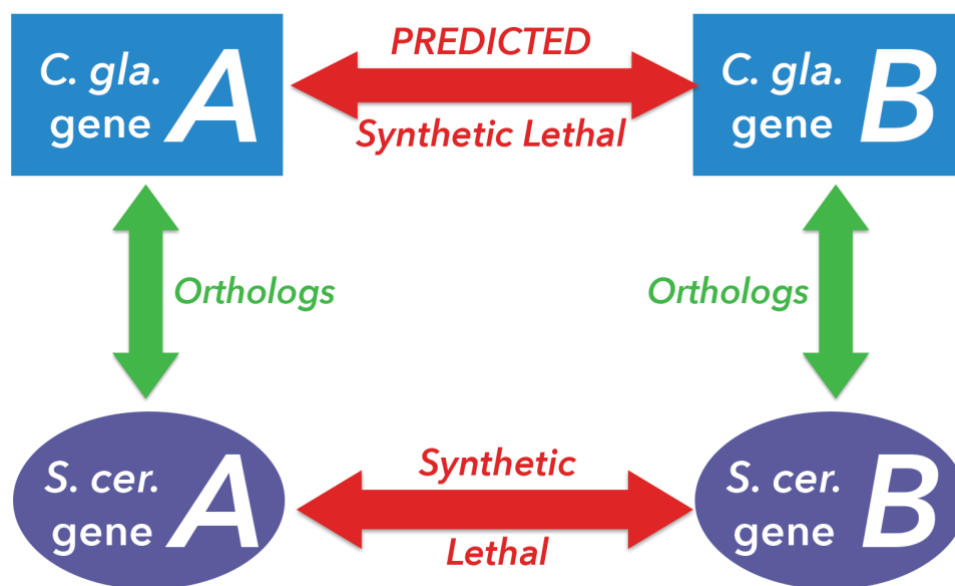


Using *S. cerevisiae* Orthologs to Predict Fungal Pathogen Biology

Antifungal agents such as azoles are used to treat infections with *Candida* species. Unfortunately, the opportunistic fungal pathogen *C. glabrata* possesses a relatively high intrinsic resistance to azoles, and also becomes resistant to azole treatment quickly.

Mitochondrial dysfunction and loss of the mitochondrial genome have been proposed as mechanisms by which *C. glabrata* acquires azole resistance. To exploit the loss of mitochondrial function in resistant *C. glabrata* isolates, researchers may be able to target proteins or pathways that become essential only when the mitochondrial genome is absent. This is based on the idea of synthetic lethality—a type of genetic interaction where the loss of two or more nonessential genes in combination results in cell inviability.

Genetic interactions such as synthetic lethality are richly documented for the budding yeast *S. cerevisiae*, but not as much for many other fungal species. By examining known genetic interactions in *S. cerevisiae*, we can predict synthetic lethal relationships in *C. glabrata* and other fungal pathogens.



If conserved, these synthetic lethal interactions may reveal future antifungal targets for use against azole-resistant strains in the clinic. Using known synthetic lethal interactions in the *S. cerevisiae* genome, predict potentially conserved synthetic lethal interactions for mitochondrial genes in *C. glabrata*.

1. Obtain a list of all genes encoded in the mitochondrial genome of *C. glabrata*:

- On the CGD homepage (<http://www.candidagenome.org>), open the Search tab in the yellow toolbar and select **Advanced Search**.

Candida Genome Database

Home Search GBrowse JBrowse Sequence GO Tools Literature Download Community

BLAST
GO Term Finder
GO Slim Mapper
Text Search
Primers
PatMatch
Advanced Search

New and Noteworthy

***C. lusitaniae* strain CBS 6936 sequence and BLAST datasets now available at CGD**

The sequence and annotation of *C. lusitaniae* strain CBS 6936, described in Durrens *et al.* (2017), has been made available at CGD. We provide downloads for sequences, chromosomal features, gff files and protein domain predictions. In addition, *C. lusitaniae* CBS 6936 is included among the datasets searchable by our multi-species BLAST tool. The sequence and annotation were obtained by CGD from NCBI. (Posted February 27, 2018)

CGD Curation News

- In Step 1 of the Advanced Search, select **Candida glabrata CBS138** as your strain.
- In Step 2, check the “**Select all chromosomal features**” checkbox.
- In Step 3, specify that that you are looking for mitochondrial genes by selecting “**mito_C_glabrata_CBS138**” as the chromosome.

