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

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INTRODUCATION

Background:

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.

Objective:

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.

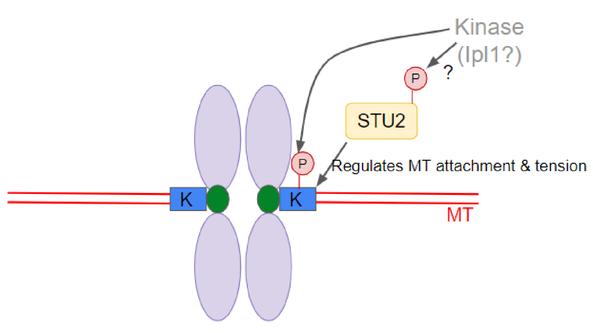

Figure 1: The spindle checkpoint is in charge of chromosomes segregation occurring accurately, as it uses kinases to alter protein function and senses microtubule and kinetochore attachment tension. STU2 regulated microtubule attachment and tension may be regulated by this same kinase as the SAC, as it may be known to phosphorylate other kinetochore proteins.

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  1 MSGEEEVDYTTLPLEERLTYKLWKARLEAYKELNQLFRNSVGDISRDDNIQIYWRDPTLF
 61 AQYITDSNVVAQEQAIVALNSLIDAFASSSLKNAHNITLISTWTPLLVEKGLTSSRATTK
121 TQSMSCILSLCGLDTSITQSVELVIPFFEKKLPKLIAAAANCVYELMAAFGLTNVNVQTF
181 LPELLKHVPQLAGHGDRNVRSQTMNLIVEIYKVTGNNSDLLEEILFKKLKPIQVKDLHKL
241 FAKVGDEPSSSKMLFEWEKRELEKKRSQEEEARKRSILSNDEGEYQIDKDGDTLMGMET
301 DMPPSKQQSGVQIDTFSMLPEETILDKLPKDFQERITSSKWKDRVEALEEEFWDSVLSQTK
361 KLKSTSQNYSNLLGIYGHIIQKDANIQAVALAAQSVELICDKLKTPGFSKDYVSLVFTPL
421 LDRTKEKKPSVIEAIRKALLTICKYYDPLASSGRNEDMLKDILEHMKHKTPQIRMECTQL
481 FNASMKEEKDGYSTLQRYLKDEVVPIVIQIVNDTQPAIRTIGFESFAILIKIFGMNTFVK
541 TLEHLDNLKRKKIEETVKTLPNFSIASGSTHSTIETNKQTGPMENKFLLKKSSVLPSKRV
601 ASSPLRNDNKSKVNPIGSVASASKPSMVAANNKSRVLLTSKSLATPKNVVANSTDKNEKL
661 IEEYKYRLQKLQNDEMIWTKERQSLLEKMNNTENYKIEMIKENEMLREQLKEAQSKLNEK
721 NIQLRSKEIDVNKLSDRVLSLENELRNMEIELDRNKKRNDTNLQSMGTISSYSIPSSTVS
781 SNYGVKSLSSALPFKEEEDVRRKEDVNYERRSSESIGDLPHRVNSLNIRPYRKNGTGVSS
841 VSDDLDIDFNDSFASEESYKRAAAVTSTLKARIEKMKAKSRREGTTRT
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Figure 2: Consensus sites previously known to be Ipl1 phosphorylation target sites on the STU2 protein.

METHODS & RESULTS

Two plasmids created by previous students, Miranda's plasmid contains a S40A mutation and Angela's plasmids has mutations at the S430A and S593A known Ipl1 consensus sites. We will combine these three mutations from these two mutated plasmid to create the final tri-mutated plasmid.

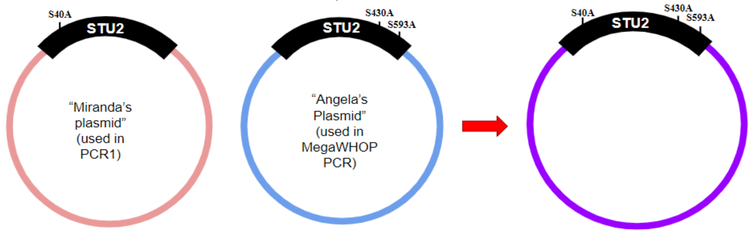

Figure 3: The intended goal is to use MegaWHOP is to combine Miranda's and Angela's plasmid to create a plasmid that contains all three mutations from the two plasmids

This would be achieved by first copying the region of in stu2 containing the S40A codon mutation using standard PCR.

