Mutating the Dsn1 Protein to Mimic Structural Phosphorylation

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Background

What is the Kinetochore?

- A complex of proteins that attaches to centromere and the spindle.
- Segregates copies of DNA to the opposite poles during mitosis.
- The malfunction of kinetochores can lead to missegregation of the DNA leading to abnormal numbers of chromosomes.

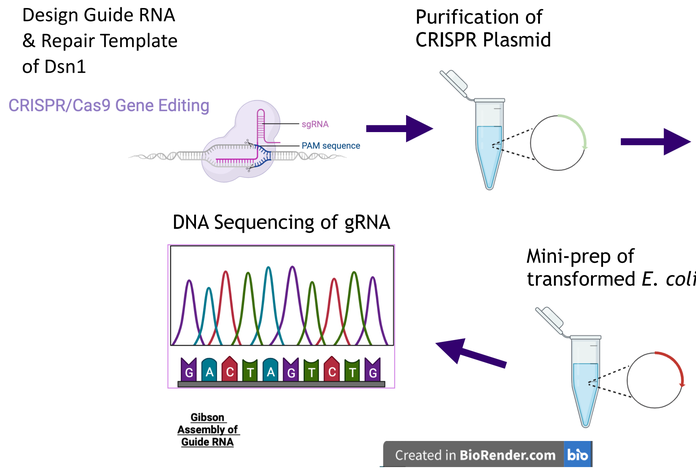
What is the Dsn1 Protein?

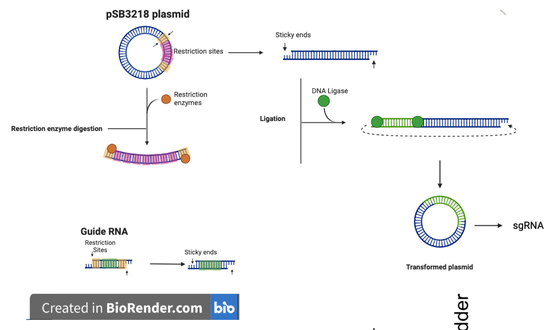
- A component of the outer kinetochore MIND complex.
- Affects the cell cycle progression and is required for the correct alignment and segregation of chromosomes.
- Dsn1p is phosphorylated at serine 546, 547, & 554 codons in the protein through mass spectrometry analysis.

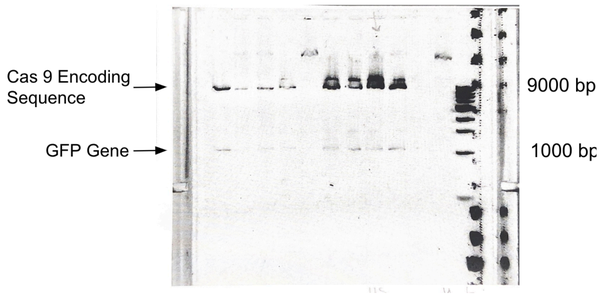
Objective:

- To measure the potential impacts of Dsn1 phosphorylation, by using budding yeast and CRISPR-cas9 system to make mutations at serine 546, 547, & 554 codons to mimic constant phosphorylation (Sue Biggins Lab).

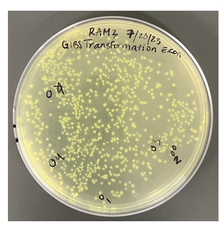
Methods & Results







Gel Electrophoresis results to check the Restriction Digest of the CRISPR Plasmid. Arrow indicates our sample.



Transformation: *E. coli* transformation on LB + G418 medium. The plate contains transformed, glowing *E. coli* colonies. Circled colonies are non-glowing, which does not contain GFP gene, and are likely to contain our guide RNA encoding sequence.

Score	Expect	Identities	Gaps	Strand
392 bits(212)	4e-114	212/212(100%)	0/212(0%)	Plus/Minus

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Query  1    CTGGAGGTCCTGTGTTCGATCCACAGAATTCGCAGATGGCCGGCATGGTCCCAGCCTCCT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  212  CTGGAGGTCCTGTGTTCGATCCACAGAATTCGCAGATGGCCGGCATGGTCCCAGCCTCCT  153

Query  61   CGCTGGCGCCGGCTGGGCAACACCTTCGGGTGGCGAATGGGAGAAAGATCTCAAGCGAAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  152  CGCTGGCGCCGGCTGGGCAACACCTTCGGGTGGCGAATGGGAGAAAGATCTCAAGCGAAA  93

Query  121  GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  92   GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGT  33

Query  181  GGCACCGAGTCGGTGCttttttttattttttGT  212
            ||||||||||||||||||||||||||||||||
Sbjct  32   GGCACCGAGTCGGTGCTTTTTTTATTTTTTGT  1
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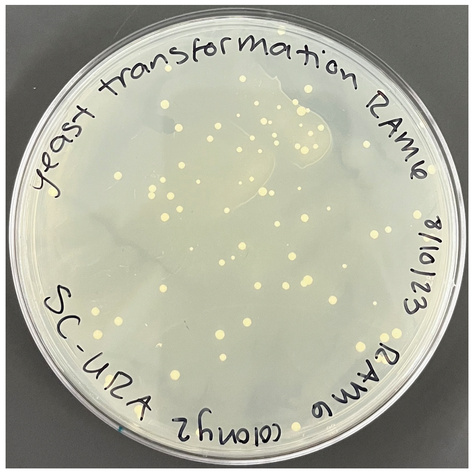
Guide RNA

Sequencing Results: The plasmid DNA purified from the *E.coli* colony showed a 100% identity match of the sgRNA. Query was our expected sgRNA, and subject was our actual sequencing results.

Conclusions & Future Experiments

- CRISPR vector that encodes for the guide RNA was successfully made. That helps cleave the target mutation sites in the *DSN1* gene, along with the repair template through homologous recombination.
- Transformation of yeast was successfully grown into colonies, indicating plasmids were induced into the yeast.
- Further DNA sequencing will make the mutation in the *Dsn1* gene.
  - Serves as a way to analyze the yeast for evidence of chromosome segregation defects.



Acknowledgement

I would like to give my special thanks to our collaborators from Sue Biggins Lab for sharing their data, and for providing us the CRISPR template, as well as the Homology Directed Repair template. Along with my classmates.