Advanced Search: Search Clear all

Step 1: Select strain (REQUIRED)
• Select a strain to limit search results

Candida glabrata CBS138

Step 2: Select chromosomal feature (REQUIRED)
• Select one or more feature types

☐ ORF ☐ repeat_region
☐ autocatalytically_spliced_intron ☐ retrotransposon
☐ blocked_reading_frame ☐ snRNA
☐ centromere ☐ snoRNA
☐ long_terminal_repeat ☐ tRNA
☐ multigene locus ☐ telomeric_repeat
☐ ncRNA
☐ not in systematic sequence
☐ pseudogene
☐ rRNA

☒ Select all chromosomal features

Step 3: Narrow results (OPTIONAL)

• Select search criteria to return specific types of genes. Results will match all selected criteria.

• Select search criteria by clicking on a checkbox, filling in a dialog box, or selecting a menu option.

• Select or unselect multiple options for Chromosomes and GO terms by pressing the Control (PC) or Command (Mac) key while clicking.

Annotation/sequence properties:

Is a feature that is **AND**

☐ Alternatively spliced
☐ Dubious
☐ Uncharacterized
☐ Verified
☐ not physically mapped
☐ transposable element gene
☐ Merged/Split
☐ Deleted
☐ Deleted from Assembly 20
☐ Deleted from Assembly 21

The default search excludes Deleted features.

Has introns (excluding UTR introns) ☐ Yes ☐ No **AND**

Is on the following chromosome or contig sequence(s): **AND**
(The "All" option includes unmapped features; to specifically exclude unmapped features, select each of the chromosomes of interest rather than "All")

ChrJ_C_glabrata_CBS138
ChrK_C_glabrata_CBS138
ChrL_C_glabrata_CBS138
ChrM_C_glabrata_CBS138
mito_C_glabrata_CBS138

- Click on “Search”. A results page will follow, listing out 37 features in the *C. glabrata* mitochondrial genome.
- Scroll to the bottom of the page and click on the “**Download All Search Results**” link.

CaglIfMt30	tRNA: Uncharacterized	tL(UAA)4mt	Mitochondrial leucine tRNA, has UAA anticodon	mito_C_glabrata_CBS138:17616 to 17697 GBrowse
				Relative Coordinates Noncoding_exon 1 to 82 Chromosomal Coordinates 17,616 to 17,697

Sort by : Systematic Name Go!

Analyze gene list: further analyze the gene list displayed above or download information for this list

Further Analysis:	GO Term Finder Find common features of genes in list	GO Slim Mapper Sort genes in list into broad categories	View GO Annotation Summary View all GO terms used to describe genes in list
	Download: Download All Search Results Download all the data retrieved by query		Batch Download Download selected information for entire gene list. Available information types include Sequence, Coordinates, GO annotations, Phenotype.

Result Page : 1 2 Next

2. Use FungiDB to find *S. cerevisiae* orthologs of *C. glabrata* mitochondrial genes:

- Open the FungiDB homepage (<http://fungidb.org/>). In the “Search for Genes” box, open the “Annotation, curation and identifiers” section and click on “Gene ID(s)”.

