Using CRISPR to induce a mutation in a MELT-like motif in *S. cerevisiae's* Stu1 protein

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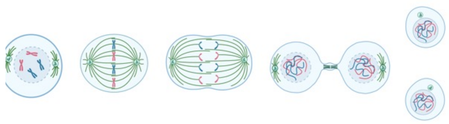
TBIOMD 495

Background

- Chromosome segregation is an essential process that is mediated by the kinetochore and microtubule spindles.

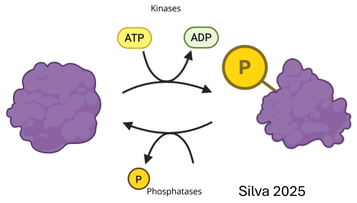


- The kinetochore is a protein structure that attaches to spindle microtubules to create kinetochore-microtubule connections for the separation of sister chromatids

- Mps1 kinase is essential for yeast kinetochore biorientation; it phosphorylates various sites in the Dam1 protein, allowing sister kinetochores to biorient and satisfy the spindle assembly checkpoint (Biggins 2013).

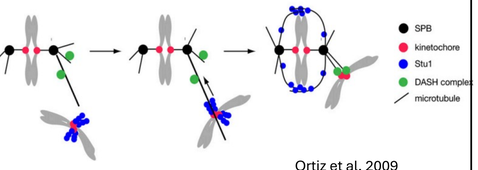
- Mps1 phosphorylation occurs at MELT motifs, short stretches of amino acids where threonine can be phosphorylated, within Spc105 to regulate the binding to kinetochores (London et al. 2012).

- Phosphorylation of proteins induces a change in surface charge, which can change the conformation of the protein
  - Phosphorylation is critical for both the assembly of the kinetochore and its binding of DNA and the microtubule



Silva 2025

- The *STU1* gene is important for proper connection to microtubules. Defects and mutations to the gene have been found to affect the spindle assembly (Pasqualone et al, 1994)
  - Known to have 2 MELT motifs
  - Recognized by Mps1 kinase
  - Involved in the polymerization of microtubules



Ortiz et al, 2009

- Using CRISPR-Cas9, we aim to induce 2 different phosphomimetic mutations at the target site, T719, in the *STU1* gene to determine if the constant phosphorylation at the IDLT716 site in Stu1 will change the phenotypic appearance of budding yeast.

Methods

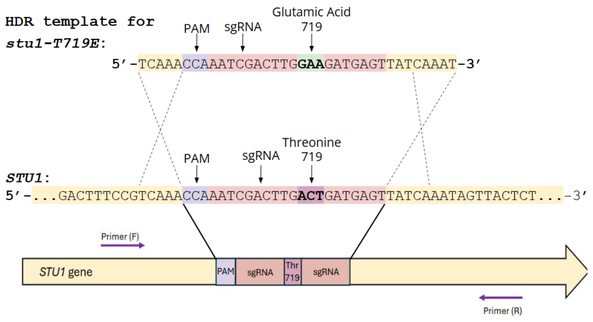


Figure 1: *stu1- T719E* HDR template from Nguyen et al (2023) showing positions of the PAM sequence, small guide RNA binding sequence, and the intended mutation locus 719 and primers used for the PCR reaction. Figure template designed by Neir and Vincent (2024).

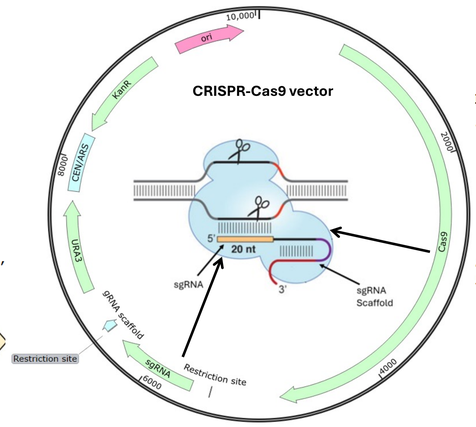


Figure 2: CRISPR:CAS9 Vector
CRISPR vector showing the positions of the relevant genes, including CAS9 and small guide RNA. The vector used in our experiment was created by Pascua and Vincent (2019)

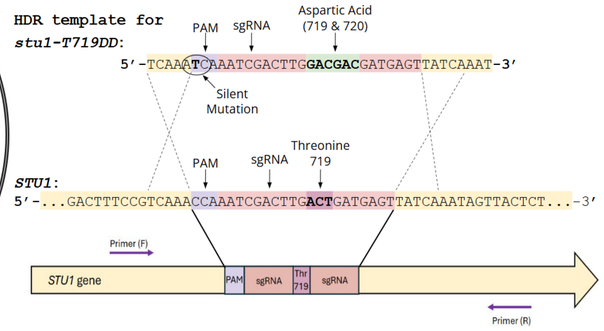


Figure 3: *stu1- T719DD* HDR template from Gombio and Vincent (2023) showing positions of the PAM small guide RNA binding sequence and the intended mutation locus 719, and primers used for the PCR reaction. Figure template designed by H. Neir and J. Vincent.

Figure 5: Yeast Transformation on SD URA-

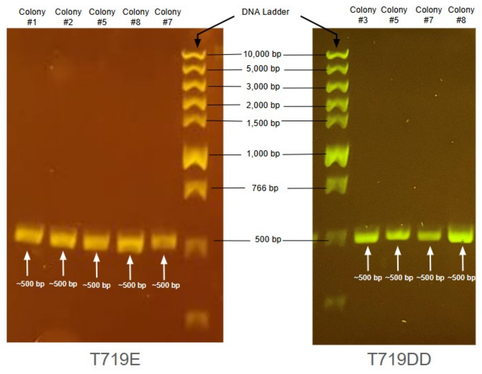


Figure 5: PCR of the middle of *STU* 1 gene
Transformants were isolated by pure culture, then we performed a genomic DNA prep and conducted a PCR reaction to copy the area surrounding our intended mutations. The PCR products were run on a gel alongside a 1 kb DNA ladder. The expected band size for successful PCR was ~500 bp.

