Phospho-null mutation to eliminate a phosphorylation site on Stu1, a protein of the kinetochore complex in *Saccharomyces cerevisiae*, using the CRISPR- Cas9 system.

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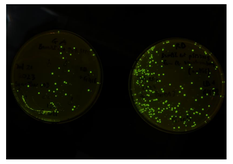

BACKGROUND

Chromosomal segregation requires coordination between the kinetochore protein complex and mitotic spindles which is essential for accurate genetic division between two daughter cells. The kinetochore is a protein complex that is located at the centromere of the sister chromatids. During mitosis, it is observed that the kinetochore actually "walks" sister chromatids toward the opposite poles of elongating cells with the guide of mitotic spindles. It has been suggested that Stu1, a small protein in the kinetochore complex, helps delay anaphase in budding yeast, *Saccharomyces cerevisiae,* until each chromosome is attached to the mitotic spindle. Stu1 interacts with the spindles and synchronously moves with the spindles when they elongate. Phosphorylation may play a big role in regulating Stu1 function. In yeasts, MELT has been known to be a common phosphorylation site, hence, the removal of a threonine amino acid on the MELT motif on Stu1 might affect the ability of sister chromatids to separate properly which may cause a reduction in yeast viability. MELT is a well-conserved sequence in fungi and has been known to be a phosphorylation site in other homologs of Stu1. Taking advantage of the CRISPR-Cas9 enzyme, we will introduce a phospho-null mutation into the budding yeast *STU1* gene to replace the threonine 719 codon with a valine codon at the MELT sequence. We hypothesize that this mutation will produce a malfunction in the Stu1 protein, which could possibly hinder its ability to coordinate spindles and kinetochore attachment and furthermore prevent chromosomal segregation altogether during mitosis.

INTRODUCTION

- Used CRISPR Direct to analyze *STU1* sequence to which suggestive Cas 9 enzyme cut sites following a PAM sequence are found.

Figure 1. *STU1* sequence analyzed via SnapGene viewer. The highlighted site in red contain codons of the MELT motif upon which a null mutation would be inserted at the threonine codon.

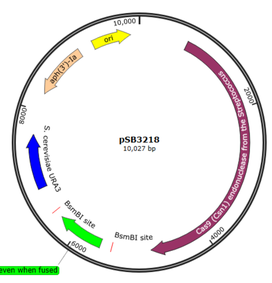

Figure 2. pSB3218 plasmid from Sue Biggins lab has been engineered to contain the Cas9 enzyme gene and designated sites for restriction enzyme BsmBI-v.2 to cut.

- Wild-type template (2071-2268) (198 nucleotides)

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GCC CCT CCT TCT TCT ACT GCC GCC ACA AAA GTA TCT GAA
AAT TAC ACA AAT TTT GAT GAC TTT CCG TCA AAC CAA ATC
GAC TTG ACT GAT GAG TTA TCA AAT AGT TAC TCT AAC CCG
TTG ATA AAG AAA TAT ATG GAT AAA AAT GAT GTT TCG ATG
TCA TCT TCT CCA ATC TCA TTA AAA GGC AGT AAT AAA CTT
GGT
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- Replace template for our sgRNA (change codon Thr to Val)