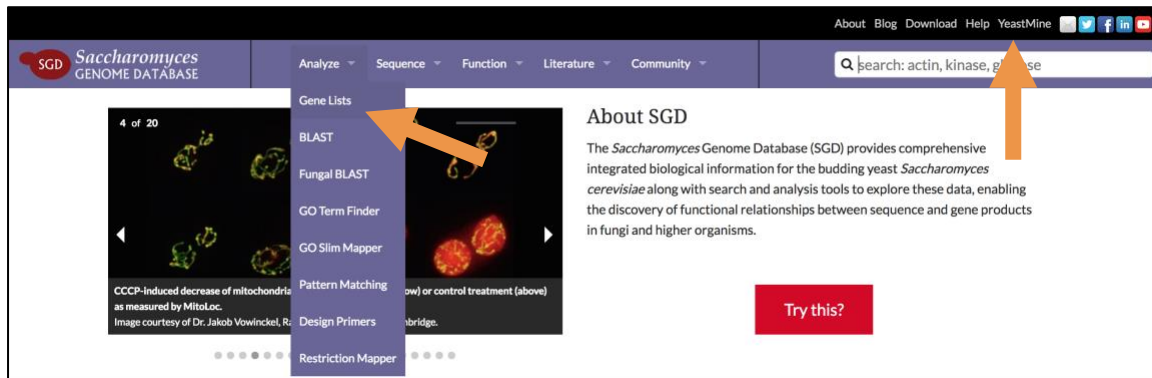
- Using your exported file from CGD, copy and paste the ORF names of the *C. glabrata* mitochondrial genes into the box. Click on “Get Answer”.
- In the Search Strategy panel, click on the “Add a Step” button. In the resulting pop-up window, click on “Transform into related records > Orthologs”.
- In the “Organism” list, search for “cerevisiae”. Select “Saccharomyces cerevisiae S288C”, and then hit “Run Step”.
- 12 orthologs in *S. cerevisiae* will be returned. Download this list by clicking on the “Download” link on the top right side of the table.

Gene ID	Transcript ID	Organism	Product Description	Input Ortholog(s)	Ortholog Group	Paralog count	Ortholog count
Q0045	Q0045.t26.1	<i>Saccharomyces cerevisiae</i> S288C	cytochrome c oxidase subunit 1	CaglIfMp04,CaglIfMp06,CaglIfMp07	OG6_102770	2	58
Q0060	Q0060.t26.1	<i>Saccharomyces cerevisiae</i> S288C	intron-encoded DNA endonuclease	CaglIfMp05	OG6_181774	0	2

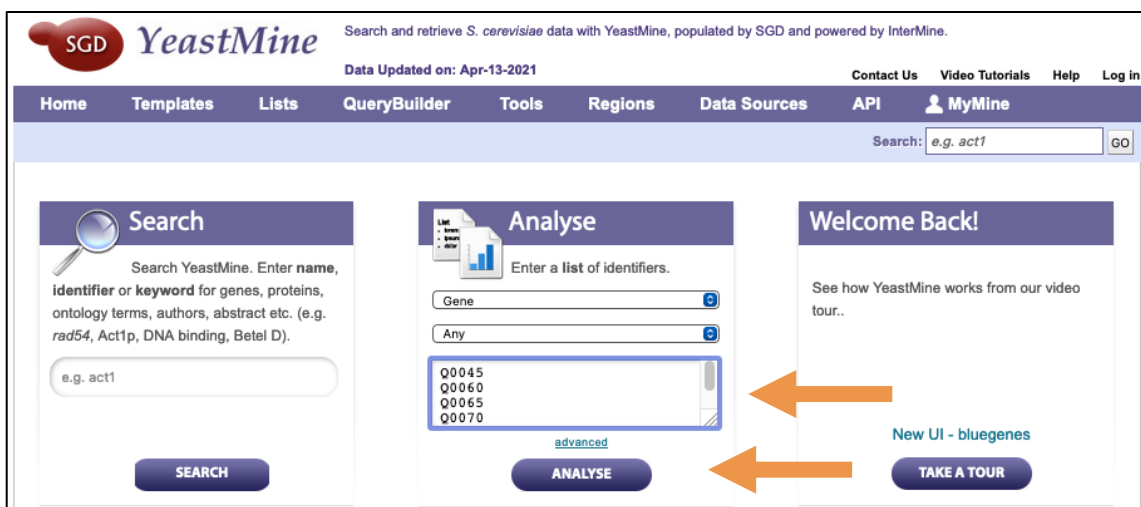
- In the download options menu, select “**Tab delimited (Excel) – choose a pre-configured table**”. Set the Download Type as **Excel File**, then hit **Get**.

