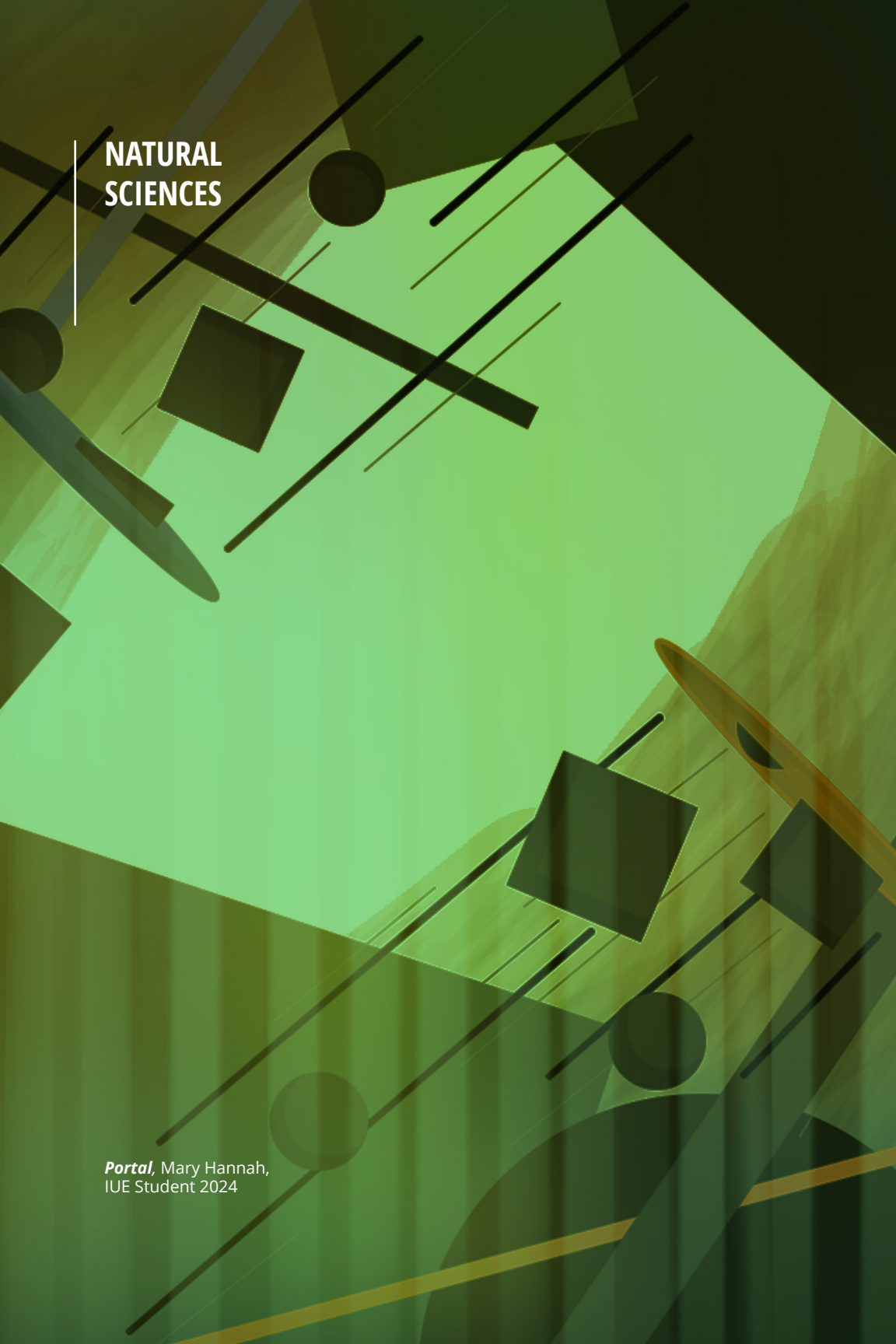
NATURAL SCIENCES



Portal, Mary Hannah,
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EFFECTS OF *YJR118c* GENE DELETION ON GROWTH OF *S. CEREVISIAE*

Gracie Russell and Korinna Schindler

Abstract

To determine effects of a gene deletion of *YJR118c* in the *S. cerevisiae* yeast strain and the function of this gene within yeast, several techniques were utilized. *YJR118c*, a part of the ILM1 family, was replaced with the *URA3* gene to observe effects at the cellular level. Bioinformatics research and wet lab procedures were completed to observe this gene further. Gene deletion, confirmation PCR, two rounds of spot assays, and morphological analysis were all utilized to obtain results. Our results suggest that *YJR118c* is cytoplasmic with a chance of being localized to the endoplasmic reticulum of the cell, specifically relating to ER retention signals.

