethal reaction with the nutritional marker present on the inserted plasmid. In order to survive in the presence of FOA, the *A. nidulans* must evict the plasmid, which repairs the disruption and generates the wild type Ku80 gene again.

As part of this project, we were also interested in the localization of Ku80 in the cell. To explore this, we tagged Ku80 with green fluorescent protein (GFP), which causes the protein to glow under certain light conditions. Preliminary microscopic analysis shows that Ku80 appears to localize in the nucleus during interphase, is released from nuclei during mitosis then returns back to nuclei when mitosis is complete.