FungiDB: Performing GO Enrichment analysis

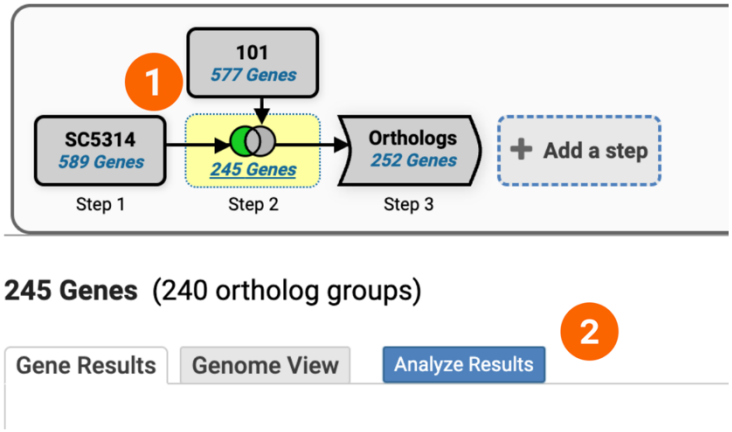
Learning objectives:

- Perform a GO enrichment analysis
- Create complex search strategy using both FungiDB and SGD

- **Use a previously created search strategy to perform Gene Ontology enrichment analysis on genes upregulated (identified by RNA-Seq) in *C. albicans* SC5314 only.**

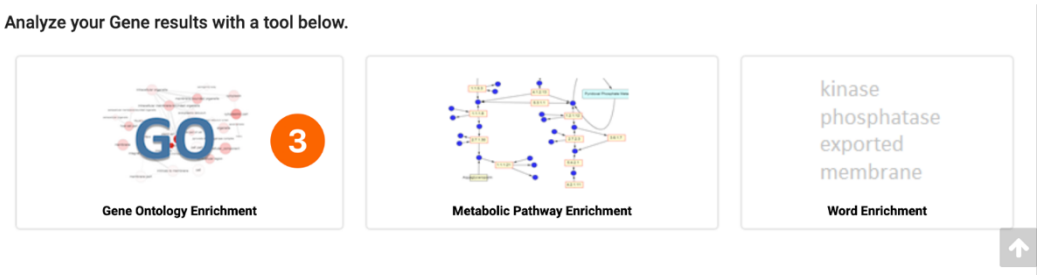
  Strategy URL:
  https://fungidb.org/fungidb/app/workspace/strategies/import/802d9f2b606fc1fa

1. Click on the Step 2 to identify upregulated gene in *C. albicans* SC5314 only.
2. Click on the "Analyze Results" tab to bring up enrichment analysis options.

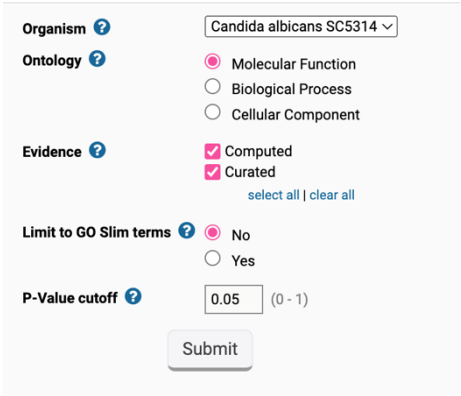


The enrichment analysis tools can be accessed under the blue Analyze Results tab and it includes Gene Ontology, Metabolic Pathway, and Word Enrichment tools. The three types of analysis apply Fisher's Exact test to evaluate ontology terms, over-represented pathways, and product description terms. Enrichment is carried out using a Fisher's Exact test with the background defined as all genes from the organism being queried. P-values corrected for multiple testing are provided using both the Benjamini-Hochberg false discovery rate method and the Bonferroni method.

