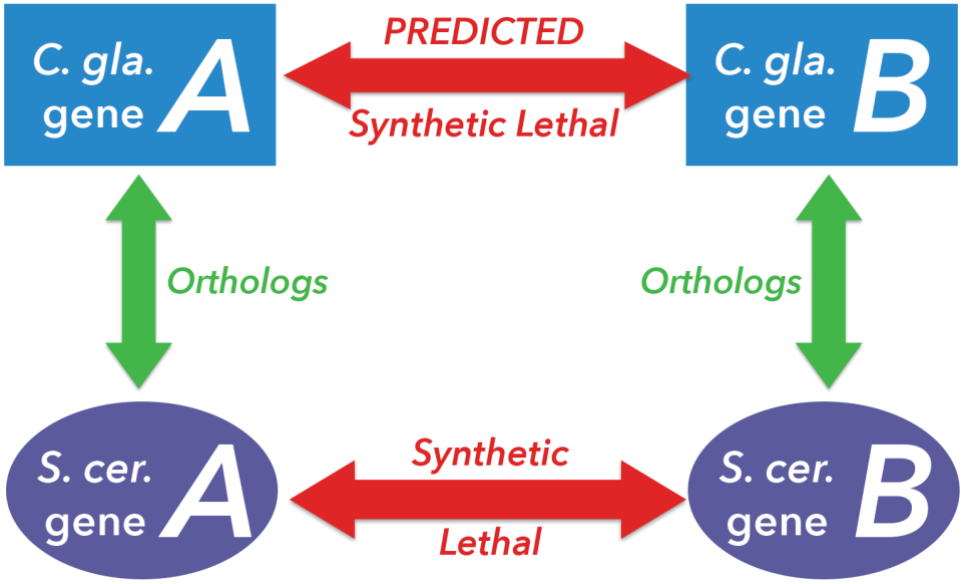
Using S. cerevisiae Orthologs to Predict Fungal Pathogen Biology

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.

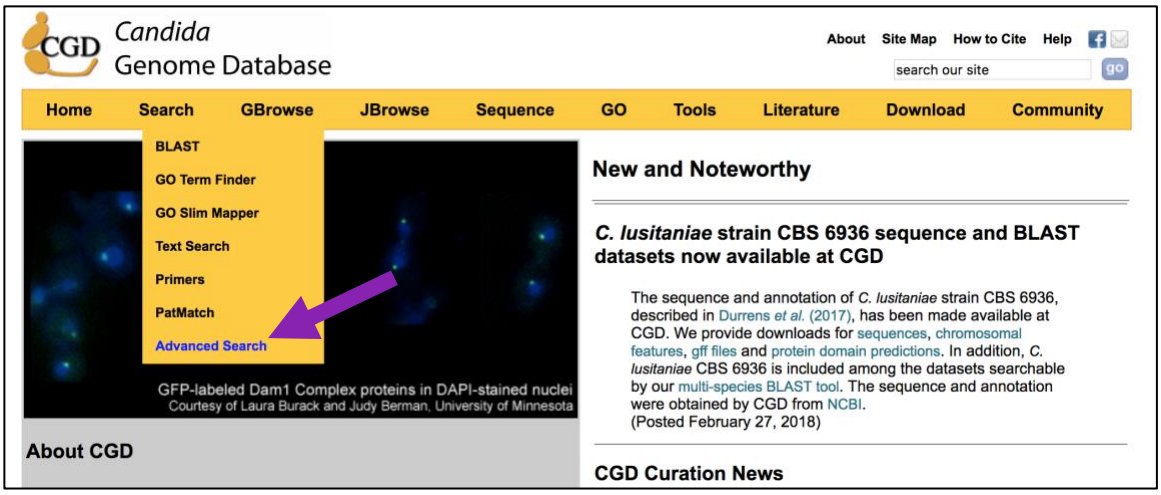
Genetic interactions such as synthetic lethality are richly documented for the budding yeast *S. cerevisiae*, but not as much for many other fungal species. By examining known genetic interactions in *S. cerevisiae*, we can predict synthetic lethal relationships in *C. glabrata* and other fungal pathogens.



