Mutating The Dsn1 Protein to Mimic Structural Phosphorylation

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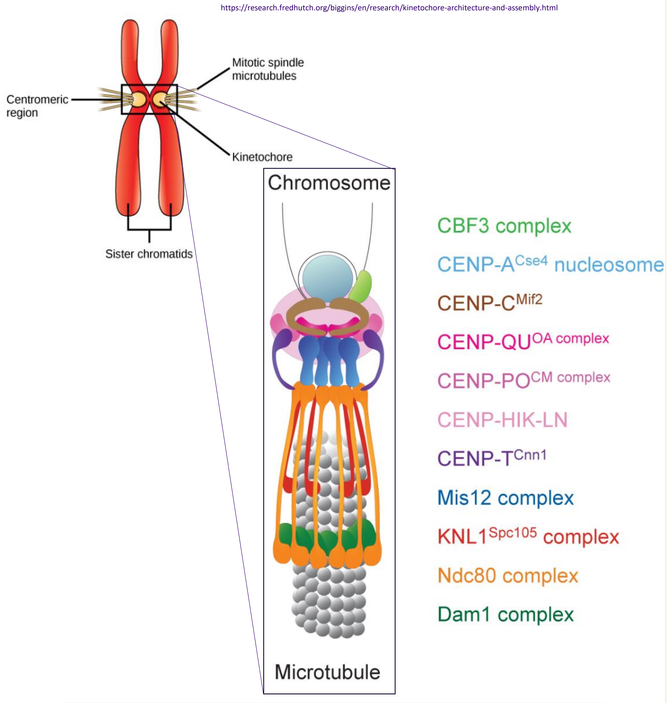
BACKGROUND

The kinetochore is a protein complex that aids in maintaining successful DNA separation. Many proteins present in the kinetochore have phosphorylation sites that help to regulate processes such as microtubule attachment. Phosphorylation is used to help regulate protein function and transmit signals throughout a cell. It is the addition of a phosphate group to an organic molecule. In budding yeast, phosphorylation helps to promote interaction between the inner and outer kinetochore. We will be investigating these processes and their importance to the kinetochore using the Dsn1 yeast protein. Dsn1p is a part of the MIND protein subcomplex and affects cell cycle progression. Phosphorylation sites have been found on amino acids S546, S547, and S554 within Dsn1p. We will be mimicking structural phosphorylation on these sites using CRISPR-Cas9. Using this enzyme, we will be changing these codons from serine to aspartic acid, which mimics phosphorylation with its negative charge.

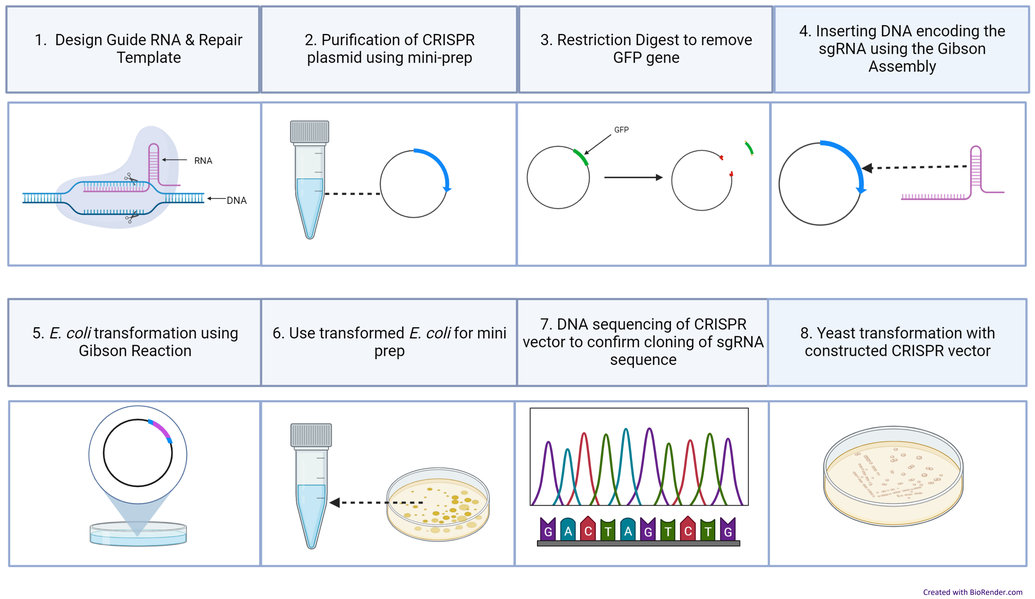
Images from: https://www.nursinghero.com/study-guides/biology1/the-cell-cycle/
https://research.fredhutch.org/biggins/en/research/kinetochore-architecture-and-assembly.html



