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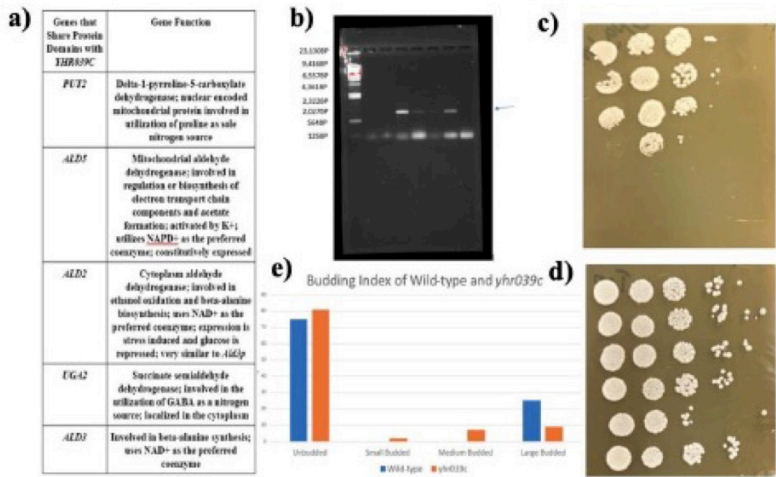
## ERGOSTEROL TRANSPORTATION AND *YHR039c* IN *SACCHAROMYCES CEREVISIAE*

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### Abstract

The budding yeast *Saccharomyces cerevisiae* is a great model organism because it is a single-celled eukaryote, in which the genome has been sequenced entirely. Though the whole genome has been sequenced, there are still genes that have unknown functions. The goal of this study is to find the potential function of the gene, *YHR039c*. Through a series of experiments the function of *YHR039c* was examined using bioinformatic searches and wet lab experiments. Using *YHR039c*'s DNA and protein sequences, many databases were used to find information like protein domains and cellular localization. The results from the bioinformatic investigations showed *YHR039c* is in the endoplasmic reticulum and affects structure. Then using a series of PCR reactions, the strain that had the knock-out of *YHR039c* was created, and then the disruption of *YHR039c* was confirmed using another PCR reaction. Lastly, using spot assays and morphological analysis, the growth of *YHR039c* yeast was compared to the wild-type strain.

Key words: *Saccharomyces cerevisiae*, Ergosterol, *YHR039c*, Molecular Biology Laboratory course, GUF-gene of unknown function, budding yeast, *S. cerevisiae*, single-celled eukaryotic organism, knock-out gene



(a) A table containing the interacting proteins that share a protein domain with YHR039C from GeneMANIA. (b) Gel image obtained from the confirmation PCR from the YHR039c knockout strain. Gel was ran using a Lambda DIII marker at 110 volts for 30 minutes. The blue arrow represents a PCR product that results from the successful knockout of YHR039c with URA3. (c) A plate containing 5  $\mu$ M of Nystatin with the WT strain and the YHR039c knockout strain. The top three rows contained dilutions of the WT strain. The bottom three rows contained dilutions of the YHR039c knockout strain. Plate was incubated at 30°C for three days before being imaged. (d) A YPD control plate containing the Wild Type strain in the top three rows and the YHR039c knockout strain in the last three rows. (e) A budding index comparing the number of budded/unbudded cells between the Wild Type Strain and the YHR039c Deletion Strain. 100 cells of each strain (WT and YHR039c) were measured.

## Methods and Description

### Bioinformatics

Utilizing various biological databases, the interactions YHR039c has with other genes in the *S. cerevisiae* genome were able to be identified. *Saccharomyces* Genome Database was used to learn YHR039c's genetic sequence. Genemania.org was utilized to plot out the various genetic and physical interactions that YHR039c has; which was then used to hypothesize the GUF's involvement with the production of sterol for use in the cell membrane (Figure 1a).

### **Gene Deletion PCR**

During gene deletion PCR, a PCR product was created that would be used to delete the *YHR039c* gene and replace it with the *URA3* gene. Three reagents were then added to a tube. Then the reagents were run in the thermocycler. Then 1  $\mu$ /mL of DpnI; a restriction enzyme that would eliminate background transformants of the plasmid DNA. Since the *YHR039c* is unique, specific forward and reverse primers were used to target the first 22 base pairs and last 20 base pairs of *URA3*. To check if the gene deletion PCR worked, the PCR products were run using gel electrophoresis using Lambda dIII as the marker.

### **Yeast Transformation**

A yeast transformation with the previous PCR product was used to replace the *YHR039c* gene with *URA3*. This began by preparing two frozen competent yeast cell aliquots with the appropriate reagents. The most important reagent added was the PCR product. The mixture was then added to the cell pellets and each were incubated for at 42°C (Gietz, et al). The tubes were then removed from incubation and centrifuged. YPD liquid media was added to both tubes and then incubated again for 2 hours at 30°C (Gietz, et al). The tubes were centrifuged again, and the cell suspension was added to petri dishes (-URA) and left to incubate at 30°C for 4 days (Gietz, et al). When the plates were observed after their incubation, it was clear that colonies only formed on the plate with the PCR product added to it. More than 90 transformant colonies were recorded on the plate. The negative control plate showed no visible colonies.