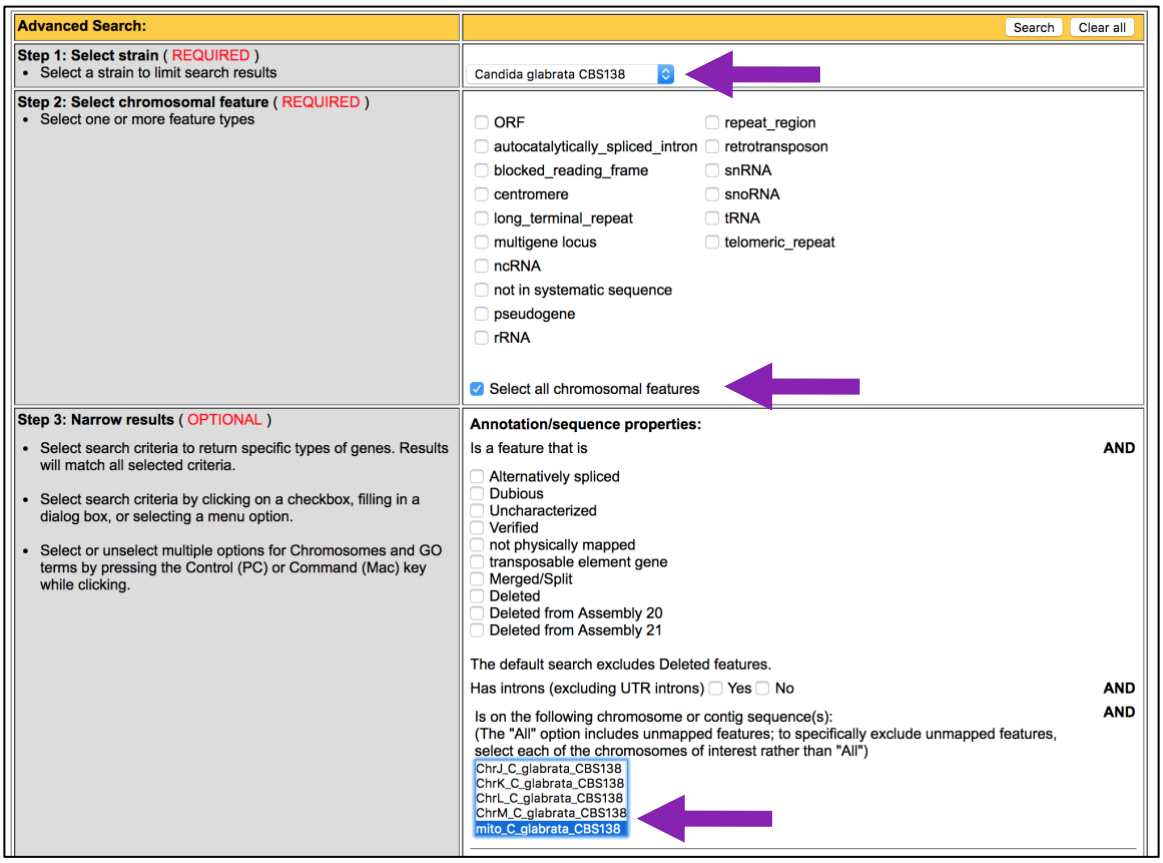
If conserved, these synthetic lethal interactions may reveal future antifungal targets for use against azole-resistant strains in the clinic. Using known synthetic lethal interactions in the *S. cerevisiae* genome, predict potentially conserved synthetic lethal interactions for mitochondrial genes in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

