UNIVERSITY *of* WASHINGTON TACOMA

# The Role of Phosphorylation in the SK-rich and C-terminal Region of the Kinetochore-Associated Stu2 Protein in Budding Yeast


**Skyler Winston, Jack Vincent Ph.D.**


## INTRODUCTION

Chromosome segregation is an important process which is involved in mitosis, if not done properly it can lead to many genetic malfunctions such as aneuploidy. Kinetochores are multi-protein complexes whose function is also a strong factor which segregates chromosomes. They require proper tension and attachment in order to meet the spindle checkpoint requirements and continue its process. Improper segregation can lead to nondisjunction and aneuploidy, which in humans can lead to a variety of genetic disorders including cancer. (Figure 1).

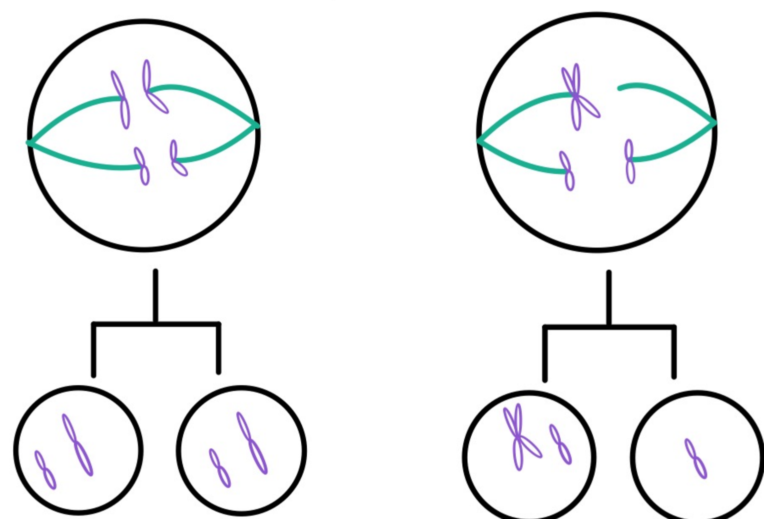

**Figure 1.** To the left is normal cell division and on the right is nondisjunction. Nondisjunction may cause aneuploidy.

In order to get a better understanding of segregation we are using yeast as a model organism to understand the composition and function of kinetochores. My project focuses on Stu2, a protein which plays an important role in microtubule function by promoting proper chromosome attachment contributing to kinetochore function.

