# Instituting Phosphonull Mutations of the Dsn1 Protein in the Kinetochores of *Saccharomyces cerevisiae*

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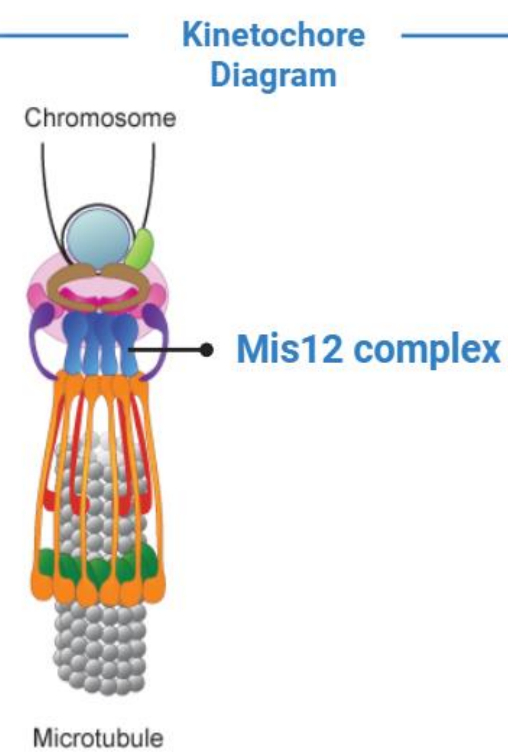
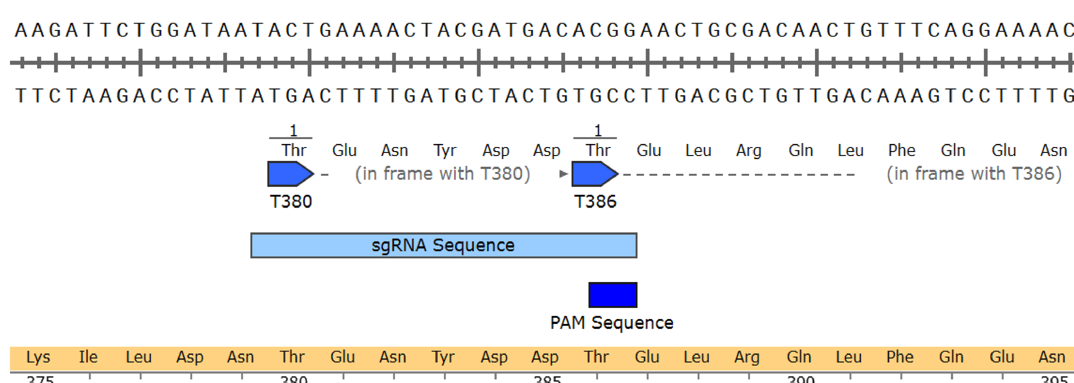
## BACKGROUND

Kinetochores are multi-protein complexes that play a vital role in chromosome segregation during cell division. Many kinetochore proteins have phosphorylation sites that regulate processes such as microtubule attachment. However, the functions of kinetochore phosphorylation are not completely understood. To investigate this problem, we are using the species, *Saccharomyces cerevisiae*, to induce mutations in the *DSN1* gene to see if its sites of phosphorylation are important for function. Dsn1p is part of the MIND protein subcomplex within the kinetochore that contributes to microtubule attachment and produces pulling forces resulting from depolymerization.



