CRISPR-Cas9 mutagenesis on suspect Dsn1 phosphorylation sites and its impact on functionality

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Background

The kinetochore is a protein complex located on the centromere of the chromosome and is responsible for proper chromosome segregation in mitosis. The kinetochore interacts with mitotic spindles to separate DNA evenly between daughter cells.

The Dsn1 protein part of the MIS12 complex, was identified in mass spectrometry as a protein involved in phosphorylation. Two sites in the Dsn1 protein serine 567 and serine 569, lie in contact with the Ndc80 complex which aids in chromosomal segregation and interacts with the microtubule.

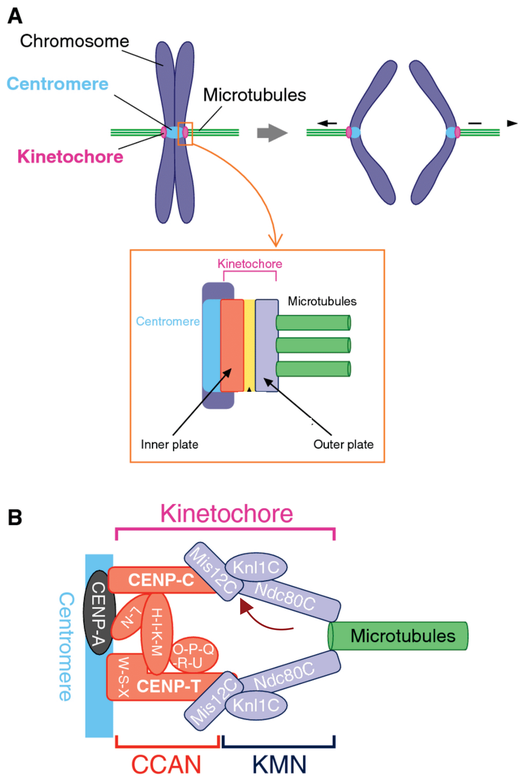

Figure 1: (a) Overview of chromosomal separation with the kinetochore and centromere labeled. (b) Close up view of the arrangement of kinetochore subcomplexes with red arrow indicating the location of the Dsn1 protein. (Hara and Fukagawa, 2020)

Using CRISPR-Cas9 gene editing technology, we aimed to introduce a mutation by replacing the serine codons with alanine amino acids so that the two sites lose the ability to accept phosphate groups.

Mutation of these sites was suspected to result in loss of function of the Dsn1 protein and prevent microtubule separation in mitosis.

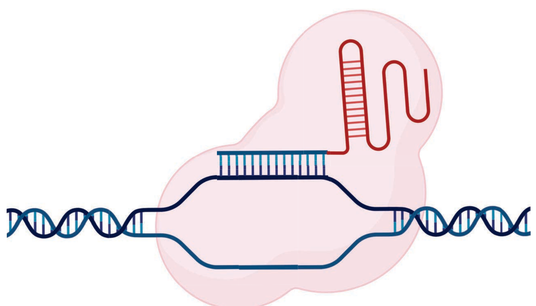

Figure 2: The CRISPR-Cas9 system. Consists of the Cas9 protein and a guide RNA that recognizes the sequence to be edited. (Created with BioRender.com)