3. Import the *S. cerevisiae* orthologs into YeastMine:

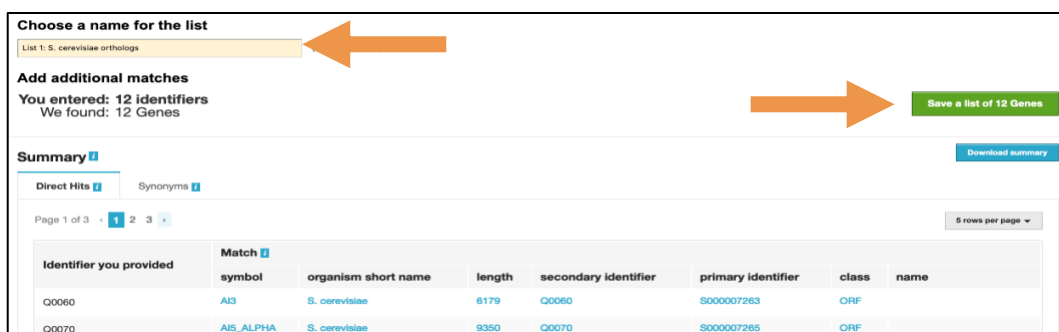
- Open the YeastMine homepage. You can access YeastMine from SGD by opening the Analyze tab and selecting **Gene Lists**, clicking the YeastMine link in the upper right corner of the homepage, or by entering the URL: <https://yeastmine.yeastgenome.org>



- Open the Excel file of *S. cerevisiae* orthologs that you downloaded earlier. To import these orthologs into YeastMine, copy and paste all entries in the **Gene ID** column of the Excel file into the “**Analyze**” box. Then, click on the purple “**ANALYSE**” button.



- A disambiguation page will be shown confirming your matches. 12 results should be shown. Name your gene list something descriptive, such as: “**List 1: *S. cerevisiae* orthologs**”. Click on the green “**Save a list of Genes**” button.

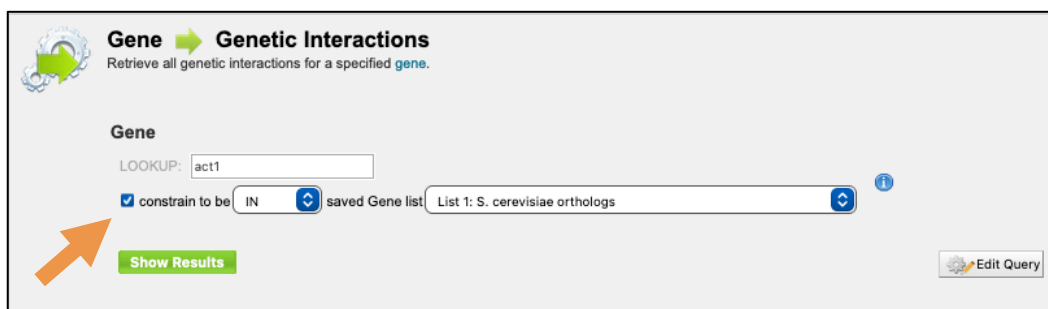


4. In YeastMine, find all synthetic lethal interactions for the *S. cerevisiae* orthologs by using the Gene → Genetic Interactions query:

- Return to the YeastMine homepage: <https://yeastmine.yeastgenome.org>
- In the “popular templates” toolbar in the middle of the page, open the **INTERACTIONS** tab and select the query **Gene → Genetic Interactions**.




- Check the “**constrain to be IN**” checkbox. This allows you to input a list of genes. From the dropdown menu, select the list of *S. cerevisiae* orthologs you saved earlier in part 3. Click on the green **Show Results** button.