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

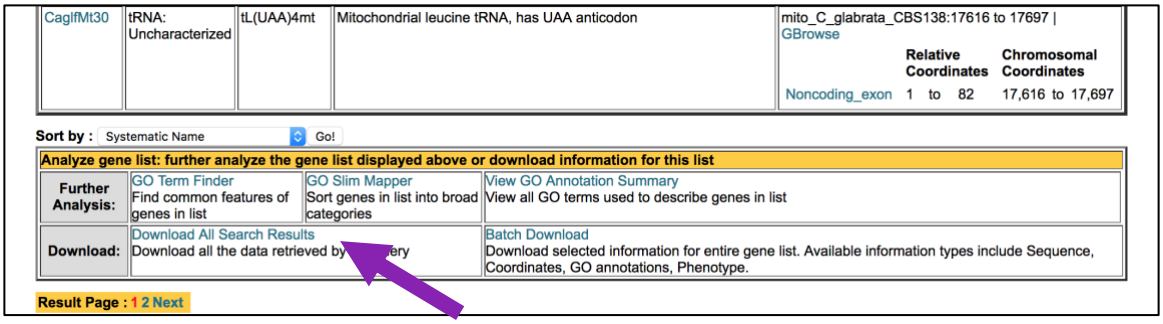
- On the CGD homepage (**http://www.candidagenome.org**), open the Search tab in the yellow toolbar and select **Advanced Search**.



- 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.

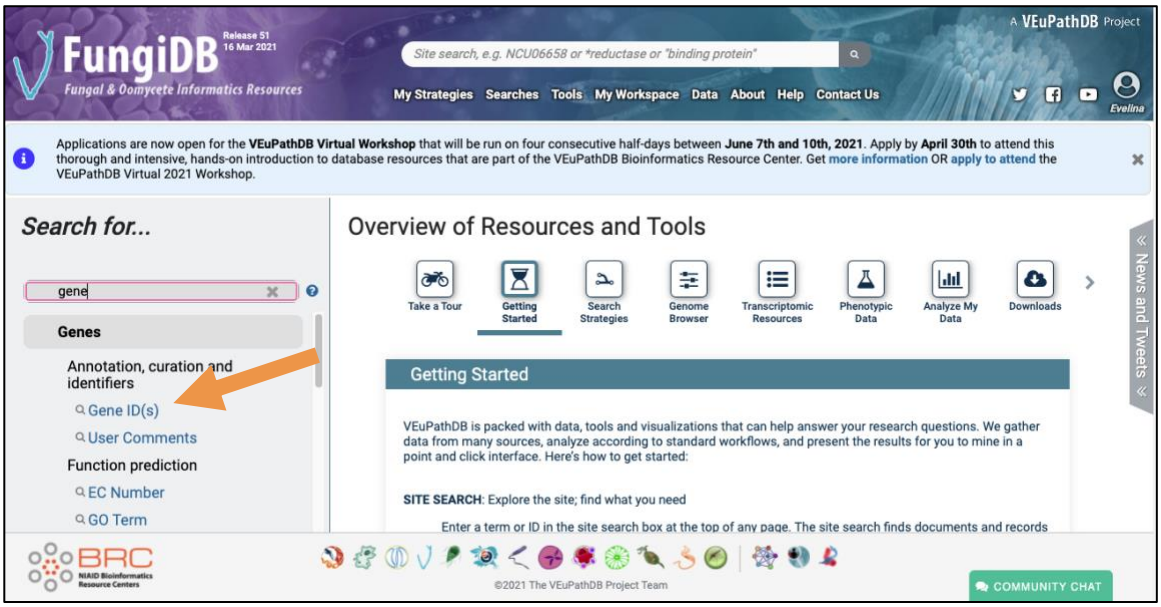


- Click on "Search". A results page will follow, listing out 37 features in the *C. glabrata* mitochondrial genome.
- Scroll to the bottom of the page and click on the "**Download All Search Results**" link.



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)**".