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GCC CCT CCT TCST TCT ACT GCC GCC ACA AAA GTA TCT GAA
AAT TAC ACA AAT TTT GAT GAC TTT CCG TCA AAC CAA ATC
GAC TTG GAA GAT GAG TTA TCA AAT AGT TAC TCT AAC CCG
TTG ATA AAG AAA TAT ATG GAT AAA AAT GAT GTT TCG ATG
TCA TCT TCT CCA ATC TCA TTA AAA GGC AGT AAT AAA CTT
GGT
```

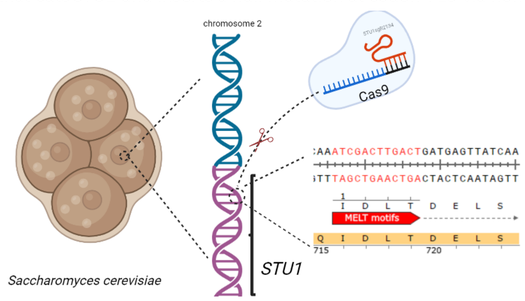
Figure 3. Replaced template is designed and analyzed by SnapGene Viewer. The original *STU1* gene codes for a threonine at the 719 position which was changed to a valine.

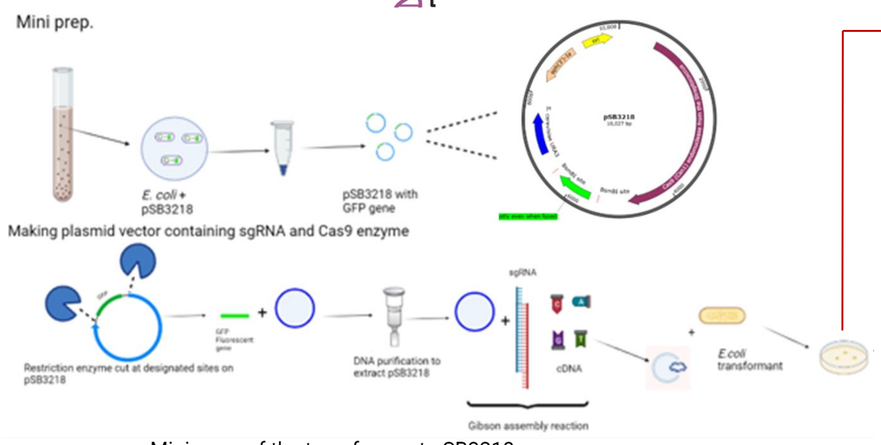
CONCLUSION AND NEXT STEP

We successfully cloned our plasmid vectors including the Cas9 enzyme by Gibson assembly reaction. We also designed the right template containing the mutation. The next step would be to transform yeast with both the plasmids as well as our template DNA that codes for valine at 719-codon in *STU1*, this combination will activate the CRISPR Cas 9 genome editing system in yeast if the transformant succeeded. A future phenotype test will be expected to see a reduction in the yeast population if the null mutation is successfully delivered into the cells, which means smaller colonies than the normal ones will be expected seen from the mutated yeast strains.

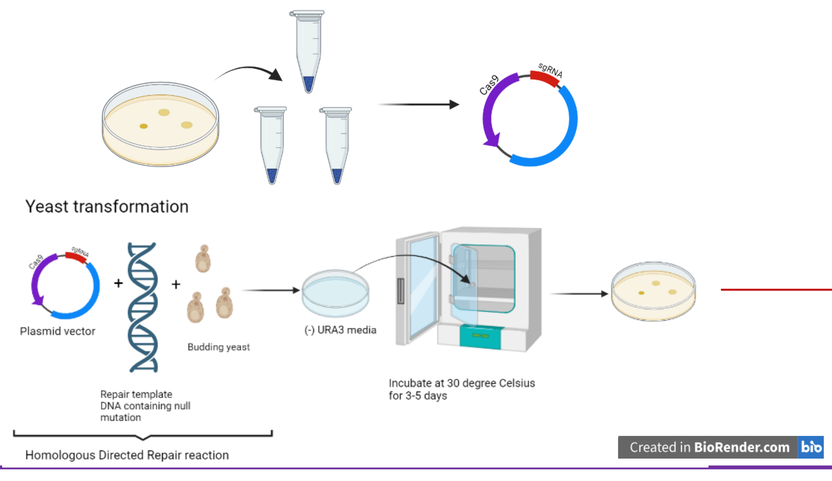
METHODS

- Designed sgRNA that cuts *STU1* and inserts a null mutation at codon-719 via homologous directed repair





Mini prep. of the transformant pSB3218



RESULTS

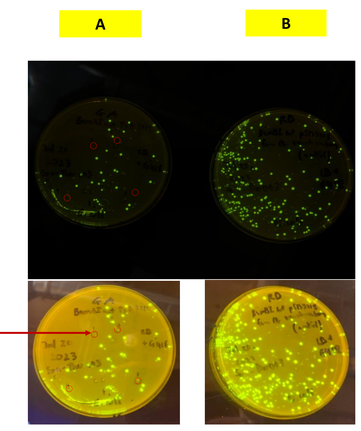


Figure 4. Result of *E.coli* transformation with pSB3218 (A) transforming *E.coli* with the product of Gibson assembly with many non-glowing colonies (red circles). (B) transforming *E.coli* with the product of restriction digest reaction.

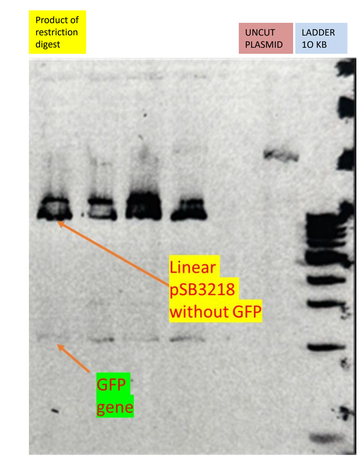
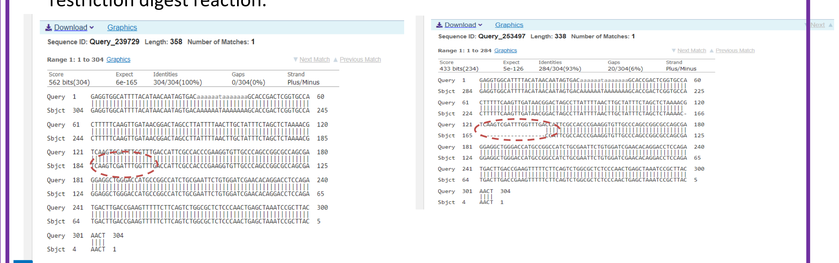


Figure 5. Results of gel electrophoresis of the restriction digest reaction



Figures 6 & 7. The results of the sequencing reaction of the cloned plasmid show a perfect match with our designed sgRNA hence the success of our transformation and Gibson assembly reaction

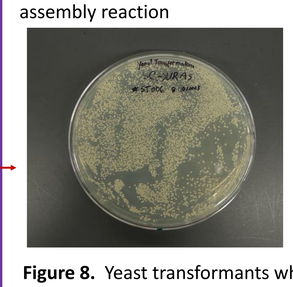


Figure 8. Yeast transformants which have successfully taken up our cloned plasmid are viable on plate lacking uracil.

ACKNOWLEDGEMENTS

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REFERENCES

SCAN ME