3. Deploy GO enrichment analysis by clicking on the "Gene Ontology Enrichment" button.

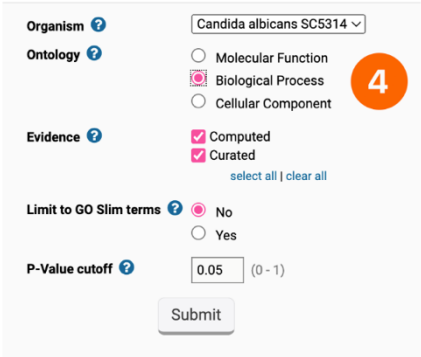


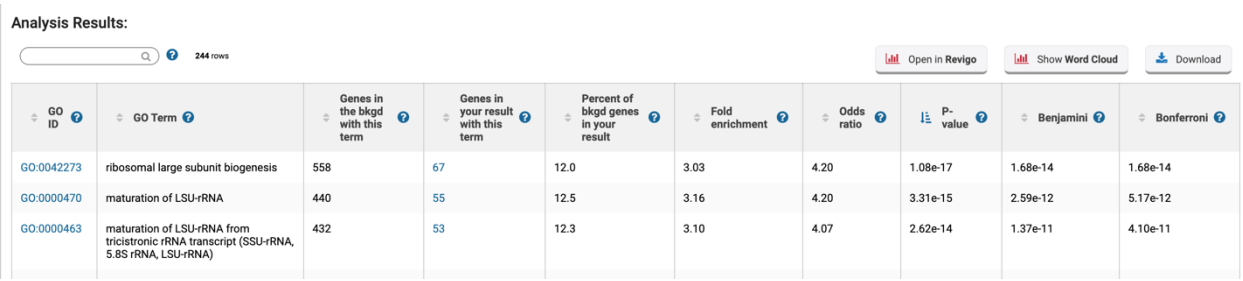
GO enrichment analysis can be performed on the following ontology groups: molecular function, cellular component, and biological processes. Also, other parameters allow users to limit their analysis on either “Curated” or “Computed” annotations, or both. Those with a GO evidence code inferred from electronic annotation (IEA) are denoted “Computed”, while all others have some degree of curation. The default P-value is set to 0.05 but can be adjusted manually.



When the GO Slim option is chosen, both the genes of interest and the background are limited to GO terms that are part of the generic GO Slim subset.

4. Perform GO enrichment analysis (Biological Process) at default selection criteria.





GO ID	GO Term	Genes in the bkgd with this term	Genes in your result with this term	Percent of bkgd genes in your result	Fold enrichment	Odds ratio	P-value	Benjamini	Bonferroni
GO:0042273	ribosomal large subunit biogenesis	558	67	12.0	3.03	4.20	1.08e-17	1.68e-14	1.68e-14
GO:0000470	maturation of LSU-rRNA	440	55	12.5	3.16	4.20	3.31e-15	2.59e-12	5.17e-12
GO:0000463	maturation of LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	432	53	12.3	3.10	4.07	2.62e-14	1.37e-11	4.10e-11

The results table includes several additional statistical measurements:

- **Fold enrichment** - The ratio of the proportion of genes in the list of interest with a specific GO term over the proportion of genes in the background with that term.
- **Odds ratio** - Determines if the odds of the GO term appearing in the list of interest are the same as that for the background list.
- **P-value** - Assumptions under a null hypothesis, the probability of getting a result that is equal or greater than what was observed.

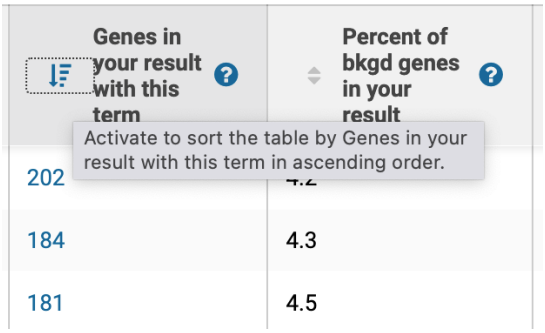
- **Benjamini-Hochburg false discovery rate** - A method for controlling false discovery rates for type 1 errors.
- **Bonferroni adjusted P-values** - A method for correcting significance based on multiple comparisons.

The GO enrichment table can be opened in Revigo, viewed as a word cloud (produced via the GO Summaries R package) or downloaded.

Notice that the table contains columns with GO IDs and GO terms along with the number of genes in the background and those specific to the RNA-Seq analysis results presented (linked in blue).