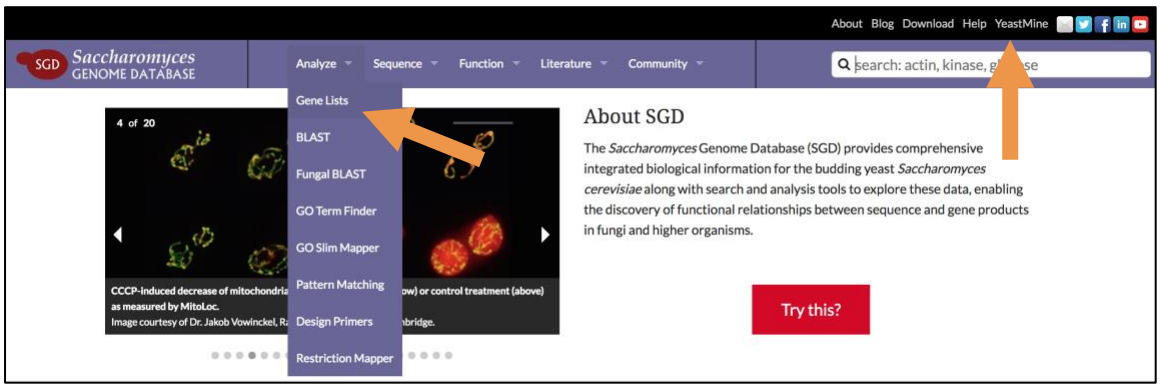


- Using your exported file from CGD, copy and paste the ORF names of the *C. glabrata* mitochondrial genes into the box. Click on "**Get Answer**".
- In the Search Strategy panel, click on the "**Add a Step**" button. In the resulting pop-up window, click on "**Transform into related records > Orthologs**".
- 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.

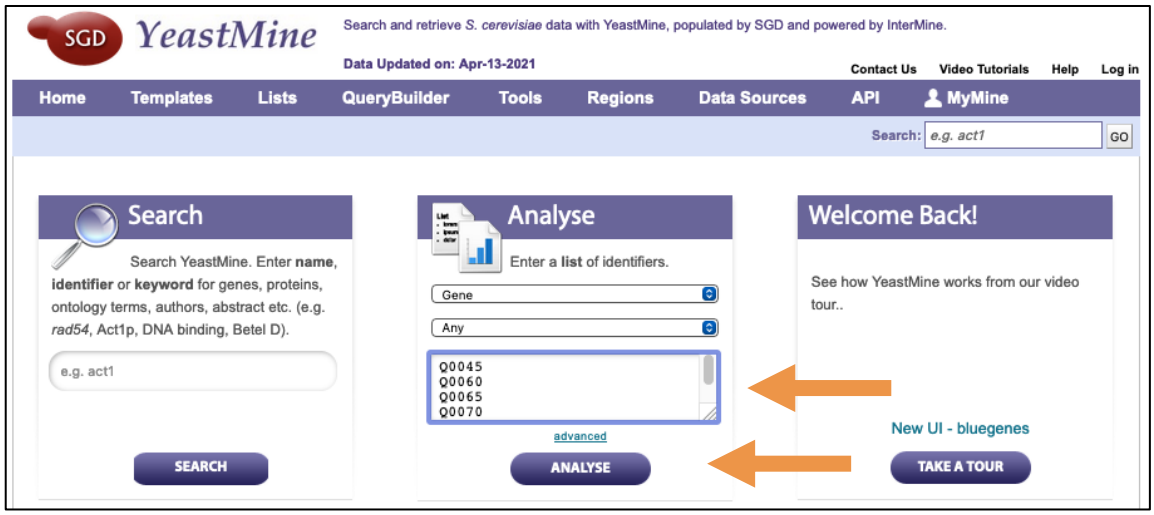
- In the download options menu, select "**Tab delimited (Excel) – choose a pre-configured table**". Set the Download Type as **Excel File**, then hit **Get**.

