Utilizing the CRISPR-Cas9 system to investigate the chromosomal segregation effects of phosphonull mutations in *DSN1* in budding yeast

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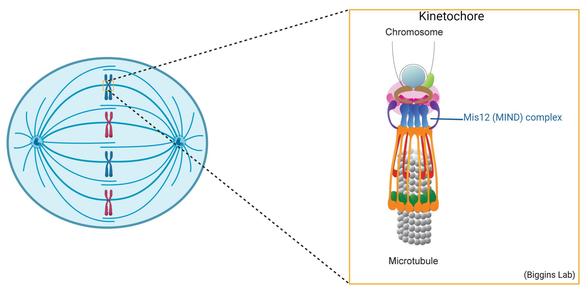
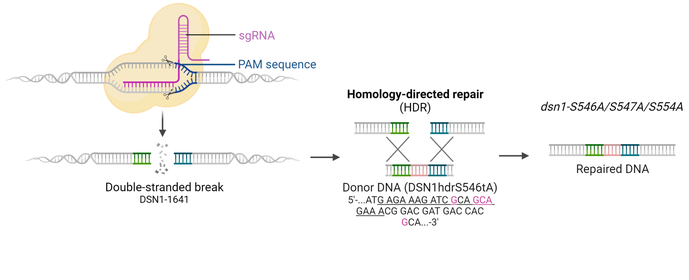
Background

The kinetochore is a multi-protein complex that mediates chromosomal attachments to microtubules during cell division. Dysfunction in kinetochore regulation leads to aneuploidy in cells. Dsn1p, a kinetochore protein, is part of the MIND protein complex; the MIND complex contributes to microtubule attachment and produces pulling forces by depolymerization. Phosphorylation of Dsn1p is part of the MIND complex regulation.

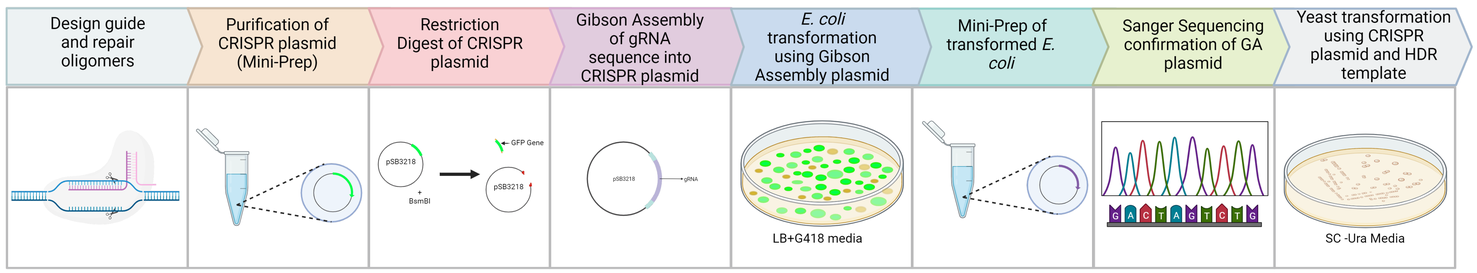


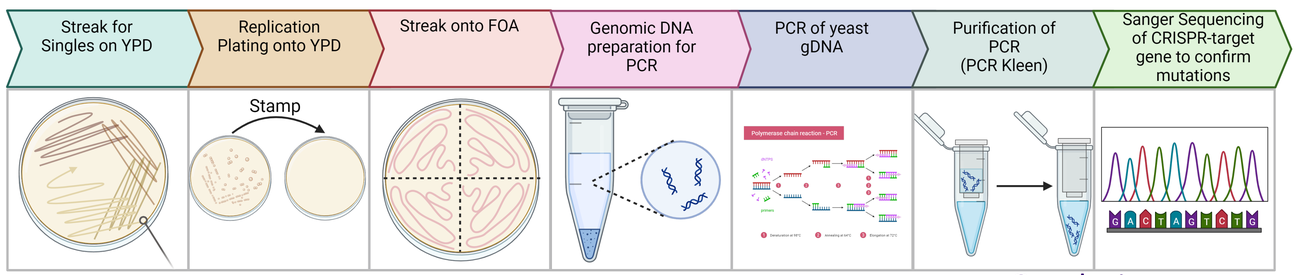
element of proteins, which changes the protein activation state. Not all phosphorylation sites affect the activity of the protein. Mutating the phosphorylation sites can determine the effect of phosphorylation on protein function; this is achieved by either making a phosphomimetic or a phosphonull mutation at the sites of interest. Mimetic mutations mimic constant phosphorylation of the site, while null mutations prevent the site from being phosphorylated.

