# Designing a CRISPR-Cas9 system for site specific mutagenesis of the Dsn1 protein in S. *cerevisiae*

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During normal cell division, chromosomal segregation is a critical step in ensuring daughter cells receive the correct number of chromosomes. A step in the chromosomal segregation process involves kinetochores, large protein complexes located on centromeres where spindle microtubules attach. Before a cell can proceed through metaphase and into anaphase, there must be correct attachment between both the kinetochore and microtubules, as well as adequate tension, through a process known as the spindle assembly checkpoint (SAC). Dsn1, a protein located within the outer kinetochore of the model organism *Saccharomyces cerevisiae*, plays an important function in the attachment of spindle microtubules and the assembly of kinetochore sub complexes involved in the SAC. Mps1 kinase, a component of the SAC, has been shown to phosphorylate specific sites on Dsn1, which are known to impact chromosome separation. Mps1 kinase is prone to phosphorylating specific sites where amino acid threonine is flanked by two acidic amino acids. We have identified site 491 of the Dsn1 protein as a potential Mps1 kinase target sequence due to this context, and known conservation of this amino acid sequence throughout closely related species. To measure whether this phosphorylation site is important to the function of Dsn1, we are designing a CRISPR-Cas 9 system to mutate codon 491 and test for functionality of that mutation. I designed a small-guide RNA (sgRNA) encoding sequence that was cloned into a CRISPR vector. I also created a homology directed repair DNA template (HDR) that was directed to target site 491 and cause a phospho-null missense mutation. I combined these designated templates into a transformation involving S. *cerevisiae* cells. Future continuation of this project will determine if the mutation at site 491 was correctly cloned into S. *cerevisiae*.