

Title: Instituting Phosphonull Mutations of the Dsn1 Gene in the Kinetochores of *Saccharomyces cerevisiae*

Kinetochores are multi-protein complexes that play a vital role in chromosome segregation during cell division. Many kinetochore proteins have phosphorylation sites that regulate processes such as microtubule attachment. However, the functions of kinetochore phosphorylation are not completely understood. To investigate this problem, we are using the species, *Saccharomyces cerevisiae*, to induce mutations in the *DSN1* gene to see if its sites of phosphorylation are important for function. Dsn1p is part of the MIND protein subcomplex within the kinetochore that contributes to microtubule attachment and produces pulling forces resulting from depolymerization. Amino acids T380 and T386 of Dsn1p were identified as phosphorylation sites by the Biggins lab using mass spectrometry analysis. We plan to use the CRISPR-Cas9 system to create mutations changing T380 and T386 codons to instead code for valine, which cannot be phosphorylated. So far, we have successfully cloned our guide RNA sequence into the CRISPR vector. Our work is currently in progress, however, we are now able to transform yeast with our CRISPR vector and repair template. We hope in the future that our transformed yeast would be able to display phenotypic differences for further experimentation.