5. Examine GO enrichment analysis results. What kinds of GO terms are enriched?

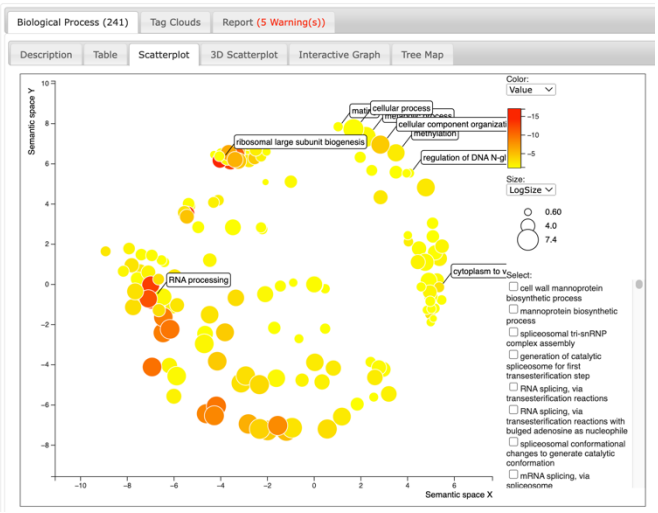
Note: you can sort genes in your results using the sort options within a column:



6. Visualize the results in Revigo by clicking on the Revigo button above the results table and leaving other parameters at default. Click the Start Revigo button below the results set and then select scatterplot.

Bubble color corresponds to the user-provided p-value (see legend in upper right-hand corner)

Bubble size represent the frequency of the GO term in the underlying database.



The table tab provides a detailed overview of the GO terms, P-values and also parent GO terms used to describe a group of related GO terms (http://geneontology.org/docs/ontology-relations/)

Creating queries across FungiDB and SGD (optional exercise)

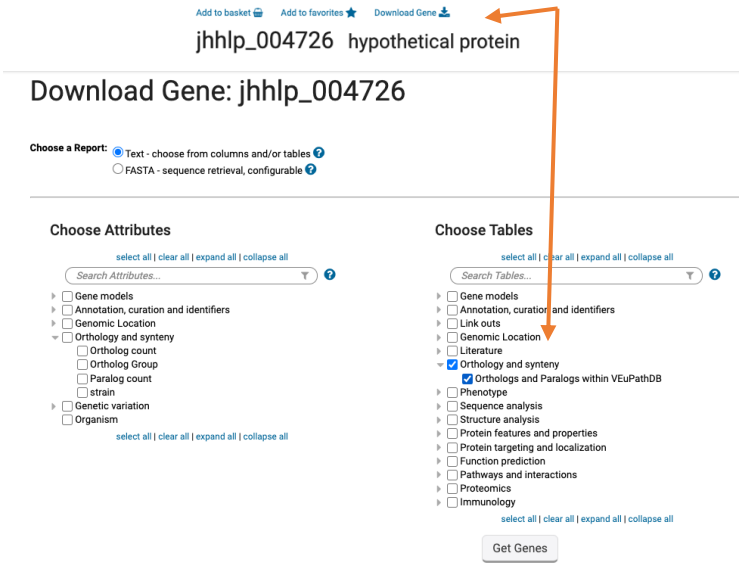
During a genetic screen in *Lomentospora prolificans*, you identified several interesting genes, including jhhlp_004726, which is a hypothetical protein. Take advantage of FungiDB and SGD records to learn more about this gene.

1. Navigate to jhhlp_004726 in FungiDB and examine available records.
https://fungidb.org/fungidb/app/record/gene/jhhlp_004726