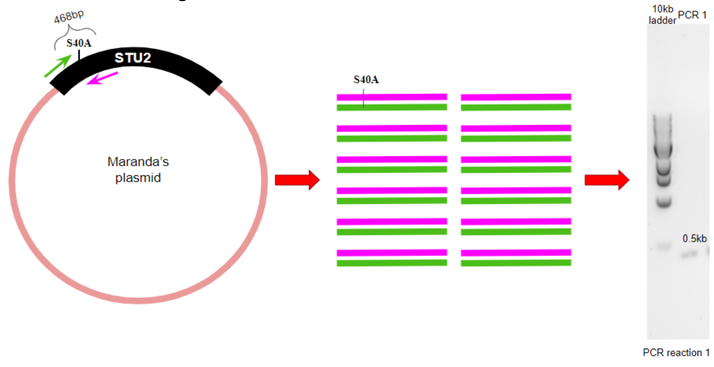

Figure 4. Maranda's primer was used to create a Megaprimer in PCR 1 reaction. PCR1 was used to amplify mutated 40th bases on the *stu2* gene which serine was turned into alanine. The Megaprimer was expected to be 460 base pairs. Electrophoresis was done to confirm the results. The electrophoresis showed base pairs roughly around the 500 base pair ladder indicating a successful PCR reaction.

Then MegaWHOP is performed using the first PCR product as "megaprimers" to create the desired mutated plasmid. We were able to successfully create plasmid sized DNA.

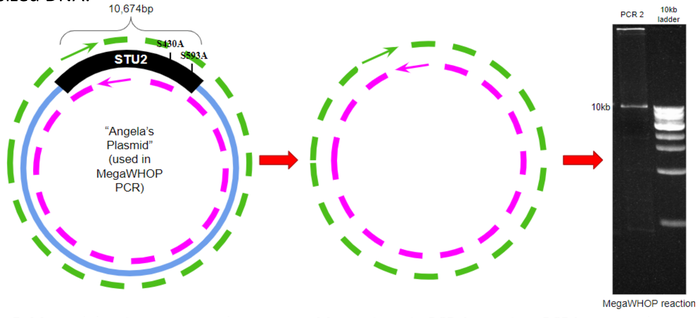

Figure 5. Maranda's primer was used to create a Megaprimer in PCR 1 reaction. PCR1 was used to amplify mutated 40th bases on the *stu2* gene which serine was turned into alanine. The Megaprimer was expected to be 460 base pairs. Electrophoresis was done to confirm the results. The electrophoresis showed base pairs roughly around the 500 base pair ladder indicating a successful PCR reaction.

This transformed PCR product with the three mutations is then transformed into E. coli produced colonies. Which are then selected for the circular MegaWHOP plasmids that are desired.

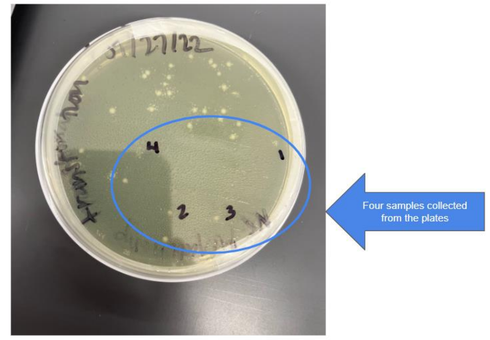

Figure 6. Transformaion of *E. coli* bacteria that were transformed with a MegaWHOP plasmid that contain three mutations. The mutations are on the 40th, 430th, and 593rd base pairs on the *stu2* gene where serine was turned into alanine. Four bacteria colonies were sent out for sequencing to see if the bacteria contained the mutations.

Then the plasmids DNA were purified using dpn1 so that the primer can be broken down to not interfere with the sequencing of the plasmids selected from the colonies and were then sent for sequencing.

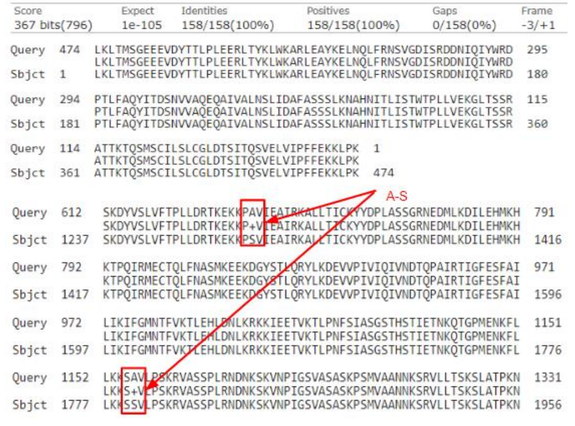

Figure 7: The transformed *E. coli* was sent for sequencing to confirm if there was any mutations found in the selected *E. coli* colonies. Using tblastx the sequences of the wildtype version of STU2 and the mutated version of stu2 were aligned and showed that there was no mutations found on the 40th codon, 460th codon, and the 593rd codon.

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. Next steps would include collecting more colonies to send for sequencing.

ACKNOWLEDGEMENTS

University of Washington-Tacoma Science lab, Miranda Makalena and Angela Mitchell for previous work in mutagenized plasmids created, and Dr. John Finke for mentorship in TBIOMED 410