Key words: *YJR118c, S. cerevisiae, yeast strain, gene deletion, ILM1 family, URA3 gene, bioinformatics, wet lab procedures, cytoplasmic, endoplasmic reticulum, ER retention signals, spot assays, confirmation PCR, morphological analysis*

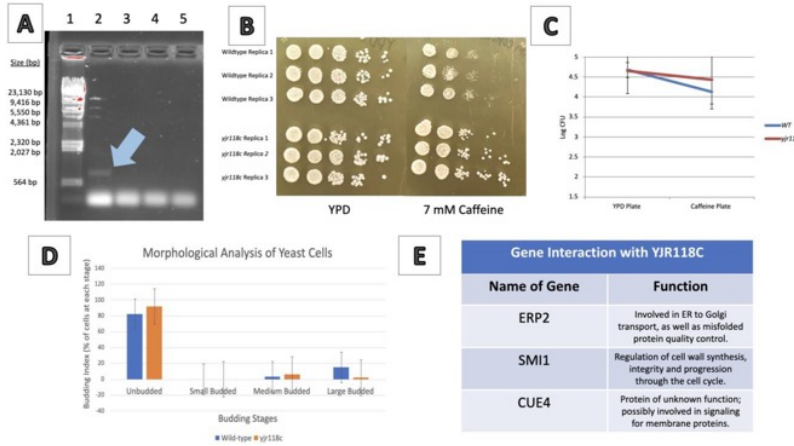

Figure 1: *Results of YJR118c genome attribute testing. A: The agarose gel image was obtained by running a sample of yjr118c confirmation PCR product on an agarose gel following the procedures of gel electrophoresis to confirm a successful disruption of the gene with URA3. An arrow indicates the location of the PCR product in well 2. B: This image presents the growth of wildtype (top three rows) and YJR118c yeast (bottom three rows) under two different conditions; the YPD as a control and the stress condition of 7 mM of caffeine to detect and observe differences in growth. Both plates were incubated at 30°C for two days. C: This image depicts the results of colony forming units (CFU) between YJR118c and wildtype under the conditions of caffeine and on YPD; results are from the spot assay imaged in B. D: Morphological data using the ImageJ software was completed; results are shown in the graph. Based on the data, wildtype cells were found to have more budded cells compared to YJR118c. E: Related genes to YJR118c are displayed in the table from data presented in our bioinformatics research. These genes were the most reoccurring and prominent; providing an increased possibility of relation with the gene under study, YJR118c. Information was found while conducting research through GeneMania.*

Description

YJR118c comes from the family domain of ILM1, which is a part of the yeast *S. cerevisiae*. This gene has no known function, but through bioinformatics research that was conducted, it was found that this gene is associated with the cytoplasm with a high chance of being localized within the endoplasmic reticulum (ER).

Results were obtained through various procedures conducted

within the lab. The gene deletion lab, which was conducted only once, demonstrated significant results with one significant band at 900 bp. This PCR product was then used for the yeast transformation procedure with the goal of deleting *YJR118C*. In one cycle of this experiment, plates were incubated for four days at 30°C, and the results were determined through examination of the plates the following week. The positive -Ura plate contained a mixture of different sized colonies ranging from small to large. All these colonies indicated that the yeast grew in the presence of –Ura. The –Ura plate was used for the confirmation PCR, which demonstrated successful results based upon lane two containing the *YJR118c* deletion strain with a band of approximately 700 bp (See Figure 1A). Additionally, two rounds of spot assays were conducted on different media, one being 7 mM caffeine which showed that *YJR118c* seemed to grow better than the wildtype strain under this stress condition (See Figure 1B and 1C). The budding index experiment concluded that *YJR118c* did not contain as many budded cells as the wild type. There could be a possible delay in the G1 phase of the cell cycle (See Figure 1D).

The wild-type strain was observed to be more sensitive to caffeine growth as its growth was stunted compared to *YJR118c* which presented little to no effect under this stress condition (Figure 1B). Without *YJR118c*, protein traffic and communication within the cell may be affected based on the research. A decrease or impairment of functionality within the cell as a whole could result from the absence of *YJR118c*. Future direction would include a repetition of morphological analysis to produce more reproducible results and the further analysis of *YJR118c* yeast strain to observe its effects on the cell membrane by conducting growth of a spot assay under the condition of 5-15% ethanol.

Methods

Bioinformatics

To obtain information about *YJR118c*, specific databases were used in regard to that specific gene. A conserved domain database was used to find the family that *YJR118c* is a part of. Additionally, GeneMania was used to determine the genes that were most dominant and prominent, indicating the possible relation to *YJR118c* (See Figure 1E). The SPELL database, along with the GO enrichment database, allowed for the determination of the types of genes or pathways that *YJR118c* interacted with. All this data combined through these different databases provided a means for the possible function of the gene *YJR118c* and how it pertains to the cellular component of the cell.

