

Using CRISPR to mutagenize phosphorylation sites of the *STU1* and *DSN1* genes in *Saccharomyces cerevisiae*

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The kinetochore is a protein complex attached to the centromere that is vital for chromosome segregation and the spindle assembly checkpoint (SAC). Specifically, the kinetochore is known for its role as a sensor for kinetochore-microtubule (KT-MT) attachment. Accurate KT-MT attachment is crucial during cell division; errors in KT-MT attachment can cause daughter cells to contain an abnormal number of chromosomes, which usually leads to cell death, and in humans is also known to be a characteristic of cancer cell development. Previous research has shown that phosphorylation of a subset of kinetochore proteins at specific sites is key to the activation and deactivation of the SAC and thus is vital to the overall mechanism of chromosome segregation. Our research objective was to better understand if phosphorylation of the *Saccharomyces cerevisiae* kinetochore proteins *Stu1* and *Dsn1* is necessary for their proper function. To accomplish this, we used a CRISPR vector to introduce mutations that mimic phosphorylation by introducing a codon for a negatively charged amino acid at a specific site of the kinetochore encoding gene. We were able to successfully create CRISPR vectors designed to introduce mutations into the *STU1* and *DSN1* genes and transform these CRISPR vectors into *Saccharomyces cerevisiae* cells. We are awaiting confirmation of mutagenesis in *Saccharomyces cerevisiae*. The construction of the CRISPR vectors allows for further analysis of the function of *STU1* and *DSN1* in KT-MT attachment and chromosome segregation, as well as offers a better understanding of the impact of phosphorylation on chromosome segregation.