

Phospho-null mutation to eliminate a phosphorylation site on Stu1, a protein of the kinetochore complex in *Saccharomyces cerevisiae*, using the CRISPR- Cas9 system.

Chromosomal segregation requires coordination between the kinetochore protein complex and mitotic spindles which is essential for accurate genetic division between two daughter cells. The kinetochore is a protein complex that is located at the centromere of the sister chromatids. During mitosis, it is observed that the kinetochore actually “walks” sister chromatids toward the opposite poles of elongating cells with the guide of mitotic spindles. It has been suggested that Stu1, a small protein in the kinetochore complex, helps delay anaphase in budding yeast, *Saccharomyces cerevisiae* until each chromosome is attached to the mitotic spindle. It has also been suggested that Stu1 interacts with the spindles and synchronously moves with the spindles when they elongate. It has been suggested that phosphorylation may regulate Stu1 function, and MELT is a known phosphorylation site in other kinetochore proteins, hence, the removal of a threonine amino acid on a phosphorylation site called the MELT motif on Stu1 might affect the ability of sister chromatids to separate properly which may cause a reduction in yeast viability. MELT is a well-conserved sequence in fungi and a known phosphorylation site in other kinetochore proteins that are homologs to Stu1. Taking advantage of the CRISPR-Cas9 enzyme, we will introduce a phospho-null mutation into the budding yeast *STU1* gene to replace the threonine 719 codon with a valine codon at the MELT sequence. We hypothesize that this mutation will produce a malfunction in Stu1, which could possibly hinder its ability to coordinate spindles and kinetochore attachment and furthermore prevent chromosomal segregation altogether during mitosis. So far, we have successfully cloned plasmids containing the guide RNA and Cas9 enzyme gene. The next step would be to transform yeast with both the plasmids as well as our template DNA that codes for valine at 719 codon in *STU1*, this combination will fully activate the CRISPR Cas 9 genome editing system in yeast.