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

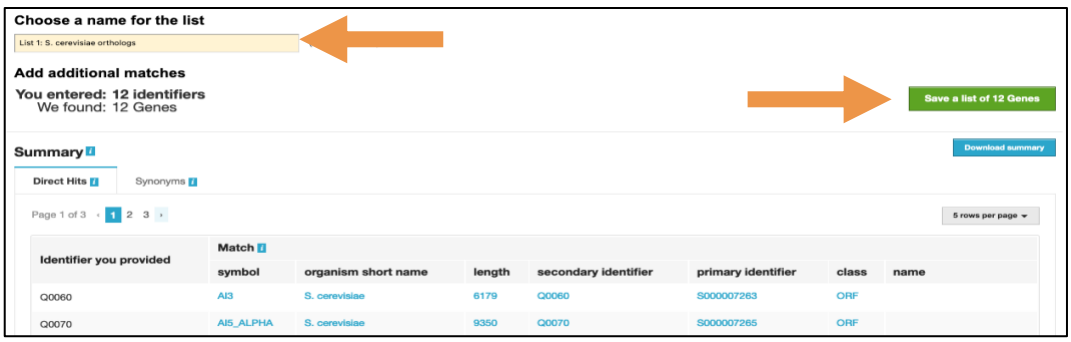
- Open the YeastMine homepage. You can access YeastMine from SGD by opening the Analyze tab and selecting **Gene Lists**, clicking the YeastMine link in the upper right corner of the homepage, or by entering the URL: https://yeastmine.yeastgenome.org



- 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 "**Analyse**" box. Then, click on the purple "**ANALYSE**" button.

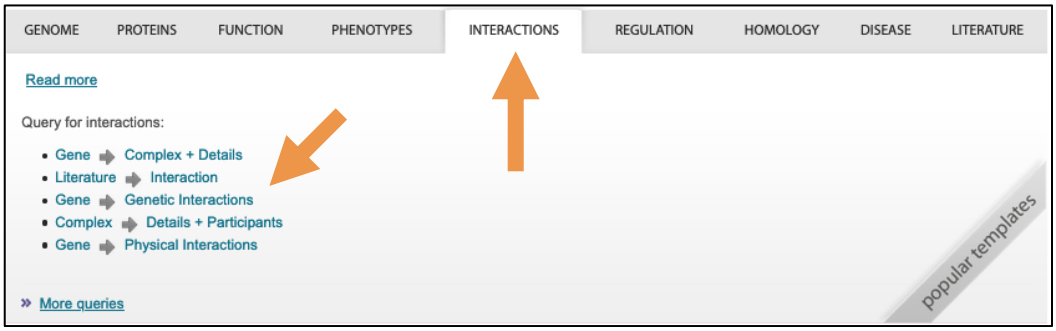


- A disambiguation page will be shown confirming your matches. 12 results should be shown. Name your gene list something descriptive, such as: "**List 1: S. cerevisiae orthologs**". Click on the green "**Save a list of Genes**" button.

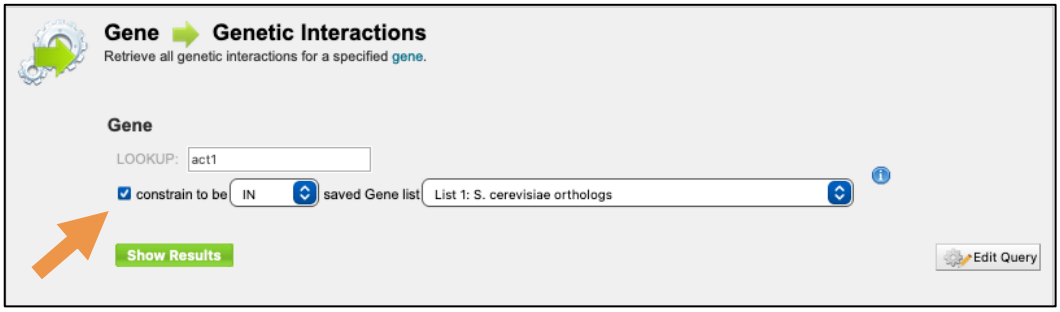


4. In YeastMine, find all synthetic lethal interactions for the *S. cerevisiae* orthologs by using the Gene → Genetic Interactions query:

