UNIVERSITY of WASHINGTON TACOMA

Using CRISPR to Make a Phospho-mimetic Mutation in the MELT domain within the *STU1* Gene in Budding Yeast

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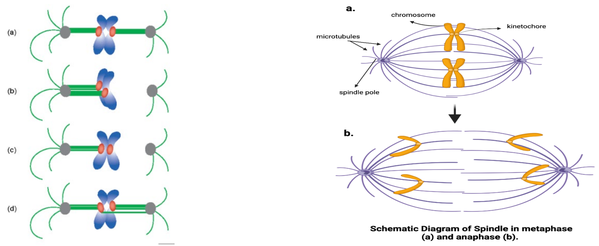
INTRODUCTION AND BACKGROUND

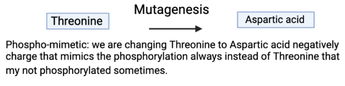
Mitosis results in the production of two daughter cells after the separation of sister chromatids that move towards opposite poles of the cell. This essential process depends on the interaction between chromosomes and spindle microtubules. The kinetochore is a group of proteins connecting the centromeric DNA to the spindle microtubules and plays a crucial role in binding microtubules and facilitating the recruitment of spindle assembly checkpoint (SAC) proteins.



Schematic Diagram of Spindle in metaphase (a) and anaphase (b).

OBJECTIVE

The induction of a phospho-mimetic mutation in the *STU1* gene can reveal the associations phosphorylation events on STU1 protein, and critical cellular processes such as cell division. This research can advance our understanding of gene regulation, cellular responses, and potential therapeutic strategies for contexts in which cell division plays a pivotal role.



Phospho-mimetic: we are changing Threonine to Aspartic acid negatively charge that mimics the phosphorylation always instead of Threonine that my not phosphorylated sometimes.

The MELT domain is crucial for accurate cell division in fungi. Its conservation across species suggests its importance in ensuring proper organization of cellular components during the process of cell splitting. The domain acts like a key fitting into different locks to guarantee proper functioning.

HYPOTHESIS

Our hypothesis is that introducing a modification in the MELT domain of the *STU1* gene to imitate phosphorylation could potentially affect the functionality of the Stu1 protein. This alteration may lead to complications with cell proliferation, potentially resulting in aneuploidy, a condition characterized by an abnormal number of chromosomes in cells.

METHOD

1. Creating effective guide RNAs (gRNAs) for CRISPR vector cloning.
2. Create the HDR (Homology Direct Repair) template illustrated in Fig 1.

stu1-T1034DD Phospho-mimetic Mutation

Forward sgRNA: 5′-gctgggcaacaccttcgggtggcgaatgg GATTAATCCCTTCAAAAACT-3′

Reverse sgRNA: 5′-attttaacttgctatttctagctctaaaac AGTTTTTGAAGGGATTAATC-3′

HDR Template: STU1HRD1034dd:
Mutation (2 aspartic acid)
PAM
Silent PAM mutation

5′-AAGTTCTGTAAGCTTCACTCCCATCGACAA
TAAAAAATCTGAAGGGGATGAGGAATCCGAC
GATGCTGTAGACGAAAATGATGTTAAGAAATG
C**ATGGAAATGGACGAT**ATGATTAATCCC**TTTA**
AAAACTTGGAAACTGATAAAACACTAGAGTTG
AAGAATAACGTTGGAAAAAGAACATCAAGCAC
AGACAGCGTAGTTA-3′

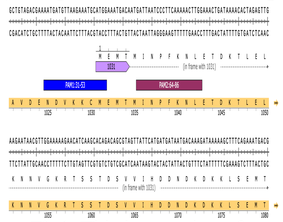
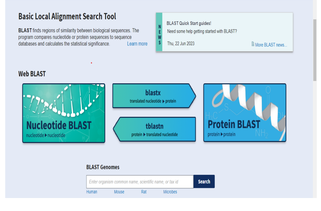


Fig1. This is a section of the *STU1* gene showing the MELT domain that we targeted. And in the blue and red are the nearest possible sequences for the design of our sgRNA. The threonine on 1031 is the one that we are going to be changing to aspartic acid.

3. Insert the sgRNA encoding sequence into the CRISPR vector through cloning.
4. Perform a restriction enzyme digestion on the CRISPR vector and utilize Gibson Assembly to substitute the GFP gene present on the vector with the cloned strain.

5. Examine tblastn search results from the protein sequence and sequencing results of non-glowing *E.coli* colonies using the Blast website.



https://blast.ncbi.nlm.nih.gov/Blast.cgi

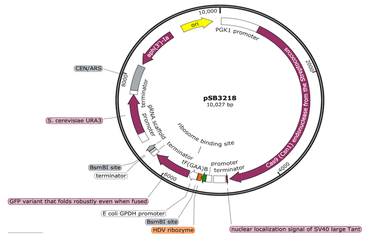
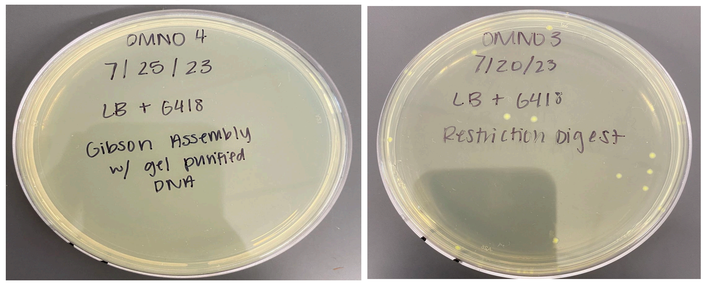


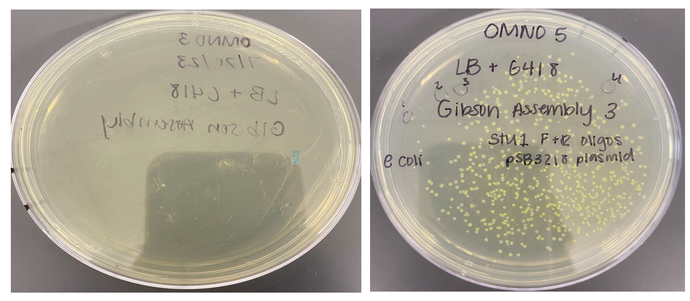
Fig 2. A plasmid Map created in SnapGene of plasmid pSB3218 10,027 bp consist of Ori= E-Coli origin of replication, GFP= green fluorescence protein gene and aph(3')-Ia= G418 resistance gene (antifungals). Replacing the GFP with our gene.

RESULTS

1.Restriction Digest, Gibson Assembly, & *E. coli* Transformation



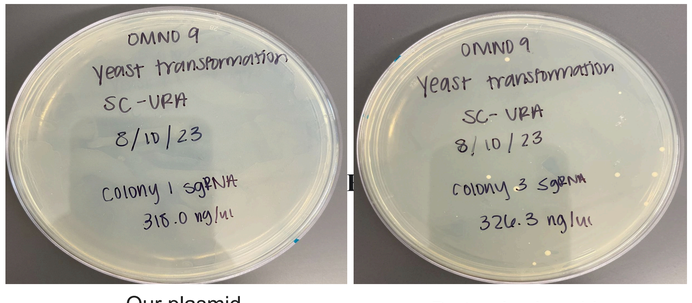
2.Restriction Digest, Gibson Assembly, & *E. coli* Transformation (Third transformation attempt)



Non-glowing colonies (don't contain GFP)

Glowing colonies (contain GFP)

3. Two Yeast Transformation Results



Our plasmid
T'form 1 CRISPR vector

Partner's plasmid
T'form 2 CRISPR vector

Referenc