Stu2 phosphorylation from asynchronous cultures

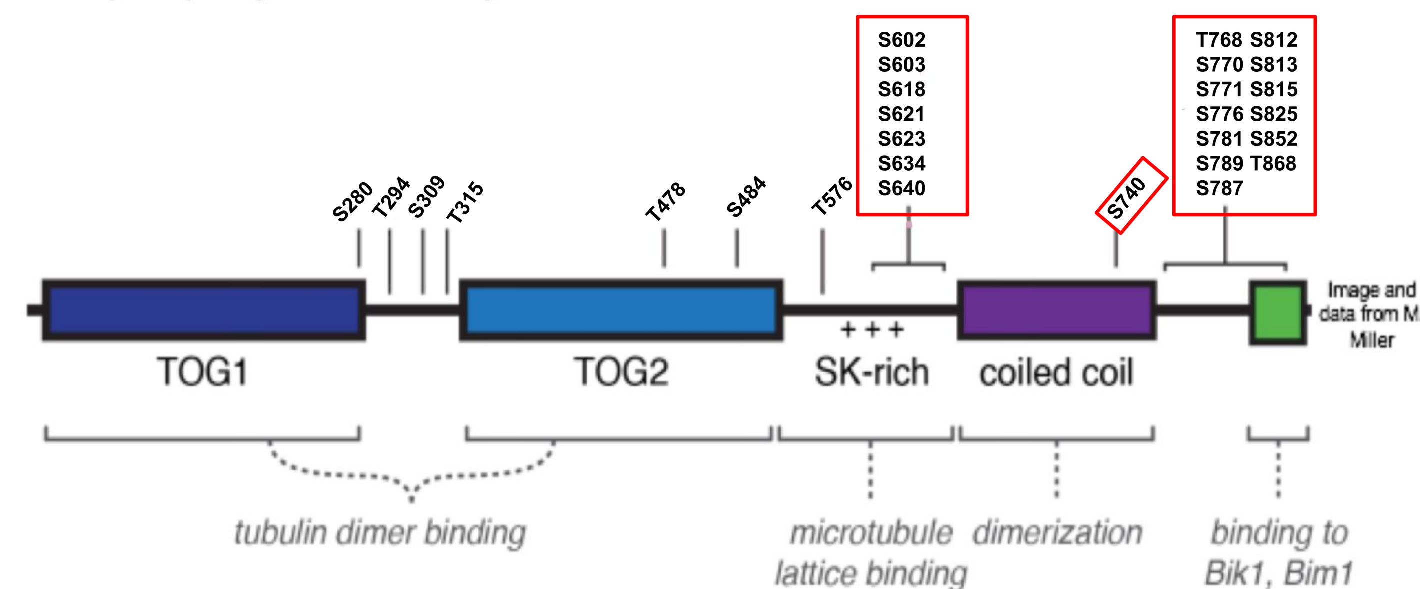


**Figure 2.** Domains and phosphorylation sites in the Stu2 protein (courtesy Matthew Miller). The rectangular regions in red indicate the regions to be mutated within the SK-rich and C-terminal regions. The red box present around the S740 amino acid represents the mismatch base present in the megaprimer.

My experimental approach focuses on the SK-rich and C-terminal regions phospho-null mutations (Figure 2). By combining these mutants, we can study whether these phospho-null amino acid sites are essential for cell growth, Previous experiments have mutated many of these sites in either the SK-rich region or C-terminal region, however those mutations have not noticeably affected the *stu2* function in yeast.

## METHODS AND MATERIALS

In order to generate a new *stu2* plasmid with the SK-rich and C-terminal mutations, I amplified a megaprimer from a previously constructed single-integration vector which had the C-terminal mutations. The forward primer I created contains a mismatch base at the S740A site shown in Figure 2. MegaWHOP PCR integrated the megaprimer into a previously constructed *stu2* plasmid which contains the SK-rich regions phospho-null mutations. This process created an entirely new plasmid with all mutations present which can be confirmed by the results in Figure 4 and Figure 5.

### Megaprimer Amplification & MegaWHOP PCR

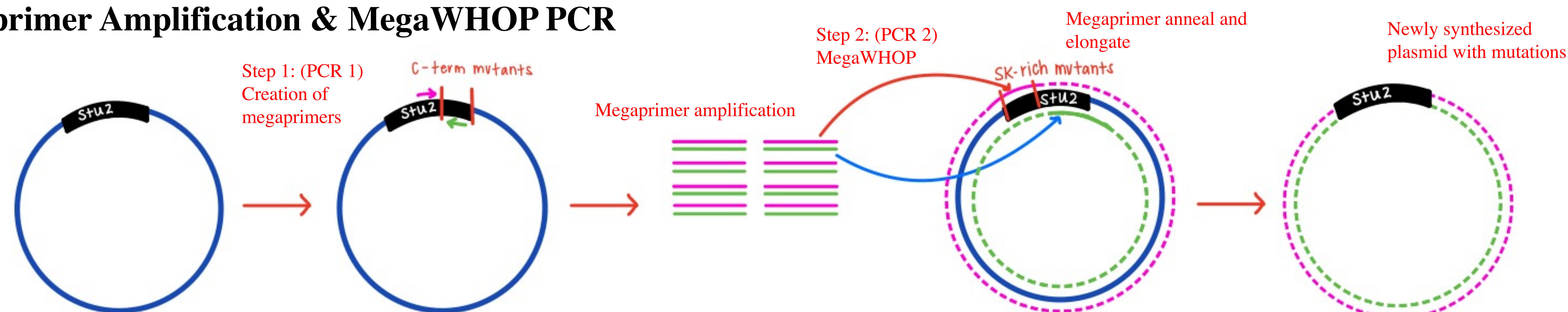


**Figure 3.** MegaWHOP PCR cloning. Megaprimer was amplified with desired C-terminal mutations (courtesy Khalil Deveaux). Template plasmid used contained SK-rich mutations (courtesy Fiona Ng).

## RESULTS

**Gel electrophoresis** was performed to check the results of the mismatch-base megaprimer designed and the MegaWHOP PCR products.

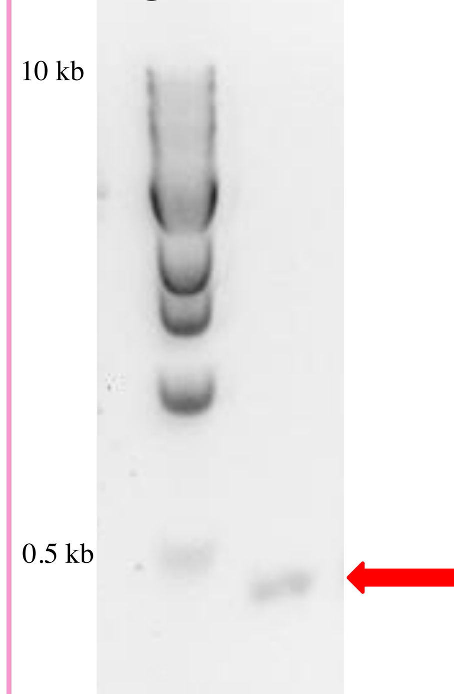


**Figure 4.** Result of gel electrophoresis of megaprimer amplification. A 1 kb ladder was used. Our PCR product is the expected band size confirming the PCR reaction was successful.