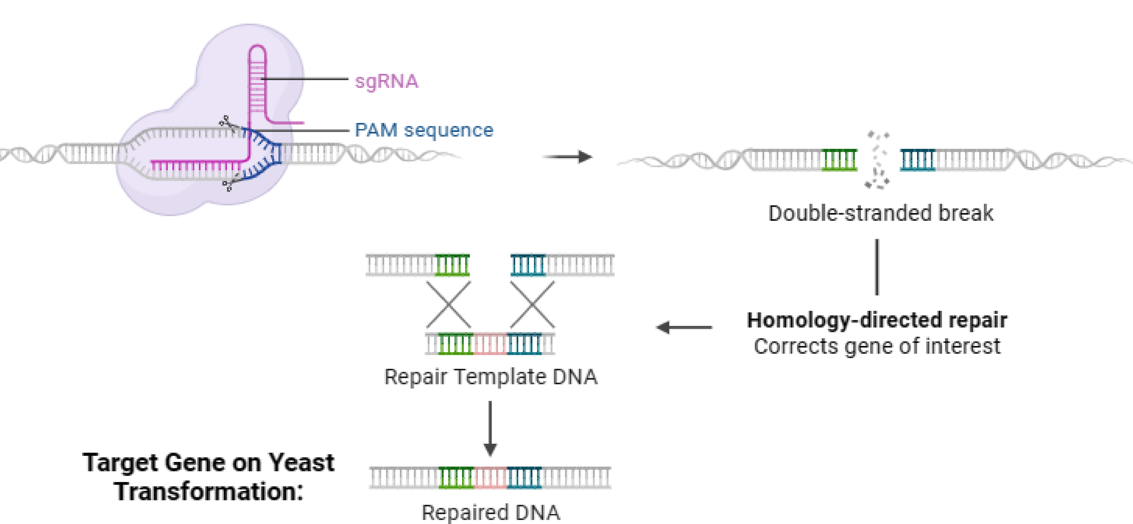

Amino acids T380 and T386 of Dsn1p were identified as phosphorylation sites by the Biggins lab using mass spectrometry analysis.

We plan to use the CRISPR-Cas9 system to create mutations, changing T380 and T386 codons to instead code for valine, which cannot be phosphorylated.

