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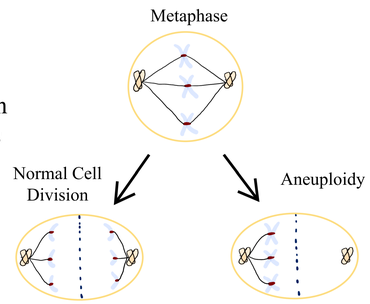
Analysis of Ipl1 Kinase Phosphorylation Sites on the Kinetochore-Associated Protein Stu2 Through Mutagenesis

Kalean Salcedo, Dr. Jack Vincent

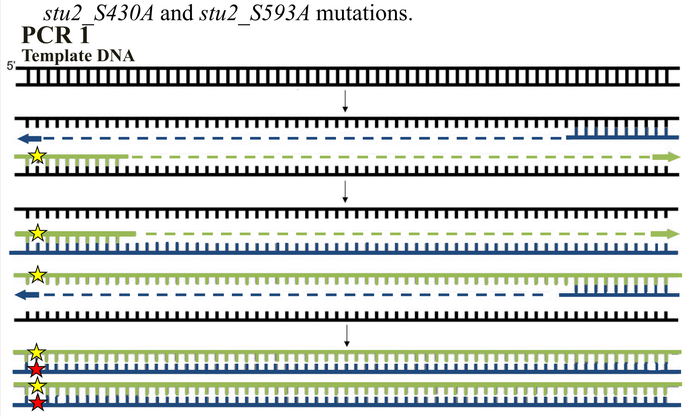
Introduction



Kinetochores are protein structures that play a significant role in biorientation of chromosomes and serves as attachment points for microtubules in chromosome segregation[1,2]. Other proteins collaborate with kinetochores to ensure proper connections are made, strong connections are stabilized, and weaker connections are destabilized to delay chromosome segregation. Previous studies have found evidence that the Stu2 protein and Ipl1 kinase in the model organism *Saccharomyces cerevisiae* have similar functions in regulating tension between kinetochores and microtubules[1,2]. This study aimed to determine if the function of Stu2 is dependent on phosphorylation by Ipl1 kinase by creating mutations at Ipl1 target sites on the *STU2* gene and observing phenotypic consequences in *S. cerevisiae*.

STU2 site-directed mutagenesis

- A forward primer containing a *stu2_S684A* mutation and a reverse primer were designed to introduce a mutation the gene.
- PCR 1 was performed to amplify the region of DNA with our desired mutation, yielding megaprimers.
- Megaprimer-whole-plasmid PCR (MEGAWHOP)[3] was performed to create a mutated version of pJV046, a plasmid containing *STU2* with *stu2_S430A* and *stu2_S593A* mutations.



Images above modified from [4]Mitchell et al.

Transformation in *E. coli*

- The *STU2* containing plasmid with *stu2_S430A, stu2_S593A* and *stu2_S684A* mutations was transformed into *E. coli* to propagate more cells.
- Propagated cells were sent off for DNA sequencing.

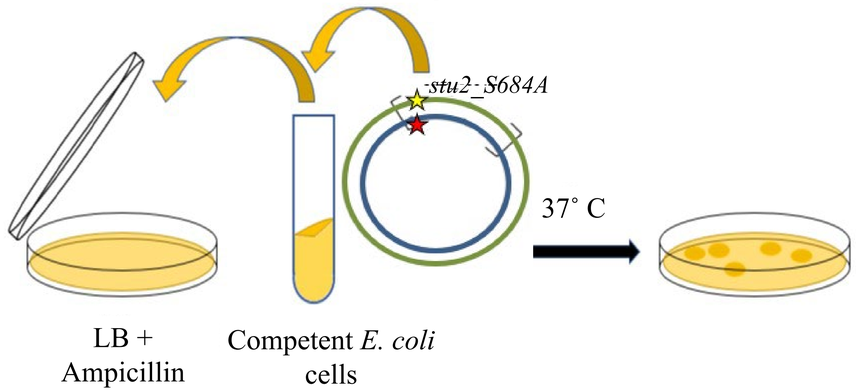


Image above modified from [4]Mitchell et al.