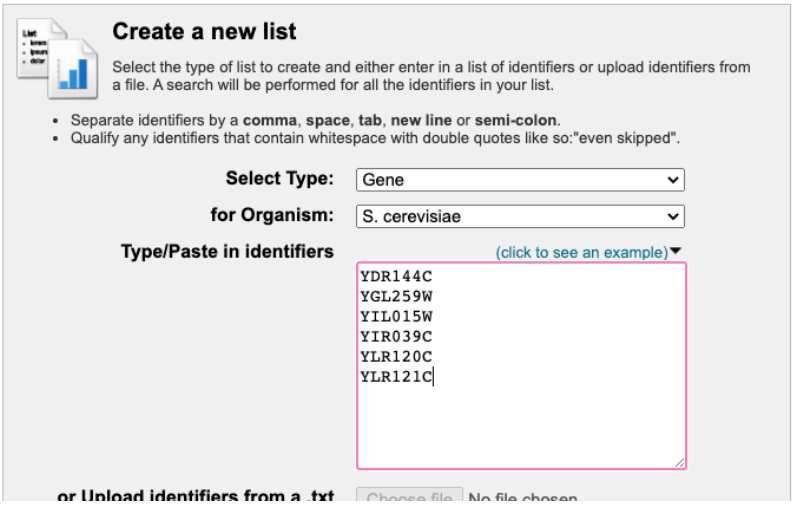
- Run an InterPro search and a GPI anchor prediction tool. What did you learn about this protein?

Hint: InterPro and GPI search tools can be found in the Protein features and properties section of the gene record page.

- Export orthologs of this gene.
Click on the Download gene link and select to export orthologs in VEuPathDB option



- Navigate to the SGD gene lists search and copy and paste *S. cerevisiae* orthologs for jhhlp_004726: https://www.yeastgenome.org/locus/YDR144C



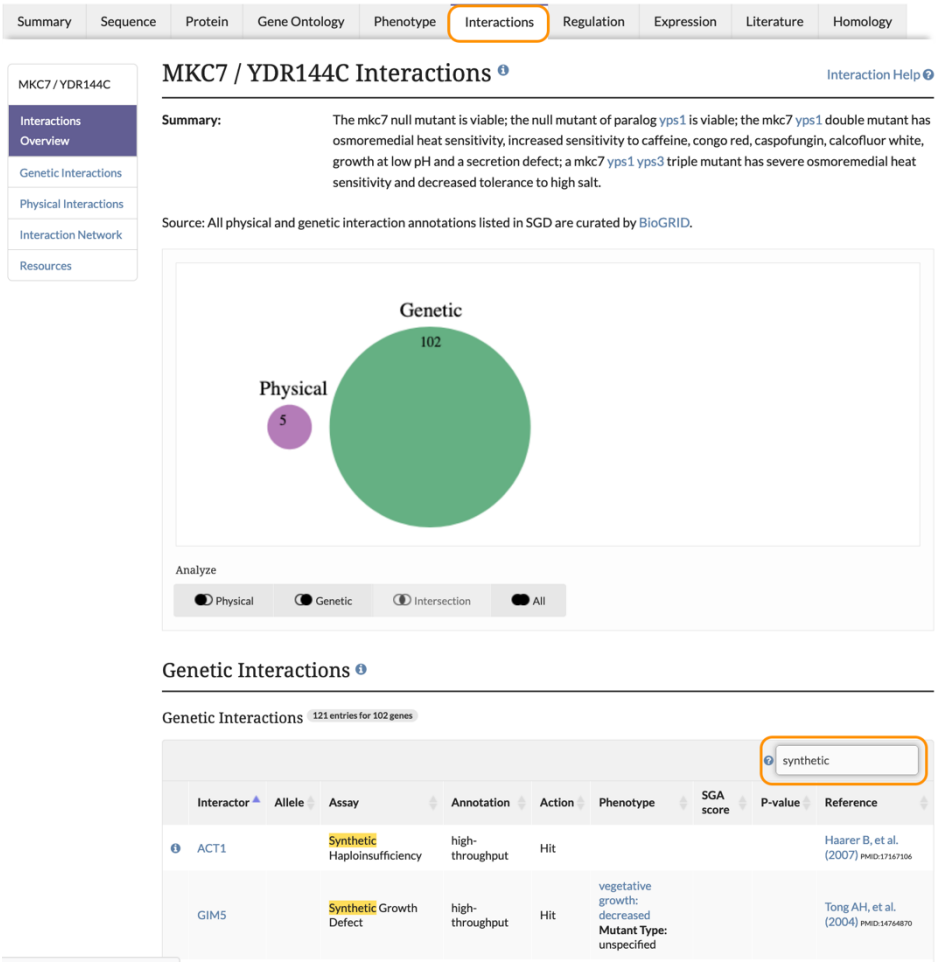
- Give your list a name such as 'Yeast orthologs 1'.
- Click on the GeneIDs to examine *S. cerevisiae* genes. What is the function of MKC7 (YDR144C) in *S. cerevisiae?* Does it encode a protein with enzymatic activity? Where in the cell does the protein execute its function? What biological process? Hint: see the **Gene Ontology** section on the locus page or click on the Gene Ontology tab at the top of the page.