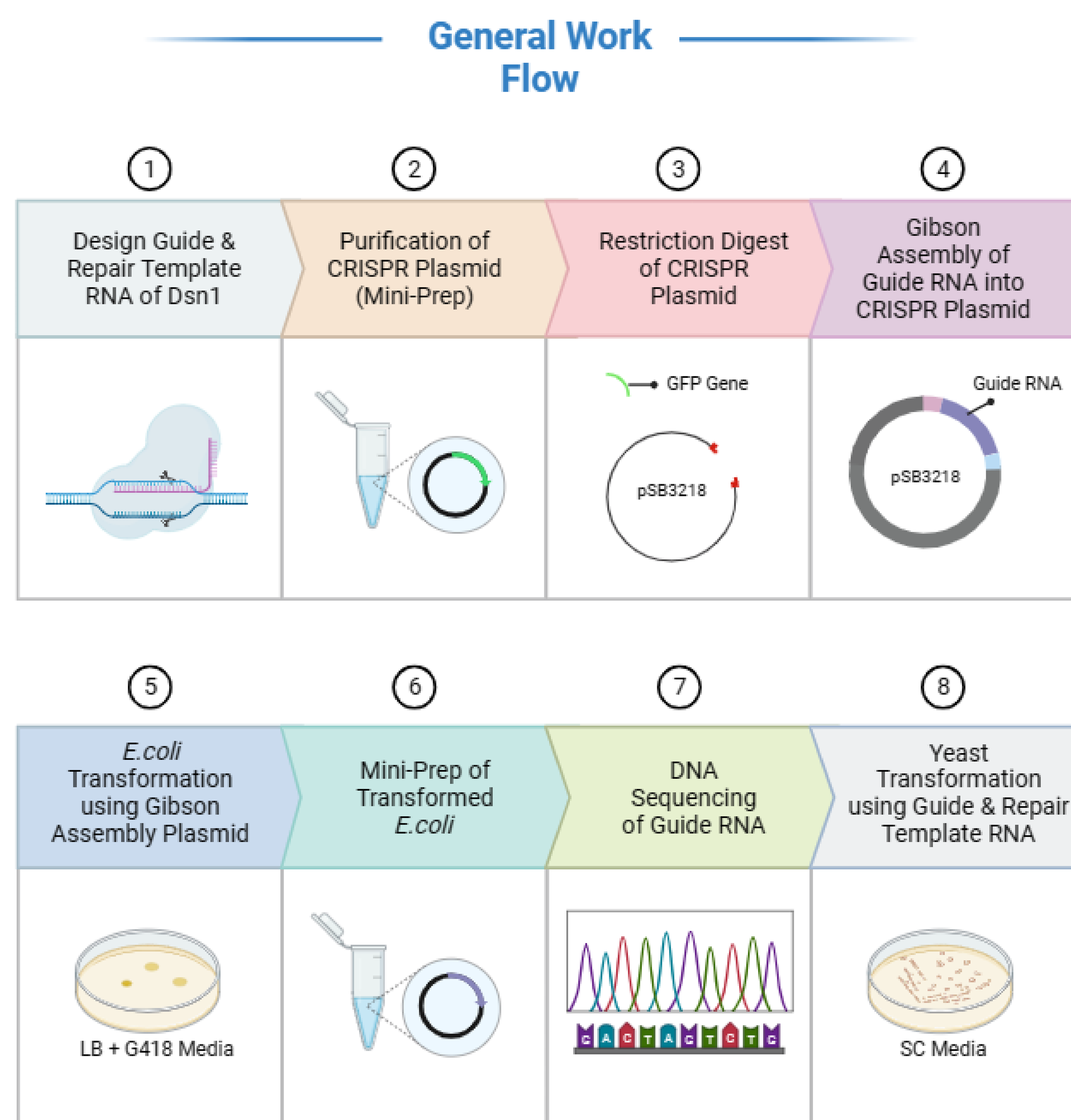


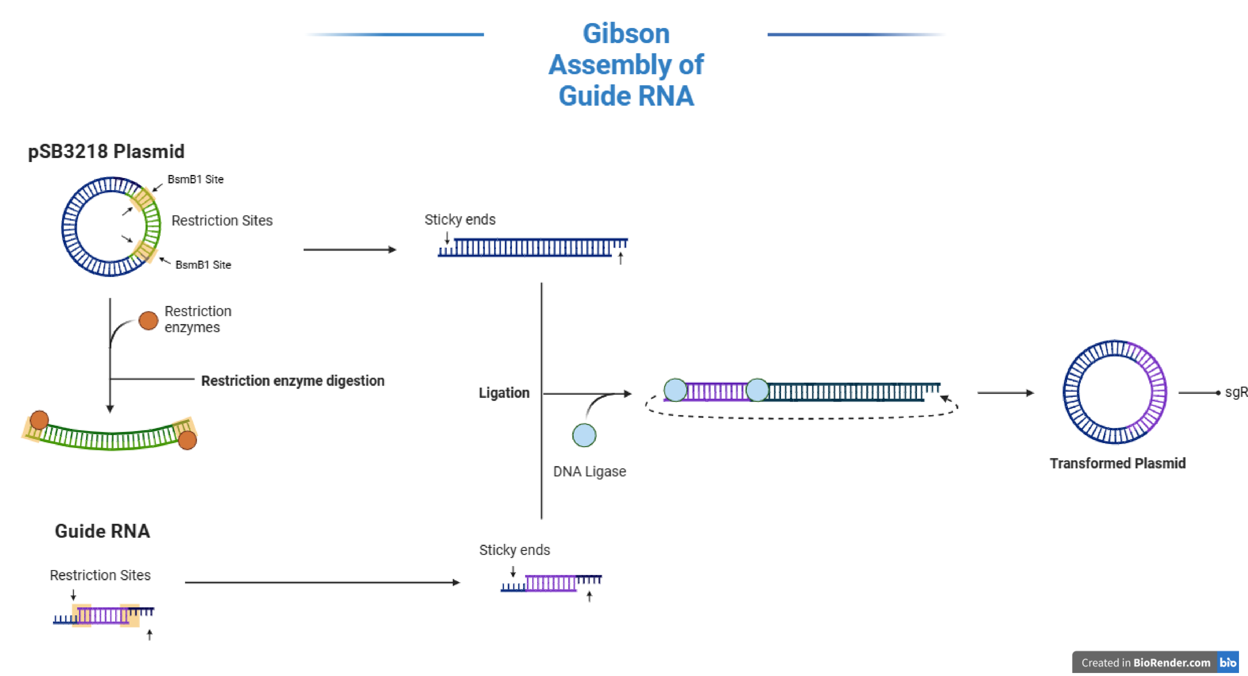

The locations of Amino Acids T380 and T386 in the *DSN1* gene of *Saccharomyces cerevisiae* and the sgRNA Sequence that we will be using for CRISPR.