- The results table contains all genetic interactions for the input list of *S. cerevisiae* orthologs. To filter for only **synthetic lethal** interactions, find the **Interaction Detection Methods Identifier** column. At the top of this column is a set of small blue icons. Click on the rightmost **View Column Summary** icon, which looks like a bar graph.

Gene

 Genetic Interactions

Retrieve all genetic interactions for a specified gene.

Manage Columns

Manage Filters

Manage Relationships

Save as List

Generate Python code

Export

Showing 1 to 25 of 68 rows

Rows per page:

25

Previous

Next

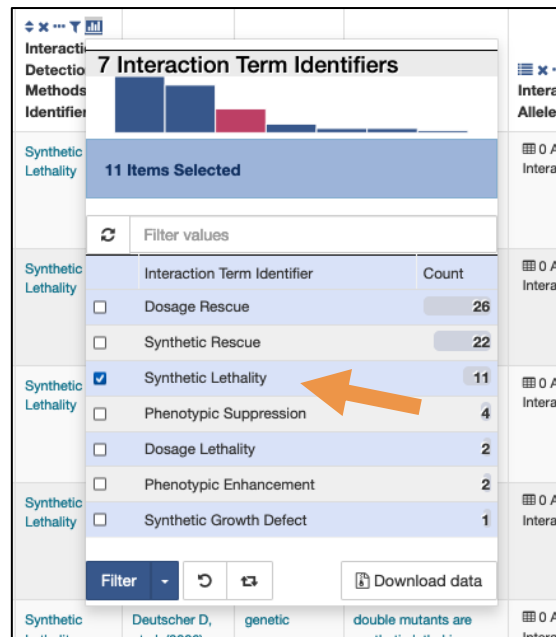
Page 1

Previous

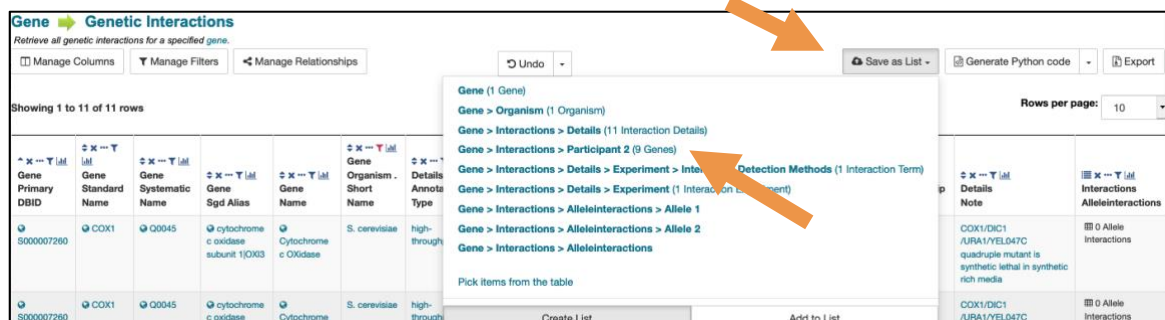
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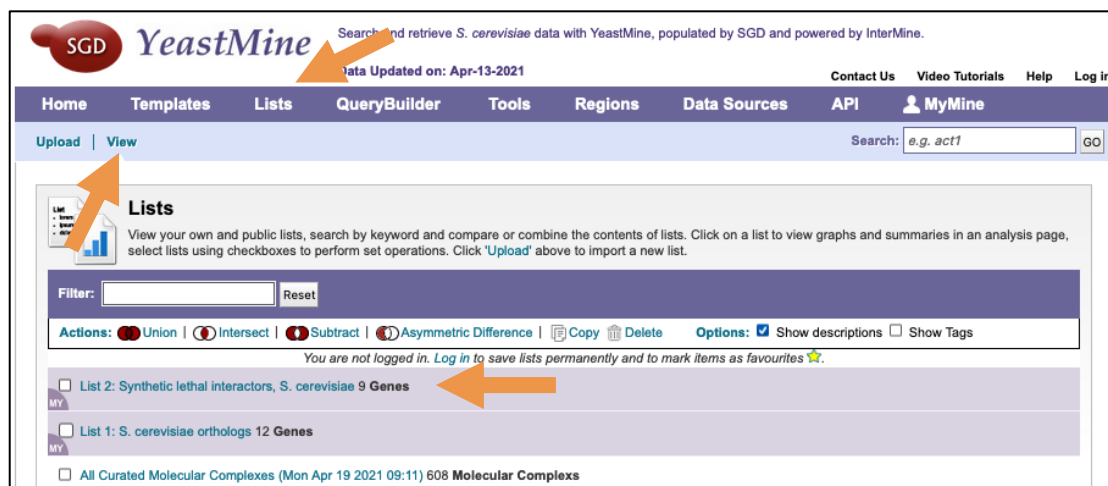
- A window summarizing all entries for this column will open. Check the entry for **Synthetic Lethality** and hit **Filter**.