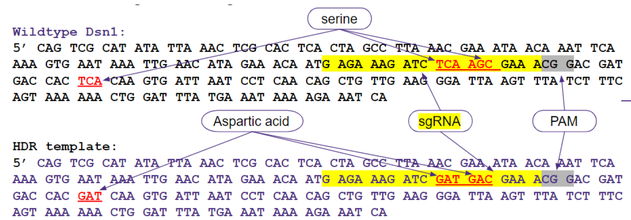
Objective:

We will be investigating the importance of phosphorylation on Dsn1p. This will be done using budding yeast as a model organism for kinetochore function and the CRISPR-Cas9 system to insert mutations at the S546, S547, and S554 codons. These mutations will change the amino acids from serine to aspartic acid, mimicking phosphorylation.

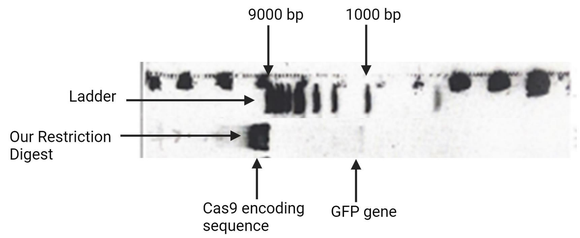
METHODS



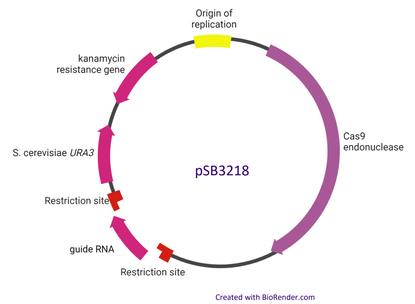
RESULTS



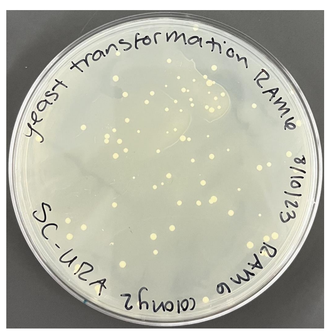
Sequence from the wildtype *DSN1* gene shown in the area surrounding codons S546, S547, and S554. The small guide RNA sequence that we designed is shown in yellow, The codons we plan to modify are in red, and the PAM sequence is shown in grey. Below that is the HDR template we designed to induce changes in the relevant areas.



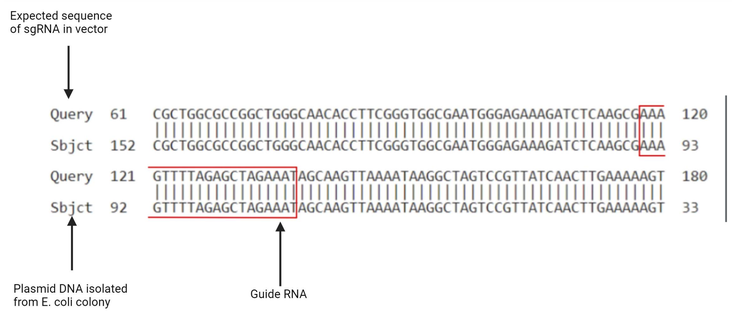
Gel analysis of restriction enzyme digestion to remove the GFP gene from the CRISPR vector in preparation for Gibson Assembly.



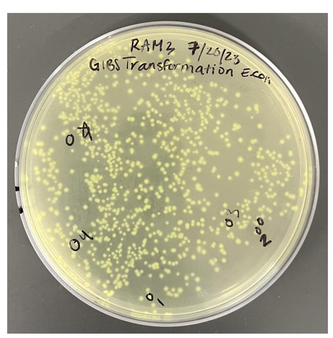
Plasmid structure showing the CRISPR Cas9 enzyme, URA3 gene, and where the plasmid was cut for the gRNA encoding sequence to be inserted via Gibson Assembly.



We transformed yeast with our CRISPR vector containing sgRNA that will target a double strand break to the *DSN1* gene. Its growth on a plate without Uracil indicates that it successfully took up the CRISPR vector.



DNA blast sequencing shows that the sgRNA has successfully been cloned into the CRISPR vector.



These *E. coli* were transformed with our Gibson Assembly reaction between the CRISPR vector and our sgRNA encoding sequence. Non-glowing colonies were selected for further analysis.

CONCLUSIONS AND FUTURE EXPERIMENTS

During this experiment, we were able to successfully make a CRISPR vector that encodes for our guide RNA. This vector helps to cleave our target sites on the *DSN1* gene and our repair template using homologous recombination. Since we successfully transformed our yeast, we know that we have input our plasmids into the yeast. Presently, we are confirming the presence of mutations using PCR and Sanger sequencing. Once mutations are confirmed, we will examine cell division phenotypes on the resulting mutants.

Acknowledgments:

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