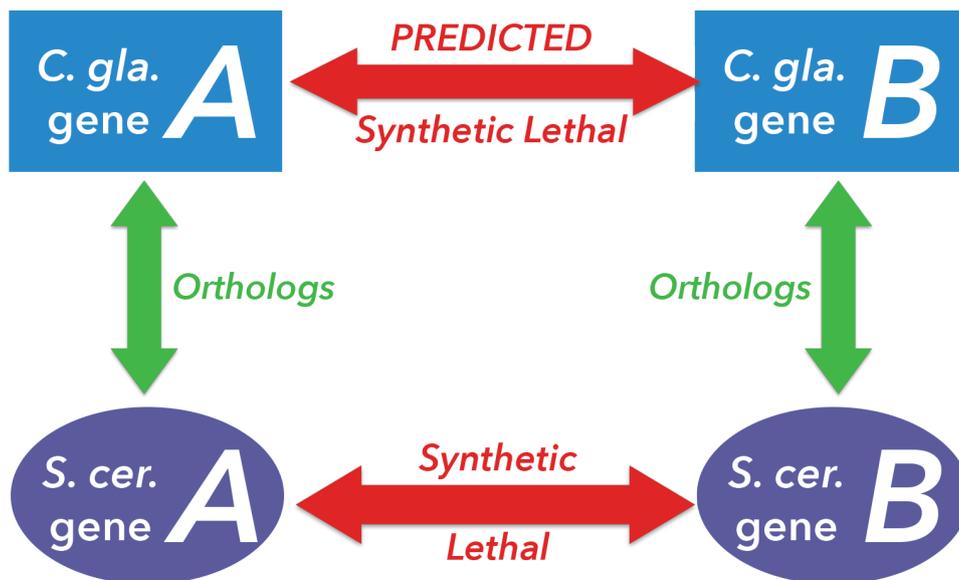


## Using *S. cerevisiae* Orthologs to Predict Fungal Pathogen Biology

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**.

- 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.

- 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.

CaglIfM30	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

<b>Further Analysis:</b>	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
<b>Download:</b>	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)”.

Release 35  
19 Feb 2018

Gene ID: NCU06658 Gene Text Search: synth

Home New Search My Strategies My Basket (0) Tools Data Summary Downloads Community Analyze My Experiment My Favorites

**Data Summary**

News and Tweets

- 19 February 2018 FungiDB 36 Released
- 1 December 2017 FungiDB 35 Released
- 7 September 2017 FungiDB 34 Released

Tweets by @fungidb

FungiDB Retweeted

Jos Houbraken @JHoubraken

Today making photo's for the new edition of our Food and Indoor course book. Next edition will also contain Aspergillus

Tweets by @eupathdb

EuPathDB @eupathdb

**Search for Genes**

expand all | collapse all

Find a search...

- Text
- Gene models
- Annotation, curation and identifiers
  - Gene ID(s)
  - User Comments
- Genomic Location
- Taxonomy
- Orthology and synteny
- Phenotype
- Genetic variation
- Transcriptomics
- Sequence analysis
- Structure analysis
- Protein features and properties
- Protein targeting and localization
- Function prediction
- Pathways and interactions
- Proteomics
- Immunology

expand all | collapse all

**Search for Other Data Types**

expand all | collapse all

Find a search...

- Popset Isolate Sequences
- Genomic Sequences
- Genomic Segments
- SNPs
- ESTs
- ORFs
- Metabolic Pathways
- Compounds

expand all | collapse all

**Tools**

**BLAST**  
Identify Sequence Similarities

**Results Analysis**  
Analyze Your Strategy Results

**Sequence Retrieval**  
Retrieve Specific Sequences using IDs and coordinates

**Companion**  
Annotate your sequence and determine orthology, phylogeny & synteny

**EuPaGDT**  
Eukaryotic Pathogen CRISPR guide RNA/DNA Design Tool

**PubMed and Entrez**  
View the Latest Pubmed and Entrez Results

**Genome Browser**  
View Sequences and Features in the genome browser

**Searches via Web Services**  
Learn about web service access to our data

- 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 red “Add Step” button. In the resulting pop-up window, click on “Transform by orthology”.
- In the “Organism” list, search for “cerevisiae”. Select “Saccharomyces cerevisiae S288C”, and then hit “Run Step”.
- 11 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 Results Genome View Analyze Results

