Using MegaWHOP to combine mutations of Ipl1 recognition sites within the *STU2* gene

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Accurate segregation of chromosomes is essential to ensuring the daughter cells are complete with genetic information. Kinetochores are attached to centromeres where the microtubules connect to pull the sister chromatids away from each other. Kinetochores also put the chromosomes in a bioretention which is essential chromosome segregation. These processes are regulated by checkpoints called Spindle Assembly Checkpoints (SAC) to help pause cell division until all the required processes are completed. SAC communicates via the phosphorylation of target proteins. The *STU2* gene is a potential gene to be related to SAC. The Stu2 protein helps detach microtubules from kinetochores when the tension is elevated and reattach when the tension is lessened. Our research aims to understand how point mutations within *STU2* at known target sites for SAC kinase ipl1 potentially impact *STU2* function. We can accomplish this by mutating serine codons at these known target sites in *STU2* and testing the effects of these mutations in brewer's yeast, *Saccharomyces cerevisiae*. If these mutations in these sites impact *STU2* function we expect the yeast that use this version of the *stu2* gene to have lower viability. We will create these mutations using a technique called MegaWHOP mutagenesis and use DNA sequencing methodologies to confirm the presence of the mutation prior to testing in yeast. We were able to complete many successful steps of the MegaWHOP, however, the versions of *stu2* that we have screened in E. Coli do not contain all intended mutations. However, there are other possible analyses of other transformational E. Coli colonies that can still be screened