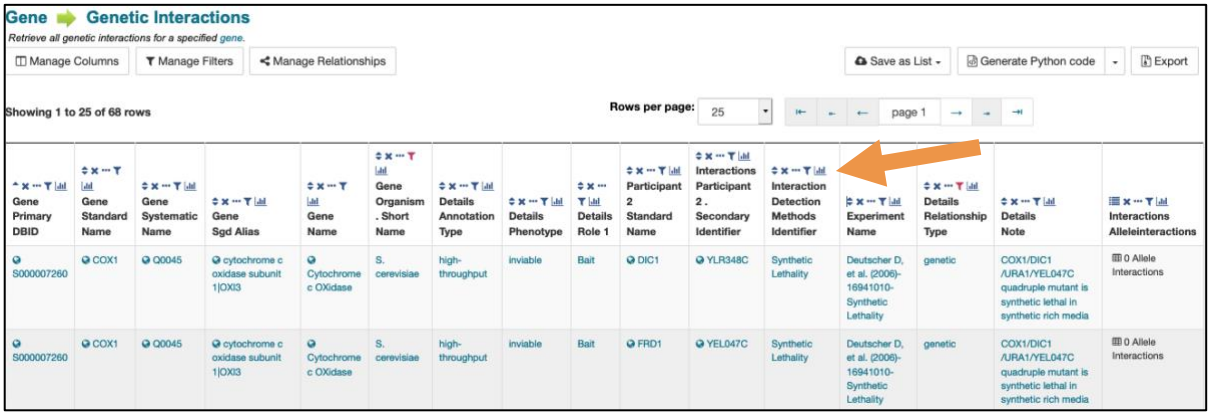
- Return to the YeastMine homepage: https://yeastmine.yeastgenome.org
- In the "popular templates" toolbar in the middle of the page, open the **INTERACTIONS** tab and select the query **Gene → Genetic Interactions**.



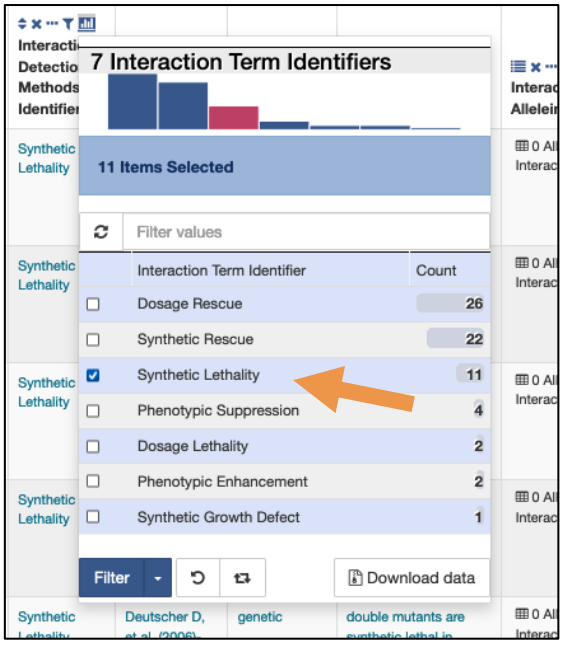
- Check the "**constrain to be IN**" checkbox. This allows you to input a list of genes. From the dropdown menu, select the list of *S. cerevisiae* orthologs you saved earlier in part 3. Click on the green **Show Results** button.



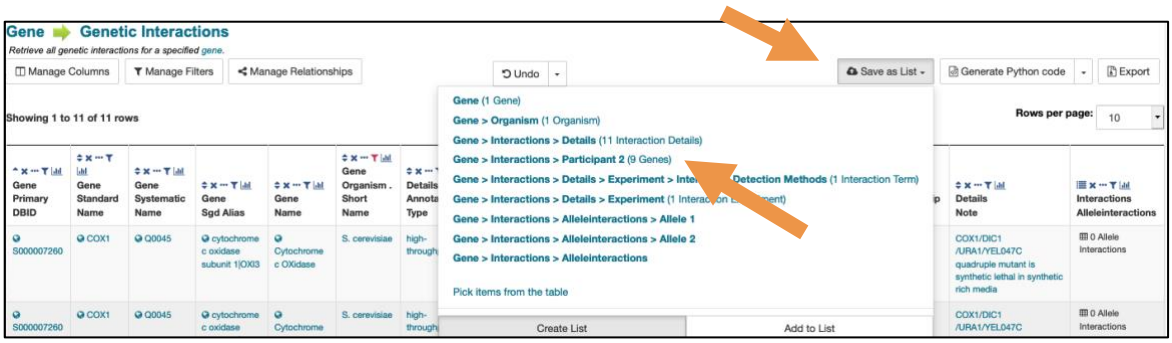
- The results table contains all genetic interactions for the input list of *S. cerevisiae* orthologs. To filter for only **synthetic lethal** interactions, find the **Interaction Detection Methods Identifier** column. At the top of this column is a set of small blue icons. Click on the rightmost **View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



