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.

CGD	<i>Candida</i> Genome	Database					About	Site Map How search our site	to Cite Help 🗗 🖂
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- 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 a	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 RNA	
 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 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 Is on the following chromosome or contig sequence(s): (The "Al" option includes unmapped features; to specifically exclude unmapped features, select each of the chromosomes of interest rather than "All") ChrL.C.glabrata_CBS138 ChrK.C.glabrata_CBS138 ChrM.C.clabarata_CBS138	ND ND

- 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.

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Download All Search Results Download all the data retrieved by very Download selected information for entire gene list. Available information types include Sequence, Coordinates, GO annotations, Phenotype.										

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 36 19 Feb 201	8		A EuPathDB Project
Home New Search - My Strategies	My Basket (0) Tools - Data Summary -	Gene ID: Ab: Downloads × Community × Analyze My Ex;	NCU06658 Gene Text Search: synth* out FungiDB Help Login Register Contact Us V F Compared to the periment NEW // My Favorites
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News and Tweets 9. 19 February 2018 FungiDB 36 Released 9. 10 Eccember 2017 FungiDB 38 Released 9. 7 September 2017 FungiDB 38 Released 9. 7 September 2017 FungiDB 38 Released March 2017 FungiDB 38 Released March 2018 Released	expand all collapse all Find a search C	expand all [collapse all Find a search Popset Isolate Sequences Genomic Sequences SNPs ESTs ORFs ORFs Compounds Expand all [collapse all	BLAST Identify Sequence Similarities Results Analysis Concentration of the second seco

- 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 popup 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.

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1	Q0045	Q0045-t26_1	S. cerevisiae S288c	KP263414:13,81826,701(+)	cytochrome c oxidase subunit 1	CaglfMp04, CaglfMp07	OG5_128358	1	43
1	Q0070	Q0070-t26_1	S. cerevisiae S288c	KP263414:13,81823,167(+)	intron-encoded DNA endonuclease al5 alpha	CaglfMp04, CaglfMp07	OG5_128358	1	43
1	Q0105	Q0105-t26_1	S. cerevisiae S288c	KP263414:36,54043,647(+)	cytochrome b	CaglfMp03	OG5_128504	1	31
1	Q0120	Q0120-t26_1	S. cerevisiae S288c	KP263414:36,54042,251(+)	intron-encoded RNA maturase bl4	CaglfMp03	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 "**Analyse**" box. Then, click on the purple "**ANALYSE**" button.

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• 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.

3efore we show you the results											
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Q0060	AI3	S. cerevisiae		6179	Q0060	S000007263	ORF				
00070	AI5 ALPHA	S. cerevisiae		9350	Q0070	S000007265	ORF				

4. In YeastMine, find all synthetic lethal interactions for the *S. cerevisiae* orthologs by using the Gene \rightarrow 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**.

GENOME	PROTEINS	FUNCTION	PHENOTYPES	INTERACTIONS	REGULATION	HOMOLOGY	EXPRESSION	LITERATURE
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• 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.

Gene Interaction Retrieve all interactions for a specified ge		
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Constrain to be IN + saved G	ene list List 1: S. cerevisiae orthologs	÷
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ee web service URL	Perl Python Ruby Java [help]	export XML

• 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.

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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".

Trail: Query Gene	Interations for	a specified ger	16.											
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• 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).

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Home	Templates	Lists	QueryBuilder	Tools	Regions	Data Sources	API	🚨 MyMine					
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Lists View your own and public lists, search by keyword and compare or combine the contents of lists. Click on a list to view graphs and summaries in an analysis page, select lists using checkboxes to perform set operations. Click 'Upload' above to import a new list. Filter: Reset Actions: Union Oplintersect Op Subtract Op Asymmetric Difference Copy in Delete Options: Show descriptions Show Tags													
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• 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)					
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@ \$00000773	@ YEL047C	S. cerevisiae	@ FRD1	Fumarate ReDuctase	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.