Constructing a CRISPR-Cas9 Vector for Mutagenesis of a Potential Phosphorylation Site of the Dsn1 Kinetochore Protein

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Kinetochores are proteins that bind to spindle microtubules and attach to chromosomes, thereby promoting proper segregation during meiosis and mitosis. The *Saccharomyces cerevisiae* Dsn1 protein is a crucial component of the kinetochore complex of the Mis12 kinetochore complex and helps mediate chromosome segregation by linking spindle microtubules to the chromosomes. Mps1 kinase is a component that helps phosphorylate kinetochore proteins in regions with acidic R-groups. Threonine 491 (T491) is a potential Mps1 target due to its neighboring two acidic amino acids. It is also in a highly conserved region of the protein in relation to other species. To investigate the potential role of phosphorylation at this site, we plan to introduce a phosphomimetic mutation through the CRISPR-Cas9 system to change Dsn1-T491 to Dsn1-T491DD (Aspartic Acid). This negatively charged amino acid mimics a phosphorylation state and allows us to investigate potential outcomes of phosphorylation at this specific site. We designed a DNA sequence that codes for a small guide RNA (sgRNA) that would direct the CRISPR-Cas9 system to cut in the *Dsn1* gene near the target mutation site. Then we inserted that DNA into a vector that encodes the Cas9 enzyme that directs CRISPR mutagenesis. We confirmed that the cloning was successful through Sanger Sequencing, and now our vector is ready to be introduced into *Saccharomyces* cerevisiae for mutagenesis of the *Dsn1* gene. The next step of this project is to examine phenotypes associated with changing Dsn1-T491 to Dsn1-T491DD.