Functional relationships between genes and pathways can sometimes be revealed by examining genetic interactions between two or more genes. Genes are described as having a genetic interaction if the simultaneous mutation of both genes produces a phenotype that is unexpected, given the phenotypes of the single mutants.

- **Find known genetic interactions for MKC7.**

    - In SGD, find the MKC7 locus page and navigate to the **Interactions** tab, which is listed in the Quick Links panel near the top. The interactions are divided into separate physical interactions and genetic interactions tables below the summary.

    - Filter the **Genetic Interactions** table on “synthetic”. This table will show only the genetic interactions where some sort of synthetic growth defect, haploinsufficiency, or lethality is produced.



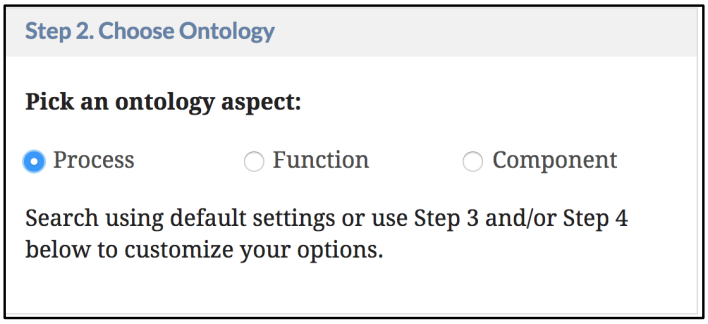
- Click on the **Download** button, which is located under the results table, and save this gene list. *Rename the file to* ***synthetic.txt***.

Note: Rename the file to ***synthetic.txt*** *so that we can find it easily later.*

- Click on the **Analyze** button, then on **GO Term Finder.**

- Run a **process** enrichment for the MKC7 genetic interaction genes.

Hint: GO Term Finder finds common Gene Ontology (GO) annotations between genes. To run a Biological Process enrichment, select the Process button as shown below, then submit the form. More ways to customize your GO Term Finder query can be found in the GO Term Finder exercise.



- Scroll down the results page to see the table of enriched biological processes. What kind of processes are associated with the genes we analyzed? What do these results suggest about MKC7's functional relationships in the cell?

- Click on any of the genes shown for a biological process of interest to visit the gene's page on SGD. Use the gene page to uncover how the respective gene is involved in the biological process you were interested in.

Result Table

Terms from the Process Ontology of gene_association.sgd with p-value <= 0.01

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR	False Positives	Genes annotated to the term
tubulin complex assembly	3 of 9 genes, 33.3%	10 of 7166 genes, 0.1%	1.96e-05	0.00%	0.00	YML094W, YLR200W, YGR078C
protein folding	4 of 9 genes, 44.4%	121 of 7166 genes, 1.7%	0.00109	0.00%	0.00	YML094W, YLR200W, YKL117W, YGR078C
peptide pheromone maturation	2 of 9 genes, 22.2%	9 of 7166 genes, 0.1%	0.00603	0.67%	0.02	YNL238W, YLR120C
chaperone-mediated protein complex assembly	2 of 9 genes, 22.2%	9 of 7166 genes, 0.1%	0.00603	0.50%	0.02	YKL117W, YLR200W
fungal-type cell wall organization	4 of 9 genes, 44.4%	205 of 7166 genes, 2.9%	0.00878	0.40%	0.02	YHR079C, YLR120C, YLR121C, YFL039C

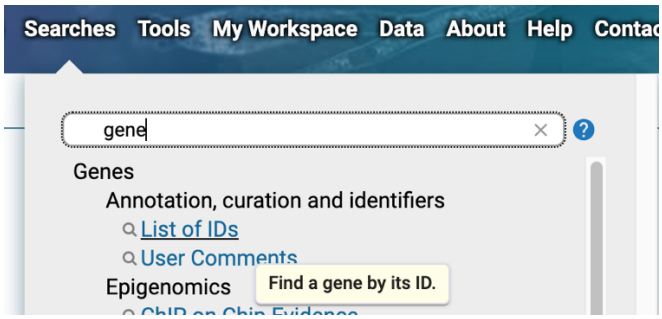
Now, let's go back to the file of MKC7 "synthetic" genetic interactors we downloaded earlier and find the orthologs of these genes in *Lomentospora prolificans*.

