Using CRISPR to Make a Phospho-mimetic Mutation in the MELT domain within the *STU1* Gene in Budding Yeast

The process of mitosis involves the division of cells in eukaryotes, resulting in the production of two daughter cells after the separation of sister chromatids that move towards opposite poles of the cell. This essential process depends on the interaction between chromosomes and spindle microtubules. The kinetochore is a group of proteins connecting the centromeric DNA to the spindle microtubules and plays a crucial role in binding microtubules and facilitating the recruitment of spindle assembly checkpoint (SAC) proteins. Protein phosphorylation is responsible for regulating the interactions between kinetochores and microtubules, and protein-protein interactions are important in maintaining proper spindle attachments and eliminating improper ones during the SAC. The phosphorylation of proteins within the kinetochore is involved in the initiation of the SAC when microtubules are not properly attached, and the identification and characterization of phosphorylation sites can aid in a better mechanistic understanding of the SAC. The *STU1* gene encodes a protein (Stu1p) that plays a role in the polymerization of microtubule spindles, relocalization of microtubules, and binding of kinetochores. Stu1p also helps in separating the spindle poles and preventing spindle collapse during bipolar spindle formation, generating a separating force. Interestingly, Stu1p contains two well conserved MELT motifs that are known areas of phosphorylation in a subset of kinetochore proteins. We intend to induce a phospho-mimetic mutation in a well-conserved MELT motif identified in the *STU1* to see if phosphorylation at this site impacts Stu1p function during cell division. We are currently in the process of developing a CRISPR vector to enable the implementation of these mutations, having created DNA sequences capable of facilitating specific mutations.