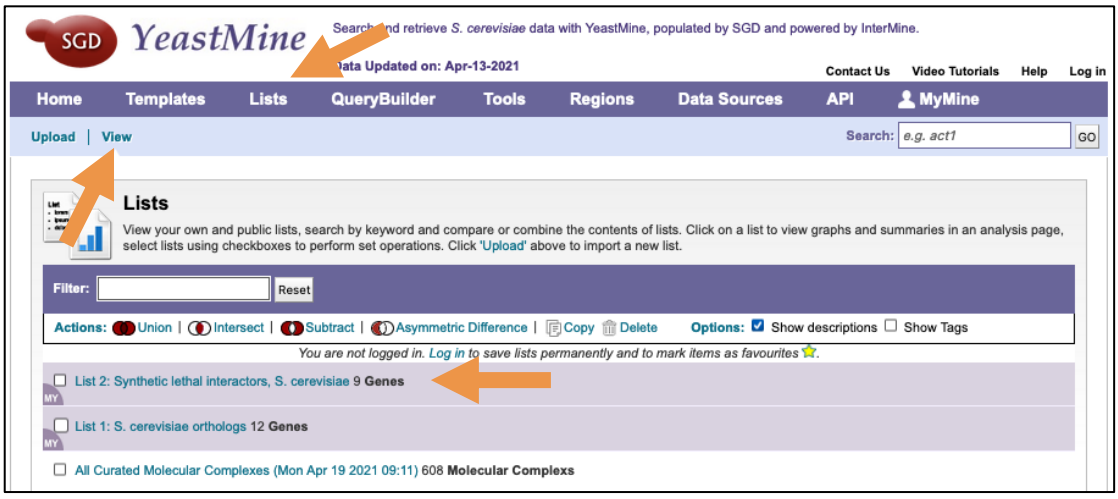
- A window summarizing all entries for this column will open. Check the entry for **Synthetic Lethality** and hit **Filter**.



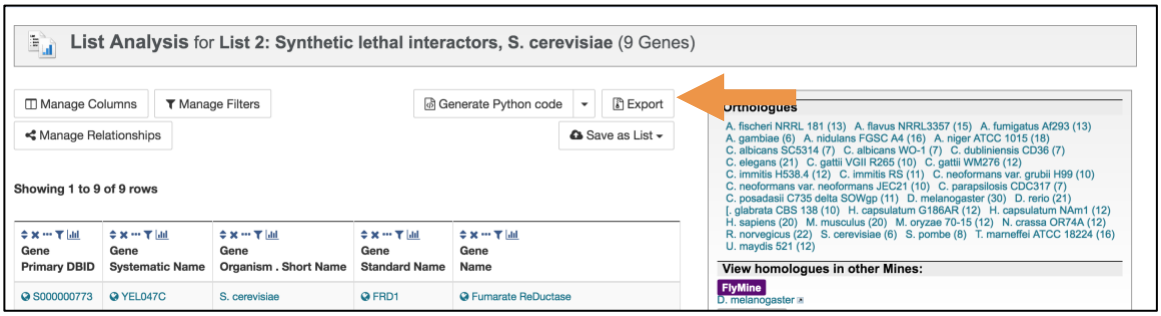
- The table now contains only synthetic lethal interactions. To save the interactors into a gene list, click on the **Save as List** button and select the entry **Gene > Interactions > Participant 2**. Give your list a descriptive name such as "**List 2: Synthetic lethal interactors, S. cerevisiae**".



- 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.



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.