- Open this file in Excel and copy the Gene IDs in the **Interactor Systematic Name** column (not including the header)

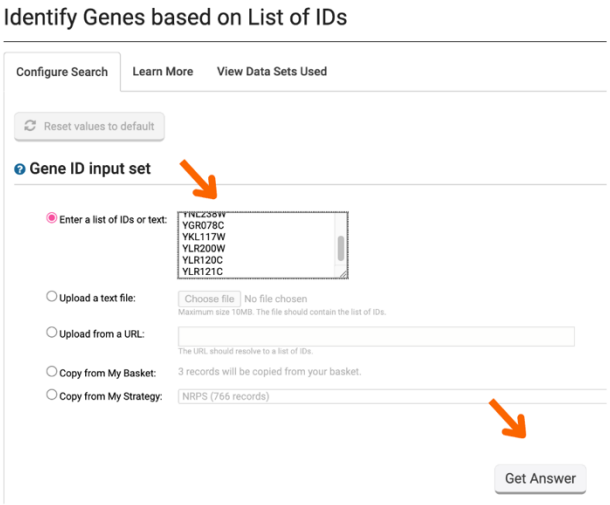
Interactor	Interactor Sy	Interactor	Interactor Systematic Name	Type	Assay	Annotation
MKC7	YDR144C	ACT1	YFL039C	Genetic	Synthetic Ha	high-through
MKC7	YDR144C	GIM5	YML094W	Genetic	Synthetic Gr	high-through
MKC7	YDR144C	IRE1	YHR079C	Genetic	Synthetic Gr	manually cur
MKC7	YDR144C	KEX2	YNL238W	Genetic	Synthetic Let	manually cur
MKC7	YDR144C	PAC10	YGR078C	Genetic	Synthetic Let	high-through
MKC7	YDR144C	SBA1	YKL117W	Genetic	Synthetic Let	high-through
MKC7	YDR144C	YKE2	YLR200W	Genetic	Synthetic Gr	high-through
MKC7	YDR144C	YPS1	YLR120C	Genetic	Synthetic Let	manually cur
MKC7	YDR144C	YPS1	YLR120C	Genetic	Synthetic Let	manually cur
MKC7	YDR144C	YPS1	YLR120C	Genetic	Synthetic Gr	manually cur
MKC7	YDR144C	YPS1	YLR120C	Genetic	Synthetic Let	manually cur
MKC7	YDR144C	YPS3	YLR121C	Genetic	Synthetic Let	manually cur

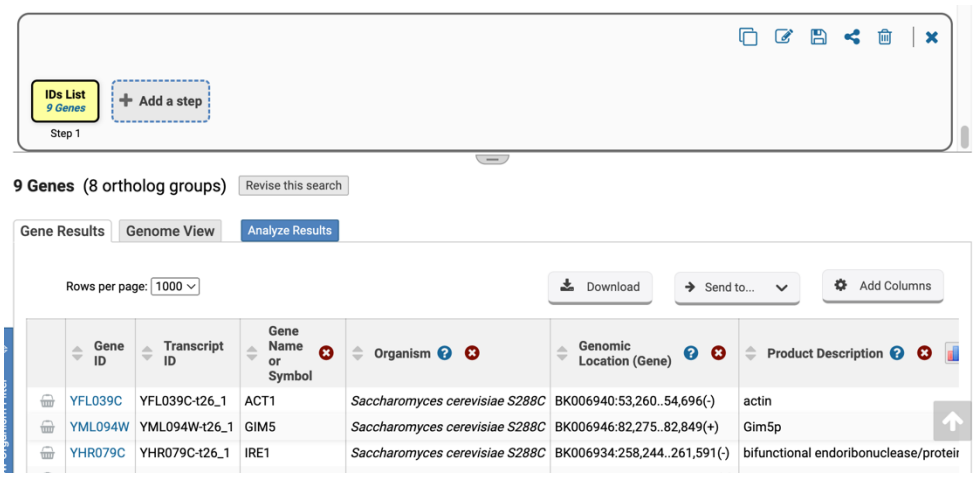
- Visit FungiDB again and initiate the List of IDs search query

The query can be deployed from the “Searches” menu at the top or the “Search for Genes” section on the main page.