Through mass spectrometry analysis, the Sue Biggins lab at Fred Hutchinson Cancer Center identified three serines in Dsn1p 546, 547, and 554 as phosphorylation sites. Utilizing the CRISPR-Cas9 system and homology-directed repair (HDR), we started the following experiments to change the serines to alanines in budding yeast to create a version of Dsn1 that cannot be phosphorylated at 546, 547, and 554, *dsn1-S546A/S547A/S554A*.



Methods





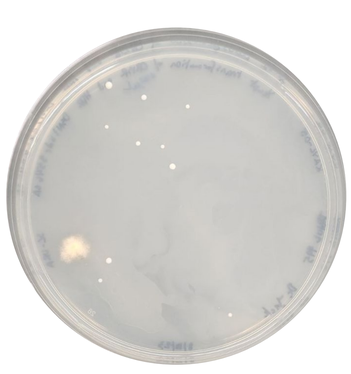
Results

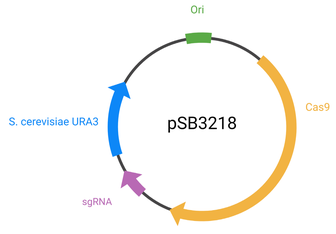
pSB3218+sgRNA expected sequence 121 AATGGGAGAAAGATCTCAAGCGAAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC 180

Plasmid DNA isolated from colony 2 sequence 244 AATGGGAGAAAGATCTCAAGCGAAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC 185

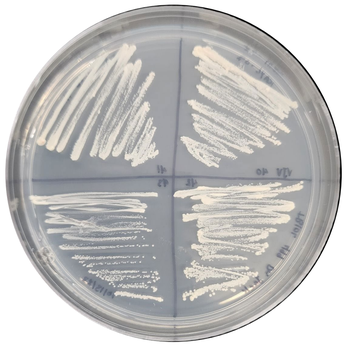
Designed SgRNA GAGAAAGATCTCAAGCGAAA

Sanger Sequencing results of CRISPR plasmid. Plasmid generated by Madison Rose, Ann Vu, and Rylee Evanger





Simplified pSB3218 CRISPR plasmid diagram. Diagram shows *URA3* gene which creates selective pressure by allowing yeast to grow without uracil (SC-URA plate), and transforming FOA into a toxin.



Successful Transformation of null *URA3* yeast by CRISPR-directed HDR on SC –URA plate. Growth shows the CRISPR plasmid is active

Successful loss of CRISPR plasmid on FOA plate. Growth shows the CRISPR plasmid is gone (no *URA3* making FOA toxic)

Expected 61 TAAATTGAACATAGAAACAATGAGAAAGATCGGCAGCAGAAACGGACGATGACCACGCACA 120

YJV 43 349 TAAATTGAACATAGAAACAATGAGAAAGATCTCAAGCGAAACGGACGATGACCACTCACA 290

DSN1 61 TAAATTGAACATAGAAACAATGAGAAAGATCTCAAGCGAAACGGACGATGACCACTCACA 120

YJV 43 349 TAAATTGAACATAGAAACAATGAGAAAGATCTCAAGCGAAACGGACGATGACCACTCACA 290

Sanger Sequencing data showing unsuccessful CRISPR mutagenesis. Other Sanger Sequence results were inconclusive.

Conclusion

The selectivity checks worked and resulted in colony growth, indicating that the CRISPR plasmid was utilized by the yeast and then lost. Sanger sequencing data is inconclusive for strains YJV 40, 41, and 42. YJV 43 shows none of the desired mutations

Future Experiments

Since there was no evidence of successful mutation next steps will focus on attempting to get successful sequence data for strains YJV 40, 41, and 42. Depending on sequence evidence of creating a *dsn1-S546A/S547A/S554A* either phenotype testing or reattempting the CRISPR mediated mutation.

Acknowledgments

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References