Mutagenesis of the *Saccharomyces cerevisiae STU1* gene to mimic constant phosphorylation of a MELT motif

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The kinetochore plays a key role during cell division, mediating the process between the mitotic spindles and the centromere. The *STU1* gene codes for a microtubule plus-end-tracking non-motor protein (Stu1) and facilitates the connection between the kinetochores and mitotic spindles. We focused on a MELT motif, which is a highly conserved sequence of amino acids that are phosphorylated and targeted by the Mps1 kinase, which plays a major role in the regulation of segregation and spindle checkpoints, and its impact of phosphorylation at this location is not known. We used a previously constructed CRISPR vector and HDR template to mutagenize the codon within the MELT motif at position 719 in the *STU1* gene of *Saccharomyces cerevisiae.* This mutation would result in a substitution of the amino acid threonine for glutamic acid within the Stu1 protein (*stu1*-T719E). Threonine is a neutrally-charged amino acid and is used in *S. cerevisiae* proteins as a phosphorylation site, but glutamic acid mimics a phosphorylation site with its constant negative charge. We transformed the CRISPR vector and HDR template DNA into *S. cerevisiae* cells and recovered transformants. To verify the success of the mutagenesis, we purified genomic DNA from transformed yeast, amplified the *STU1* gene via PCR, and then sent it for Sanger sequencing. It was found that the CRISPR vector and HDR template were correctly taken up by the yeast cells. The next steps, which include phenotypic tests, will allow us to see if the mutation we made impacts cell division of *S. cerevisiae* cells.