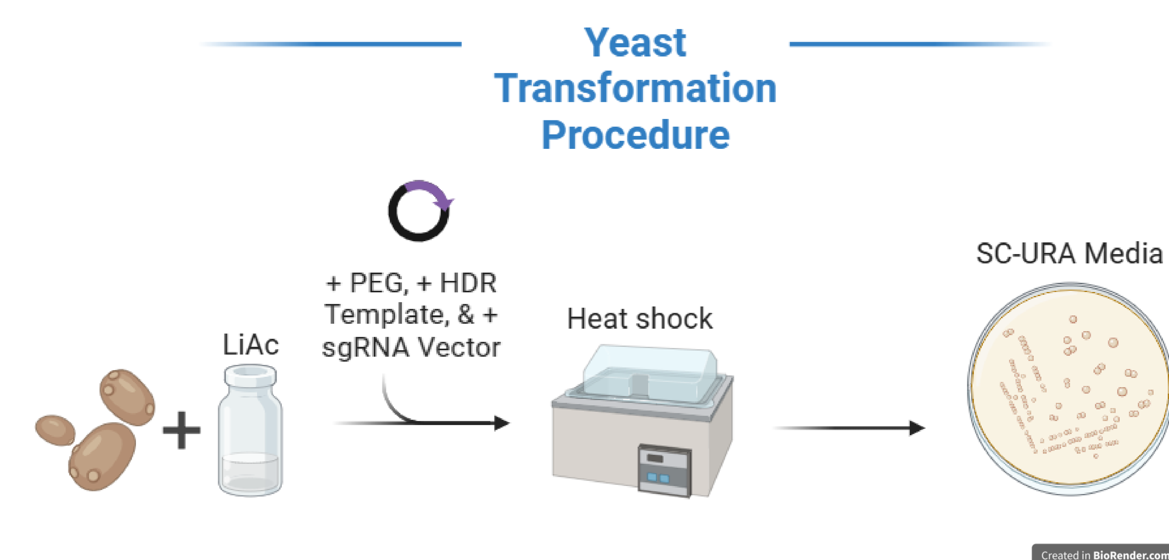
### CRISPR: Cas9 Gene Editing



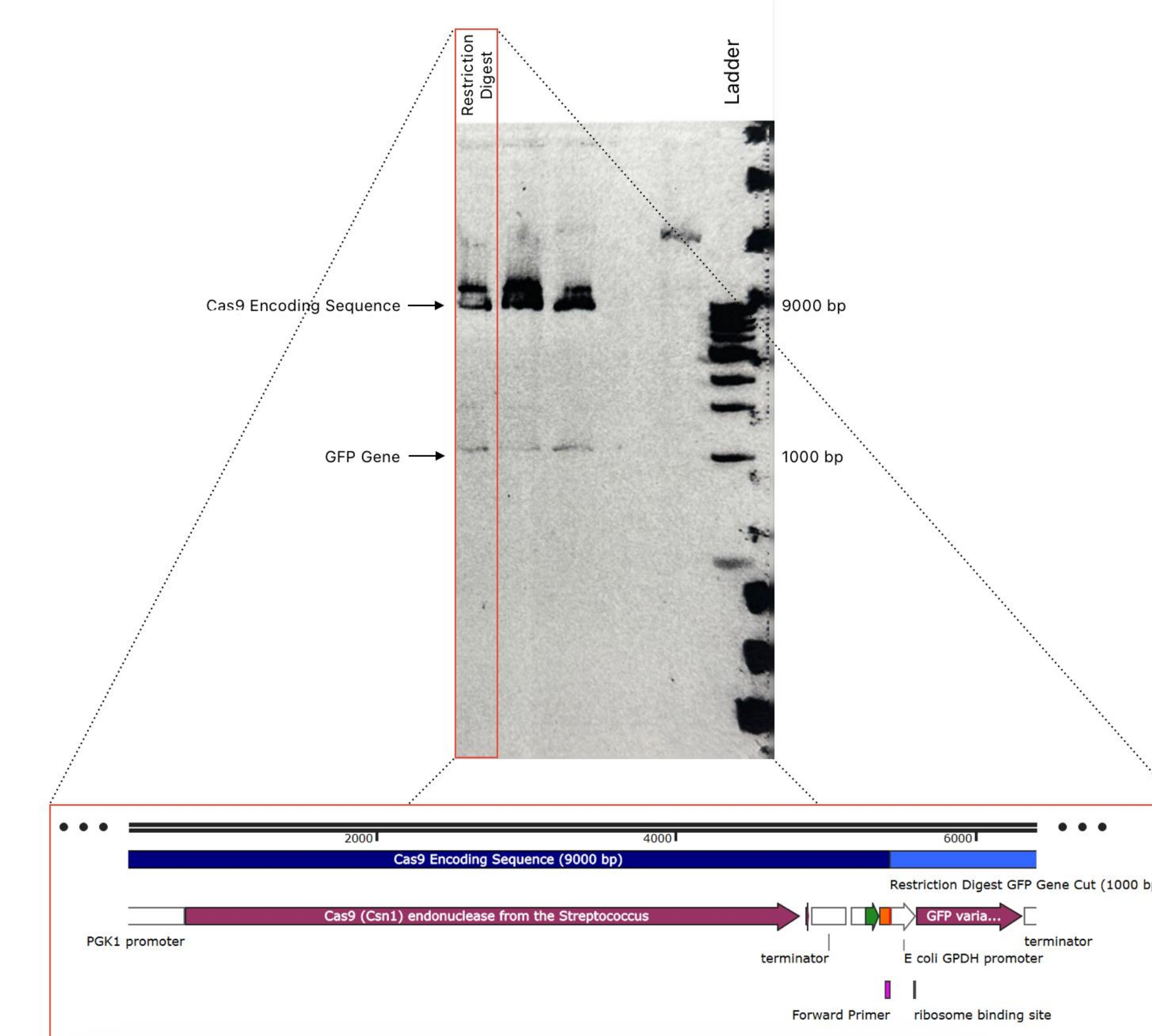

## METHODS



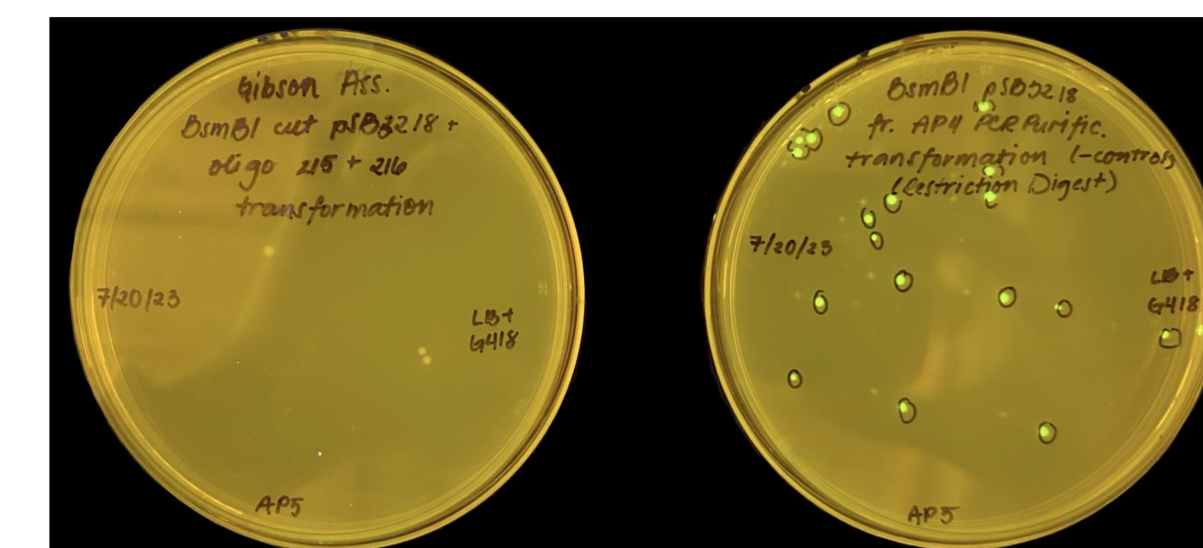



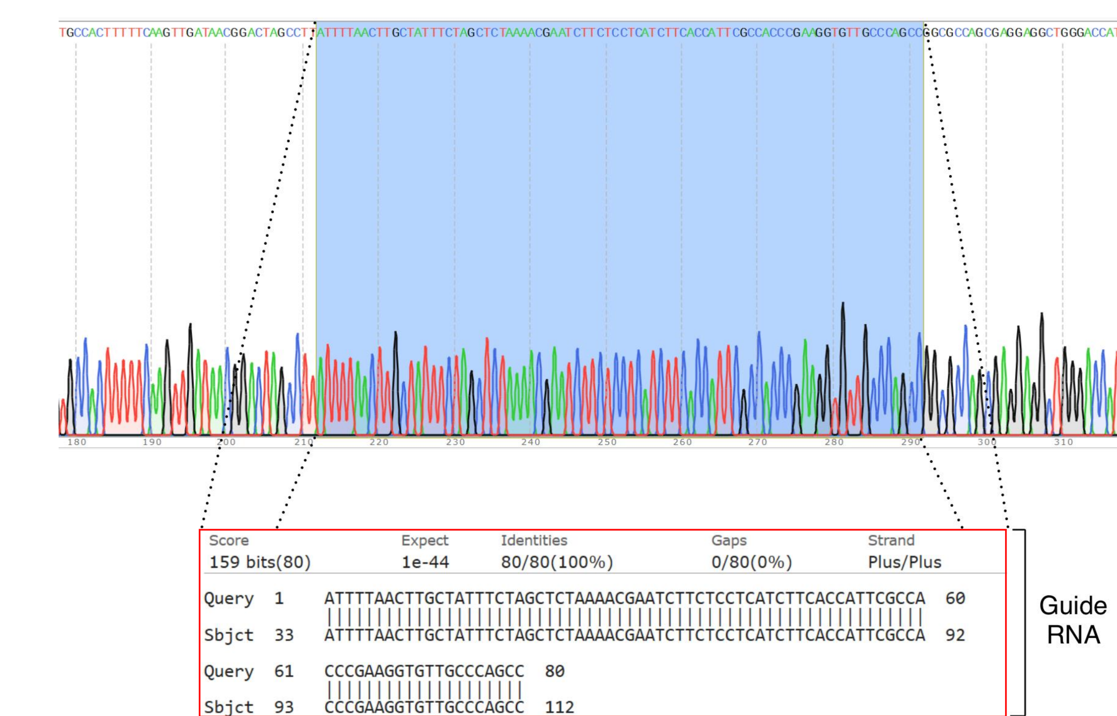

## RESULTS




Gel Electrophoresis is used to check for the results of the Restriction Digest of the CRISPR Plasmid.



*E.coli* transformation on LB + G418 medium. The plate on left contains transformed, non-glowing *E.coli* colonies with Gibson Assembly DNA, these E.coli colonies do not contain the GFP gene, indicating that we have integrated the guide RNA into the plasmid. The plate on the right contains transformed, glowing *E.coli* colonies with Restriction Digest DNA as control.




In our sequencing analysis, we can see that the plasmid DNA purified from our *E.coli* colony contains our Guide RNA.

## CONCLUSIONS & FUTURE EXPERIMENTS

So far, we have successfully made our CRISPR vector which encodes for our guide RNA. This will allow us to cleave our target mutation sites in the *DSN1* gene, along with our repair template through homologous recombination. However, in our first attempt at transformation, we have not successfully transformed yeast. If we had, it would've indicated that we have successfully introduced those plasmids into yeast and we would confirm the presence of the mutation in the *Dsn1* gene through DNA sequencing, and carefully analyze it for evidence of growth and chromosome segregation defects of *Saccharomyces cerevisiae*.

## ACKNOWLEDGEMENTS

Thank you to the Sue Biggins Lab for sending Mass Spectrometry data for experimentation and for hosting us at the lab meeting in Fred Hutch, and to my classmates and Dr. Jack for both the help and support.

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