- The table now contains only synthetic lethal interactions. To save the interactors into a gene list, click on the **Save as List** button and select the entry **Gene > Interactions > Participant 2**. Give your list a descriptive name such as “**List 2: Synthetic lethal interactors, S. cerevisiae**”.



- Access your new gene list by clicking on the **Lists** link in the top purple toolbar. Make sure that the **View** tab is open (see arrows).



- Export the list of synthetic lethal interactors by clicking on the **Export** button, and then on the **Download file** button.

List Analysis for List 2: Synthetic lethal interactors, *S. cerevisiae* (9 Genes)

Manage Columns Manage Filters Generate Python code Export Save as List

Showing 1 to 9 of 9 rows

Gene Primary DBID	Gene Systematic Name	Gene Organism - Short Name	Gene Standard Name	Gene Name
S000000773	YEL047C	<i>S. cerevisiae</i>	FRD1	Fumarate Reductase

Orthologues

A. fischeri NRRL 181 (13) A. flauus NRRL3357 (15) A. fumigatus AF293 (13)
A. gambiae (8) A. nidulans FGSC A4 (16) A. niger ATCC 1015 (16)
C. albicans SC5314 (7) C. albicans WO-1 (7) C. dubliniensis CD36 (7)
C. elegans (21) C. gattii VGII R265 (10) C. gattii WM276 (12)
C. immitis H538.4 (12) C. immitis RS (11) C. neoformans var. grubii H99 (10)
C. neoformans var. neoformans JEC21 (10) C. parapsilosis CDC317 (7)
C. posadasii C735 delta SOWgp (11) D. melanogaster (30) D. rerio (21)
C. glabrata CBS 138 (10) H. capsulatum G186AR (12) H. capsulatum NAM1 (12)
H. sapiens (20) M. musculus (20) M. oryzae 70-15 (12) N. crassa OR74A (12)
R. norvegicus (22) S. cerevisiae (6) S. pombe (8) T. marneffei ATCC 18224 (16)
U. maydis S21 (12)

View homologues in other Mines:

FlyMine D. melanogaster x

5. Import the *S. cerevisiae* synthetic lethal interaction genes into FungiDB for further analysis:

- Open the FungiDB homepage (<http://fungidb.org/>). Similar to part 2 of this exercise, in the **Search for Genes** box, open the **Annotation, curation and identifiers** section and click on Gene ID(s).
- Copy and paste all of the systematic *S. cerevisiae* gene names (YEL047C, YKL141W, etc.) from the downloaded list obtained in part 4 of this exercise. Hit **Get Answer**.
- To the right of the Gene Results table, click on the **Analyze Results** button. Select **Gene Ontology Enrichment** and run an enrichment for Biological Process. Are the results surprising? Remember that these *S. cerevisiae* genes have synthetic lethal interactions with mitochondrial genes. Do the results suggest any biological processes that, if disrupted, might possibly inhibit mitochondria-defective *C. glabrata* clinical isolates?
- Use the “Transform by Orthology” function to convert the *S. cerevisiae* genes into *C. glabrata* orthologs. These *C. glabrata* genes are predicted to have synthetic lethal interactions with *C. glabrata* mitochondrial genes.