Methods

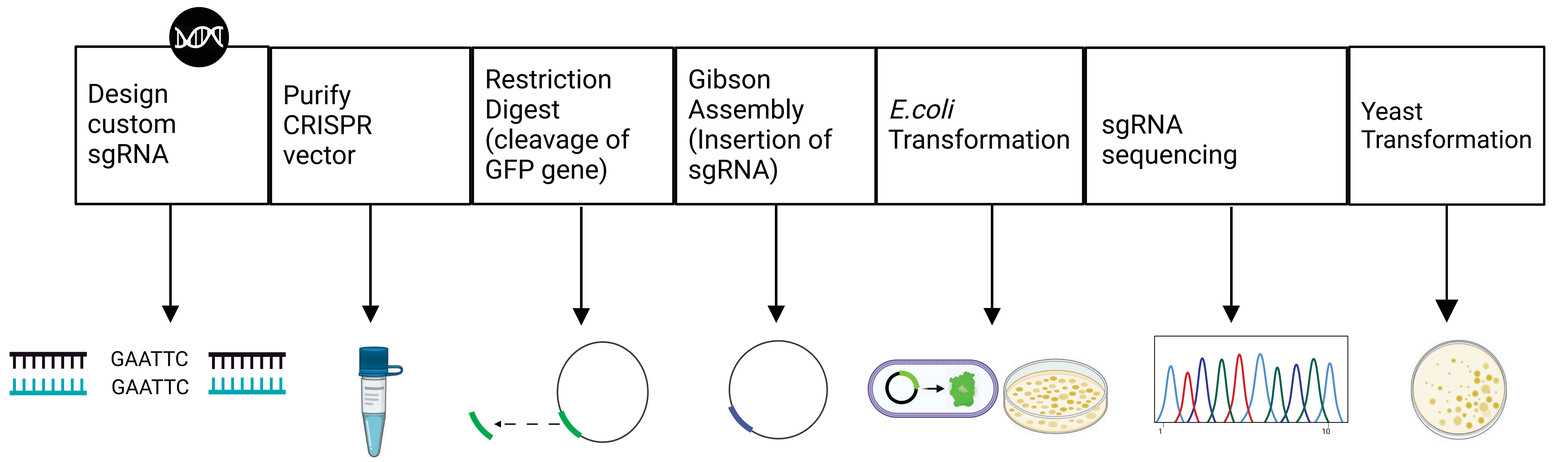

Figure 3: Image above demonstrates the protocols followed in order. (created with BioRender.com)

Results

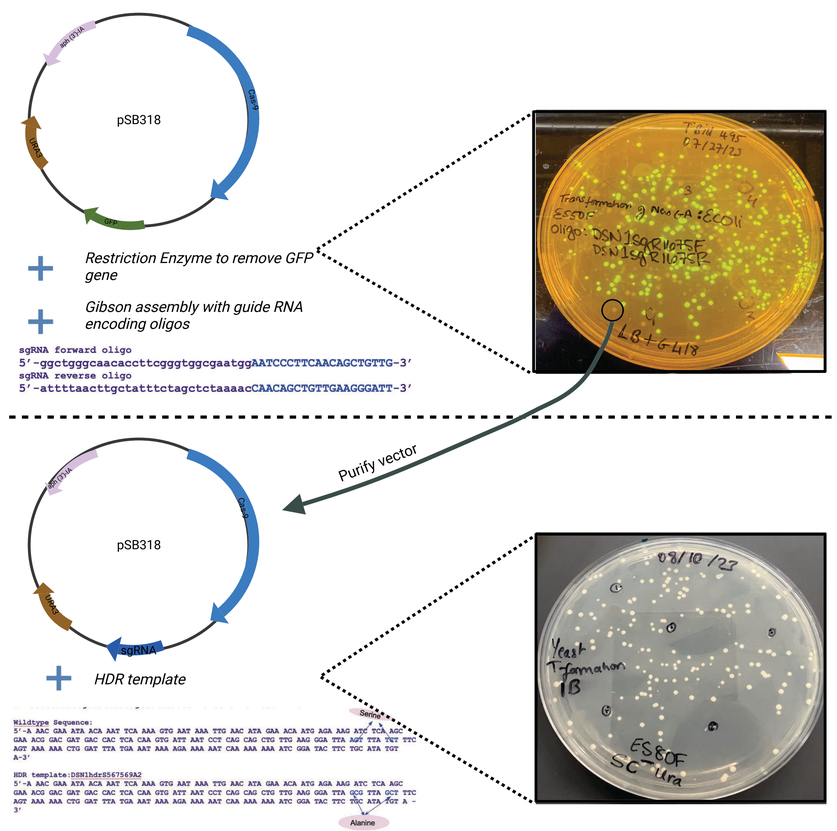

Figure 4: Illustration of the pSB3218 plasmid with transformations in *Escherichia coli* and *Saccharomyces cerevisiae* (Created with BioRender.com)

Conclusions and Future Experiments

As of now, the CRISPR vector seems to have successfully been cleaved of the GFP gene. This has been confirmed through non-glowing colonies.
Sequencing results were successful, however no evidence was found that the gRNA was taken up by the plasmid vector. Thus,
further DNA sequencing is necessary to determine confirmation in uptake of the alanine mutation

Acknowledgements

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References

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