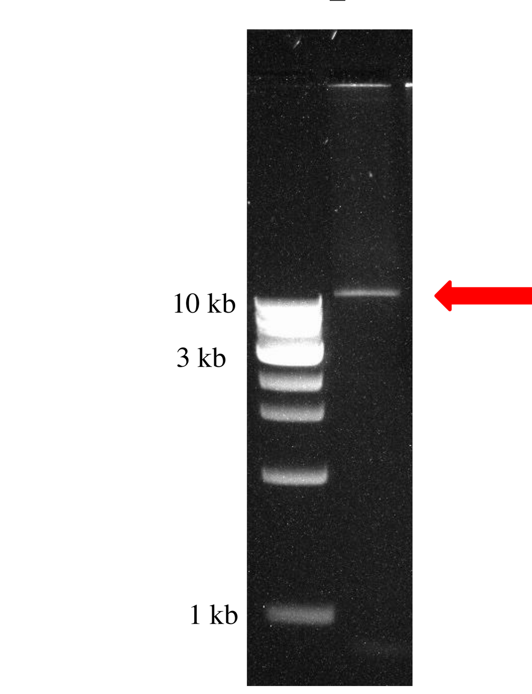


**Figure 5.** Result of gel electrophoresis of MegaWHOP product. A 10 kb ladder was used. Our PCR product is the expected band size. The PCR reaction was successful.

### Transformation Results

After successful MegaWHOP PCR, the *stu2* plasmid with all desired mutations was transformed into E. *coli*. Figure 7 below confirms successful transformation with the presence of bacterial colonies.

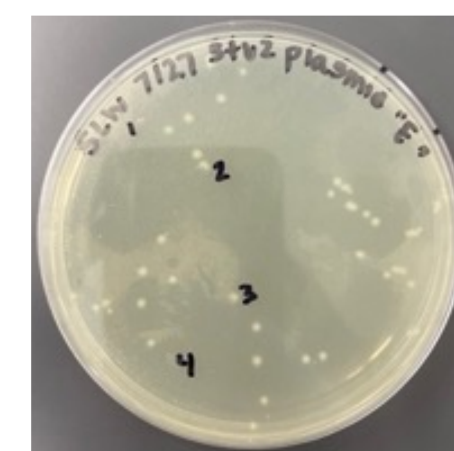

**Figure 6.** Experimental YPD plate of *stu2* plasmid transformed into E. *coli*.

Currently waiting on gene sequencing results to confirm the mutations present in the newly made Stu2 plasmid.

## NEXT STEPS

Following transformation, purified DNA must be sequenced to confirm that all mutations are present within the newly created plasmid. Once, sequencing confirms mutations the DNA should be introduced into yeast to study phenotypical results. By attempting phenotypical tests, we many be able to infer that the function of the phosphorylation sites are essential to the function of the Stu2 protein which may contribute to cell viability and further understanding of aneuploidy.

## REFERENCES


[1]Humphrey L, Felzer-Kim I, Joglekar AP. 2018 Stu2 act as a microtubule destabilizer in metaphase budding yeast spindles. MBoC. 29(3) 247-255. [2]Miller MP, Asbury CL, Biggins S. 2016. A TOG Protein Confers Tension Sensitivity to Kinetochore-Microtubule Attachments. Cell Press. 165:1428-1439. [3]Miller MP, Evans RK, Zelter A, Geyer EA, MacCoss MJ, Rice LM, Davis TN, Asbury CL, Biggins S. 2019. Kinetochore-associated Stu2 promotes chromosome biorientation in vivo. PLoS Genetics. 15(10): [p.25].
[4]Van Hooser AA, Heald R. 2001. Kinetochore function: The complications of becoming attached. Current Biology. 11(21):855-857.


## ACKNOWLEDGEMENTS


I would like to thank Fiona Ng and Khalil Deveaux for their previous mutagenesis research which allowed me to build upon the knowledge of Stu2. I would also like to thank the entire lab staff and my peers this quarter for all their help.