Using CRISPR to induce a mutation in MELT-like motif in *S. cerevisiae's* Stu1 protein Alexandra Zamora, Anu Batsuren, and Dr. Jack Vincent

Chromosome segregation is the process all cells must undergo to ensure each cell contains the right number of chromosomes upon division. It is imperative that cells receive the correct number of chromosomes to maintain proper function and prevent aneuploidy. The wrong number of chromosomes or aneuploidy usually leads to cell death, or drastically changes the phenotype of surviving cells. The kinetochore is essential for chromosomal separation, as it is a large protein complex that attaches to a chromosome's centromere at one end and to microtubules at the other end for segregation. Proper attachment of each duplicate DNA molecule to microtubules extending to both poles of the cell, biorientation, is an essential feature of cell divisions. The Mps1 kinase is essential for budding yeast kinetochore biorientation. It phosphorylates various kinetochore proteins, often at sequences called MELT motifs, to execute this role. Stul is also a protein that facilitates biorientation. Although it is not directly a part of the kinetochore, Stu1 plays an essential role in assembling the mitotic spindle in yeast. This protein is a likely target of the Mps1 kinase, as Stu1 has several known conserved MELT motifs and has overlapping roles in chromosome alignment. In this study, we investigate the potential effects of changing the amino acid within one of the Stu1 MELT motifs. We used CRISPR-Cas9 to create two different mutations that mimic constant phosphorylation. We were successful in creating the two mutations, and are currently working on phenotypic analyses to determine the effects of these mutations.