Results: Gel Electrophoresis and *E. coli* LB + Ampicillin Plate

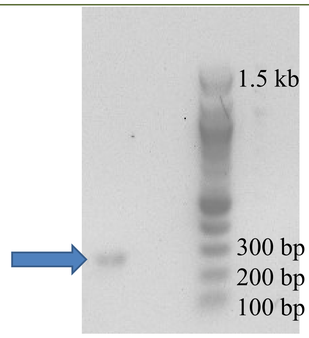


Figure 1: Gel electrophoresis of PCR 1 showed a band between 200 bp and 300 bp of the 100 bp ladder. The expected yield of primers was 264 bp. This confirms that PCR 1 was successful in creating megaprimers for PCR 2.

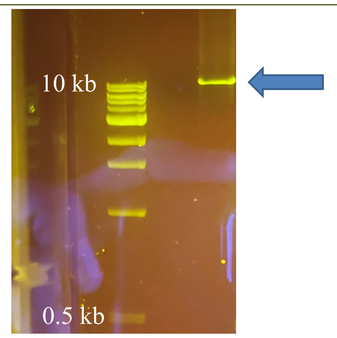


Figure 2: Gel electrophoresis of PCR 2 showed a band that was above the 10 kb mark of the 1 kb ladder. The expected yield of plasmids was 10,674 bp. This confirms that PCR 2 was successful in cloning plasmids for transformation.

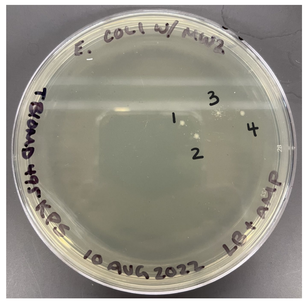


Figure 3: Four colonies of *E. coli* transformed with a *STU2* containing plasmid with *stu2_S430A, stu2_S593A* and *stu2_S684A* mutations, grown on a LB + Ampicillin agar plate after an incubation period of 19 hours at 37˚ C. The resulting colonies were small, white-opaque, round and slightly raised. Bacterial growth on the plate indicates that transformation of *E. coli* with the mutated pJV046 plasmid was successful.

Conclusions

- Sequencing showed that the intended *stu2_S684A* mutation was made onto the template DNA.

```
Query  601   TTTTCCAATAGGGCT
                ||||||||||||  |
Sbjct  2063  TTTTCCAATAGGCTT
```

- The *stu2_S430A* and *stu2_S593A* mutations of the plasmid were checked and confirmed.

stu2_S430A

```
ACAGCAGGT  1380  Query
|||  ||||
ACAGAAGGT  1284  Sbjct
```

stu2_S593A

```
GCACttttttCAGG  900   Query
|  ||||||||||||
GAACTTTTTTCAGG  1764  Sbjct
```

- Transformation into yeast was not accomplished at this time.

Additional Findings

- The blastn showed a K in the query sequence of mutated *stu2* against the subject sequence of wild-type *STU2* upstream of the *stu2_S420A* mutation.

```
Query  1981  KTTTTAAACAG
              ||||||||||
Sbjct  683   TTTTTAAACAG
```

References

[1]Biggins S. 2013. "The Composition, Functions, and Regulation of the Budding Yeast Kinetochore." *Genetics* 194 (4): 817-846.
[2]Miller, M P., Charles L. A, and Biggins, S. 2016. "A TOG Protein Confers Tension Sensitivity to Kinetochore-Microtubule Attachments." *Cell* 165 (6): 1428-1439.
[3]Miyazaki K. 2011. MEGAWHOP Cloning: A Method of Creating Random Mutagenesis Libraries via Megaprimer PCR of Whole Plasmids. Methods in Enzymology. Methods Enzymol. 498: 399-406.
[4]Mitchell A., Makalena M., Vincent J. 2019. Mutational Analysis of Potential Phosphorylation Sites on the Kinetochore-Associated Protein Stu2. Poster session presented at: Sciences and Mathematics Undergraduate Research Symposium. University of Washington-Tacoma.

Acknowledgements

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