### **Confirmation PCR**

Throughout confirmation PCR, two of the yeast colonies that were made from the yeast transformation above were used. The yeast colonies were isolated from the petri dish and labeled. They were then collected in microfuge tubes that contained lithium acetate/SDS

solution (Lööke et al., 2011). Different amounts of ethanol were then added to the microfuge tubes and removed to isolate the pellet. After the pellet was isolated, water was then placed in the tube to dissolve the pellet (Lööke et al., 2011). One  $\mu\text{L}$  of DNA from each yeast colony (1 and 2) was then added to two labeled PCR tubes each with 5  $\mu\text{L}$  of both the forward and reverse primers. The forward and reverse primers that were used were JKS138 and JKS003. The forward primer annealed to the antisense strand upstream of the START codon. Whereas the reverse primer annealed to the sense strand downstream of the STOP codon. The PCR reaction was then run for both tubes. The other half of each colony was then transferred to two -URA plates, one for each colony.

The next portion of confirmation PCR was then completed to check that *YHR039c* was successfully removed from the DNA and replaced with *URA3*. To determine if *YHR039c* was successfully removed, the PCR products were run on an agarose gel using gel electrophoresis. This was to separate the DNA fragments into clear bands. If a band was clear around 700-900 bp, the *YHR039c* gene was successfully replaced by *URA3*. The standard Lambda DIII was used in well 1. Positive results from the gel imaging were observed. Three clear bands were found between the 500 and 2000 bp standard bands, suggesting the size of between 700 and 900 bp (Figure 1b). This marked success in the removal of the *YHR039c* gene as the PCR product should have been around 700 to 900 bp. The confirmation PCR product was detected in lanes 4, 5, and 7 and was most prevalent in reaction 1 made by Claire. As a result of that product being the clearest band, the "Claire 1" colony was the one used for the spot assays.

### **Spot Assay Analysis**

The next step in the research process was to perform spot assays of both the wild type strain and *YHR039c* knockout strain on medias that contained YPD and stress conditions. During this process,

both the wild type strain and the *YHR039c* knockout strain were diluted in a tenfold serial dilution using a microtiter plate. 2  $\mu$ L of each cell suspension was then transferred from the microtiter plate to each agar plate. The control YPD plate was used, along with three other YPD plates with stress conditions: 80  $\mu$ g Fluconazole, CaCl<sub>2</sub>, and 5  $\mu$ g Nystatin. Once spotted, each plate was incubated at 30°C until a significant amount of growth was observed. This allowed the number of colony forming units for each strain to be quantified. This data allowed us to compare the growth of the wild type and *YHR039c* knockout strain to determine the affects each stress condition had on *YHR039c* knockout strain (Figure 1c-d).

### **Morphological Analysis**

The last piece in completing the project was performing a morphological analysis of the wild-type strain and the *YHR039c* knockout to compare how the cell cycle is affected in each strain. During this part of the experiment 100 yeast cells were counted in each strain. These cells were collected by suspending the log-phase yeast cells in a phosphate buffer and then making a slide to put underneath the microscope to take photos of the cells. This gave the ability to visualize how the cell cycle was affected in the wild-type and the *YHR039c* knockout strain. Some similarities observed in the budding index between wild-type and *YHR039c* is the percent of unbudded cells present. When comparing the percentages, the wild type had 74% of its cells unbudded and the *YHR039c* knockout had 81% of its cells unbudded. A noticeable difference is the percentage of large buds each strain had, the wild type strain had 25% of its cells as large buds whereas *YHR039c* has 9% of its cells as large buds. Another key difference is the wild type did not have any small or medium buds, but *YHR039c* had a small percentage of small buds (2%) and medium buds (7%). Based on the results we were able to see a shift in the amount of large, budded cells presents. There was a noticeable decrease in large,

budded cells in the yeast strain with the *YHR039c* knock-out. This leads to the conclusion that *YHR039c* affects the G2 phase of the cell cycle and mitosis. This is because there was 24% of large, budded cells in the wild-type strain and then when *yhr039c* was deleted the percent of large, budded cells present decreased to 9%, this decrease can also be visualized in the bar graph attached above (Figure 1e).

### Reagents

Strain	Genotype	Source
Wild Type Strain (BY4741)	<i>YHR039C</i>	Lab Stock
Deletion Strain	<i>yhr039c::URA3</i> in BY4741	This Study
Plasmid	pRS406	Provided by Instructor
Confirmation PCR Forward Primer	Annealed to the region immediately upstream of the START codon on the antisense strand	Provided by Instructor
Confirmation PCR Reverse Primer	Annealed to the region immediately downstream of the STOP codon on the sense strand	Provided by Instructor

### Statistical Analysis

The use of a Two-Way Anova Test was needed to compare the data from the spot assays and quantitate it. The stress conditions using CaCl<sub>2</sub> and Fluconazole had no statistically significant difference in growth between the wild type or the *YHR039c* knockout according to the Two-Way Anova Test. The stress condition of Nystatin, the colony forming units in the wild type of strain decrease only slightly by less than 0.5 CFU, whereas the colony forming units in the *YHR039c* knockout decreased by approximately 2.0 CFU. This suggested that the *YHR039c* plays a role in the integrity of the plasma membrane as there was a statistically significant decrease in colony forming units when *YHR039c* is not present. ■

## REFERENCES

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