Advanced Paging

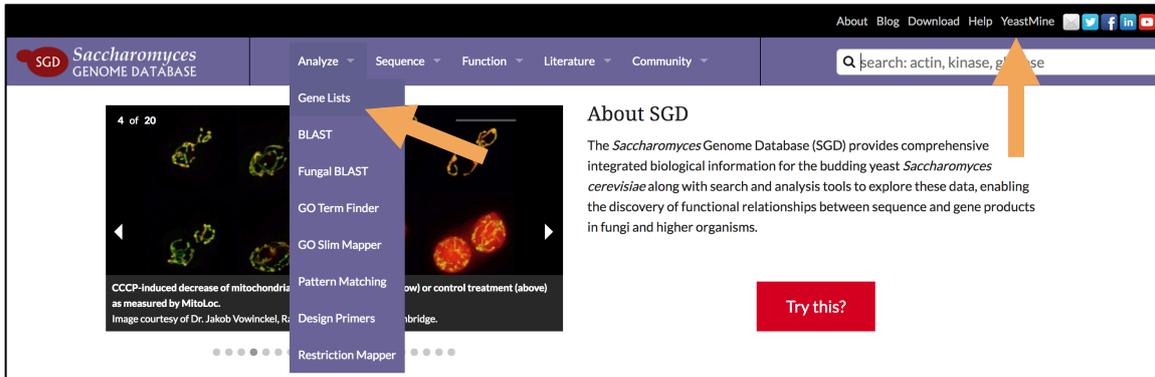
Download Add to Basket Add Columns

Gene ID	Transcript ID	Organism	Genomic Location (Gene)	Product Description	Input Ortholog(s)	Ortholog Group	Paralog count	Ortholog count
Q0130	Q0130-426_1	<i>S. cerevisiae</i> S288c	KP263414:46,723..46,953(+)	F0 ATP synthase subunit c	CaglIfMp10	OG5_126818	0	78
Q0045	Q0045-426_1	<i>S. cerevisiae</i> S288c	KP263414:13,818..26,701(+)	cytochrome c oxidase subunit 1	CaglIfMp04, CaglIfMp07	OG5_128358	1	43
Q0070	Q0070-426_1	<i>S. cerevisiae</i> S288c	KP263414:13,818..23,167(+)	intron-encoded DNA endonuclease a15 alpha	CaglIfMp04, CaglIfMp07	OG5_128358	1	43
Q0105	Q0105-426_1	<i>S. cerevisiae</i> S288c	KP263414:36,540..43,647(+)	cytochrome b	CaglIfMp03	OG5_128504	1	31
Q0120	Q0120-426_1	<i>S. cerevisiae</i> S288c	KP263414:36,540..42,251(+)	intron-encoded RNA maturase b4	CaglIfMp03	OG5_128504	1	31

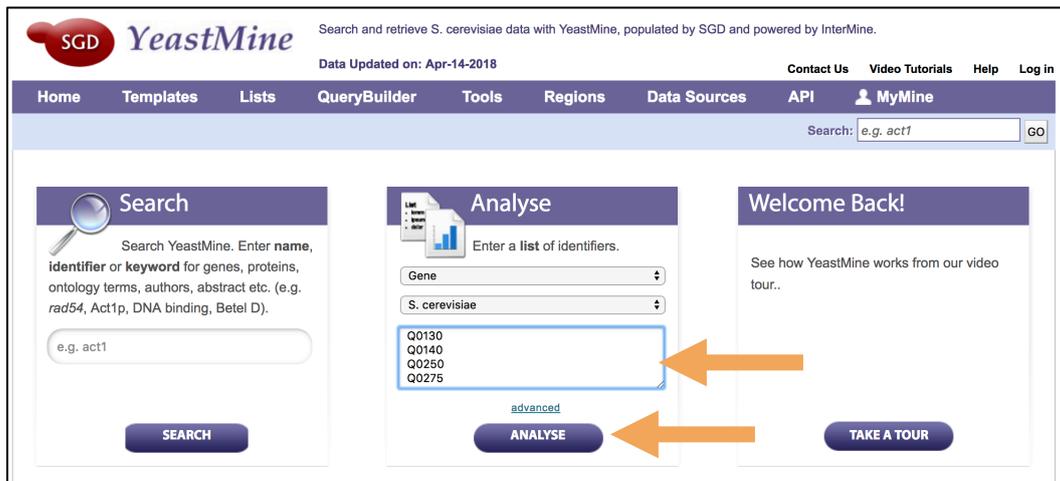
- In the download options menu, select “**Tab delimited (Excel) – choose a pre-configured table**”. Set the Download Type as **Excel File**, then hit **Get**.