Gene Deletion PCR

To amplify the *URA3* gene in this procedure in replacement of *YJR118c*, a PCR tube containing PCR mix was obtained along with a forward primer, reverse primer and template DNA which were all added. The primers will help to create complementary template DNA. Additionally, the tube then went through a thermocycler until the reaction was complete and was stored at 37°C for incubation with *Dpn*I to remove the plasmid template. Following the incubation, the samples were then stored at –20°C for one week and then used for transformation.

Yeast Transformation

According to the Gietz, et al protocol, the transformation was created using ssDNA,14 µl of the gene deletion PCR product, PEG3350, and Lithium Acetate (Gietz & Schiestl, 2007). For each plate, 200 µl of cell suspension was added to –Ura plate. Once the cell suspension was delivered, the inoculum was then spread with a glass rod. The liquid was absorbed into the medium through incubation at room temperature. Additionally, the plates were inverted and incubated at

30°C for four days.

Confirmation PCR

Two isolated colonies were extracted from 2 separate transformation agar plates from the Yeast Transformation lab and were used to create DNA samples for performing a PCR reaction. The purpose of running a PCR reaction was to detect whether the *YJR118c* was disrupted by the *URA3* gene. One µl of the DNA supernatant was obtained from a single yeast colony and used to run a confirmation PCR reaction (Looke et, al, 2011). The second part of this procedure involved streaking the other half of the two colonies used in the first part. A sterile toothpick was used to gently obtain the colony and streak it on URA plates using the streaking yeast procedure for isolated colony growth. Labeled plates were then placed in the 30°C incubator for 2 to 4 days.

To run the confirmation PCR reaction, the DNA was mixed with forward and reverse primer (JKS 144 and JKS003) which were used to create template DNA both upstream and downstream of the target sequence. These primers anneal to the complementary sequences of the template. It was then incubated in a thermocycler. Results were analyzed by agarose gel electrophoresis (Figure 1A).

Spot Assay

A 10-serial fold dilution within the wells of a microtiter plate was conducted using the first three rows for the wild- type strain and the second three rows for *YJR118c*. This experiment was carried out by moving 10 µl of the cell suspension was added to 90 µl of sterile water in the adjacent cell to get a suspension of the yeast. Serial 1:10 dilutions were made four times with a starting concentration of 0.1 OD600. From these dilutions, 2 µl were added to one non-stress condition of YPD and three stress conditions of 7 mM Caffeine, 100 mM of CaCl2, and 5 and 8 mM Dithiothreitol creating a six-by-five

spot assay. This data was quantified through counting the colonies in specific columns for the wildtype and *YJR118c* respectively, and a two-way ANOVA was used.

Morphological Analysis

Log-phase yeast culture of *YJR118c* and wildtype were microcentrifuged. The pellet was then resuspended in 450 µl of Phosphate buffer, and then 50 µl of formaldehyde was added and mixed. After sitting at room temperature for 5 minutes, it was centrifuged. The pellet was again resuspended in 500 µl of Phosphate buffer and was taken for sonication.

Prepared yeast cells were placed on glass slides and set aside to dry for five minutes. The prepared slides were observed under a microscope. Once the yeast cells were focused under the 40x objective, images were captured using a smartphone camera and then were uploaded into the ImageJ software for further observation. The size and composition of the wildtype and *YJR118c* yeast cells were then measured. The frequency of budded versus unbudded cells and the size of each budded and unbudded cell were observed. One hundred of each yeast cell type was measured horizontally and vertically, where measurements were then recorded. Budded cells were separated into three categories: small-budded, medium-budded, and large-budded to compare results.

Reagents

Yeast strain used in this study.

Yeast Strain	Genotype	Source
YJR118C	*yjr118c*::URA3	This study
BY4741	*YDL167C*	IU East Lab

	Final Concentration (mM)	Source
Caffeine	7	IU East Lab
Calcium Chloride ($CaCl_2$)	100	IU East Lab
Dithiothreitol	5	IU East Lab
Dithiothreitol	8	IU East Lab
Yeast Peptone Dextrose (YPD)	N/A	IU East Lab

Statistical Analysis

The Two-Way ANOVA test was utilized to compare quantitative data from the spot assays conducted. This was used to further determine if YPD and Caffeine affected Caffeine affected the growth of each yeast type, *YJR118c*, to compare the colony-forming units. Error bars are shown in Figure 1, panel C, to indicate any error or uncertainty within the measurements gathered and determine its significance. Additionally, the p-value for Figure 1, panel C, demonstrated a p-value of 0.632, which is insignificant due to only doing this part of the experiment a couple of times. ■

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