Figure 6: Yeast Transformation on SD URA-

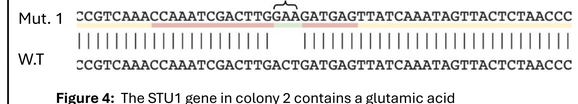
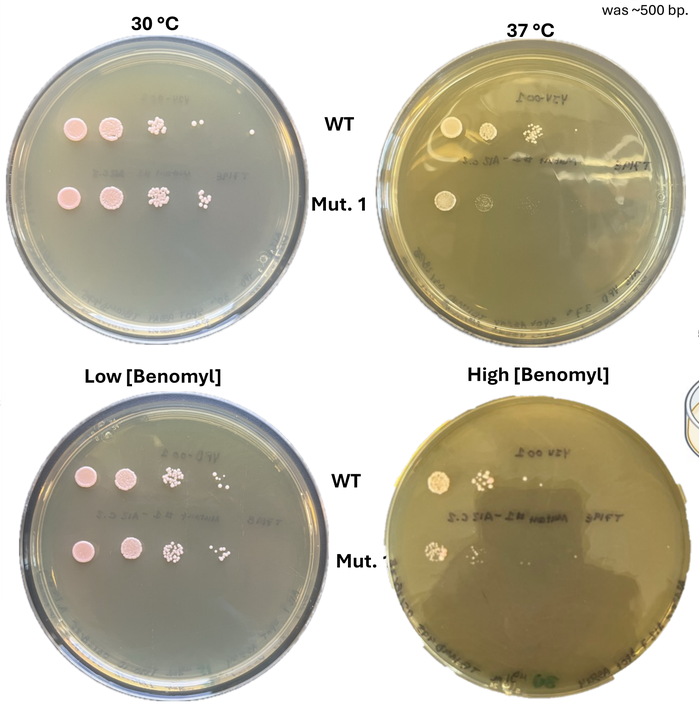
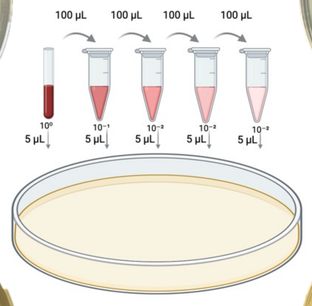


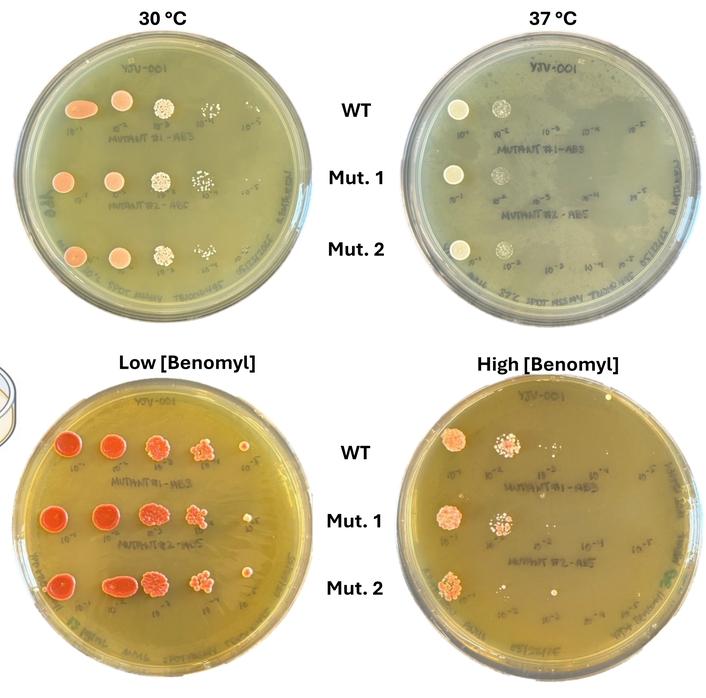
Figure 4: The STU1 gene in colony 2 contains a glutamic acid substitution mutation.

Figure 6: The STU1 gene in colony #3 contains a double aspartic acid substitution mutation



Phenotypic Testing





Conclusion

- Successful yeast transformation
  - CRISPR vector and HDR template transformed into budding yeast, indicated by the growth on the SD-URA plates

- PCR reaction
  - Conducted using forward and reverse primers. The product was the anticipated size ~500 base pairs
  - Desired mutations confirmed

- Sanger sequencing confirmed the presence of desired mutations at 719 in *Stu1*
  - Threonine → Glutamic acid
  - Threonine → 2x Aspartic acid

- Phenotypic analysis
  - No change between wild type and T719DD
  - Slow growth is seen under stress conditions in the T719E mutant

Future Directions

- Investigate T719E mutant
  - Sequence more mutants from colony #2 to identify if the desired mutation is observed
  - Examine more cellular phenotypes, pinpoint where growth is being delayed

- Design an HDR template to induce a phosphonull mutation at this locus
  - Mutagenize the locus 719
  - Threonine → Valine
  - Investigate the phenotypic effect of phosphonull mutation

- Further investigation of the complex process of cellular division

Acknowledgements

Thank you to our incredible research team for their collaboration; your guidance was immensely appreciated—Hannah Neir for designing the template for the HDR figure. Sue Biggins' lab for advising on next steps, providing reagents , strains, and project direction.

References