### 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 in 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. 11 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.

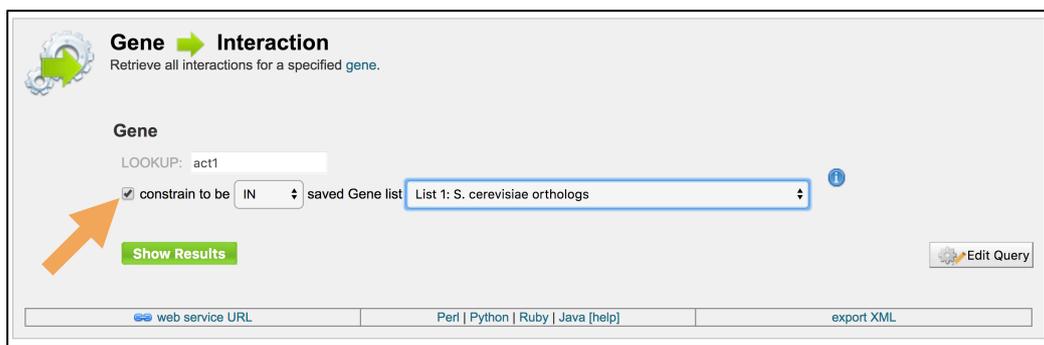


#### 4. In YeastMine, find all synthetic lethal interactions for the *S. cerevisiae* orthologs by using the Gene → Interaction query:

- 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 → Interaction**.



- 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 & physical interactions for the list of *S. cerevisiae* orthologs you inputted. 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.

Trail: Query  
**Gene → Interaction**  
 Retrieve all interactions for a specified gene.

Manage Columns | Manage Filters | Manage Relationships | Save as List | Generate Python code | Export

Showing 1 to 25 of 482 rows | Rows per page: 25 | page 1

Gene Primary DBID	Gene Standard Name	Gene Systematic Name	Gene Sgd Alias	Gene Name	Gene Organism . Short Name	Details Annotation Type	Details Phenotype	Details Role 1	Participant 2 Standard Name	Secondary Identifier	Interaction Detection Methods Identifier	Experiment Name	Details interactionType
S000007260	COX1	Q0045	cytochrome c oxidase subunit 1 OX13	Cytochrome c OXidase	S. cerevisiae	high-throughput	inviable	Bait	DIC1	YLR348C	Synthetic Lethality	Deutscher D, et al. (2006)-16941010-Synthetic Lethality	genetic
S000007260	COX1	Q0045	cytochrome c oxidase subunit 1 OX13	Cytochrome c OXidase	S. cerevisiae	high-throughput	inviable	Bait	FRD1	YEL047C	Synthetic Lethality	Deutscher D, et al. (2006)-16941010-Synthetic Lethality	genetic

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

Interaction Term Identifier	Count
<input type="checkbox"/> Affinity Capture-Western	206
<input type="checkbox"/> Affinity Capture-MS	99
<input type="checkbox"/> Reconstituted Complex	32
<input type="checkbox"/> Co-purification	29
<input type="checkbox"/> Dosage Rescue	26
<input type="checkbox"/> Co-fractionation	24
<input type="checkbox"/> Synthetic Rescue	22
<input type="checkbox"/> Affinity Capture-RNA	13
<input checked="" type="checkbox"/> Synthetic Lethality	11
<input type="checkbox"/> Protein-RNA	5
<input type="checkbox"/> Two-hybrid	5

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

**Orthologues**

A. fischeri NRRL 181 (13) A. flavus NRRL3357 (15) A. fumigatus Af293 (13)  
 A. gambiae (6) A. nidulans FGSC A4 (16) A. niger ATCC 1015 (18)  
 C. albicans SC5314 (7) C. albicans WO-1 (7) C. dubliniensis CD36 (7)  
 C. elegans (21) C. gattii VGI 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)  
 [ 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 521 (12)

View homologues in other Mines:  
 FlyMine  
 D. melanogaster

## 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.