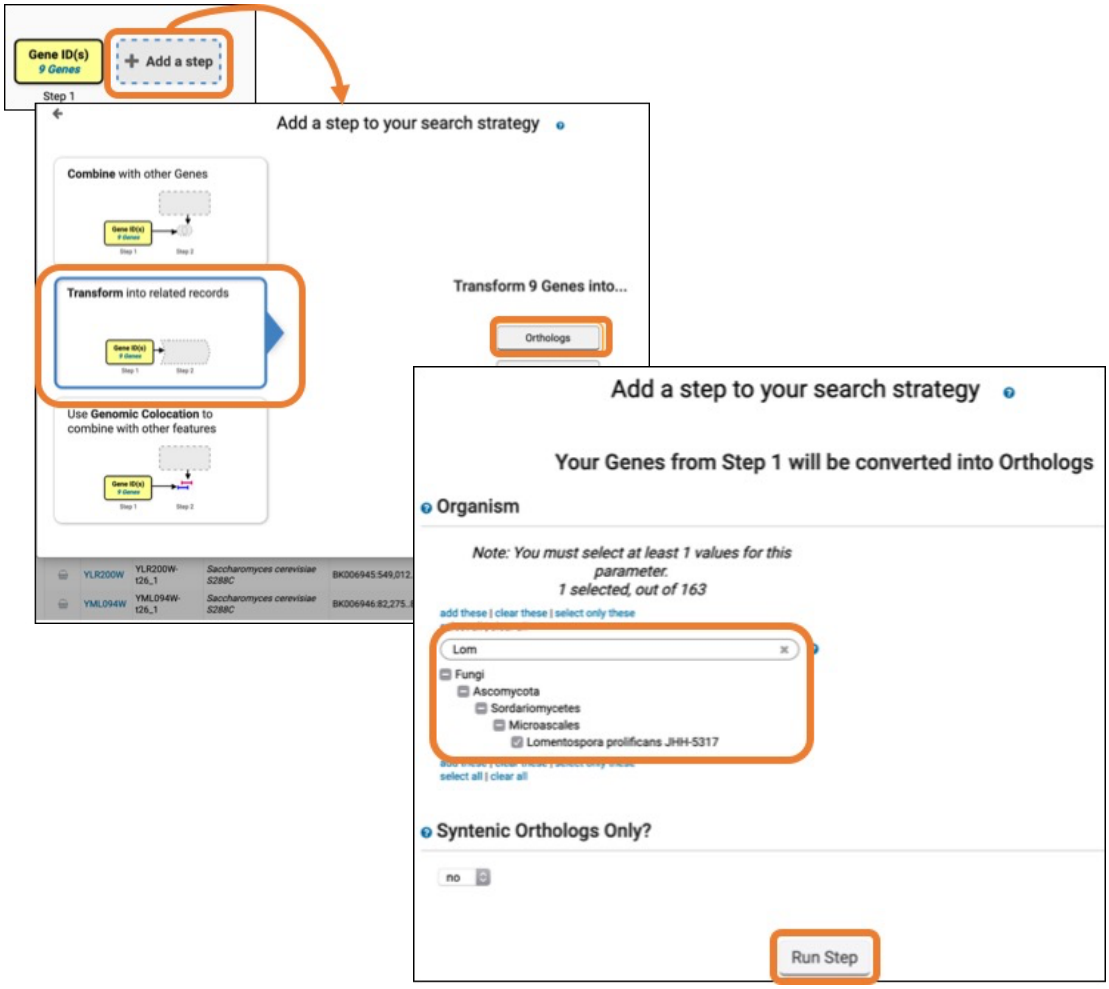
- Paste the list of Gene IDs that had the “synthetic” genetic interactions with MKC7 into FungiDB query and click on the **Get Answer** button.

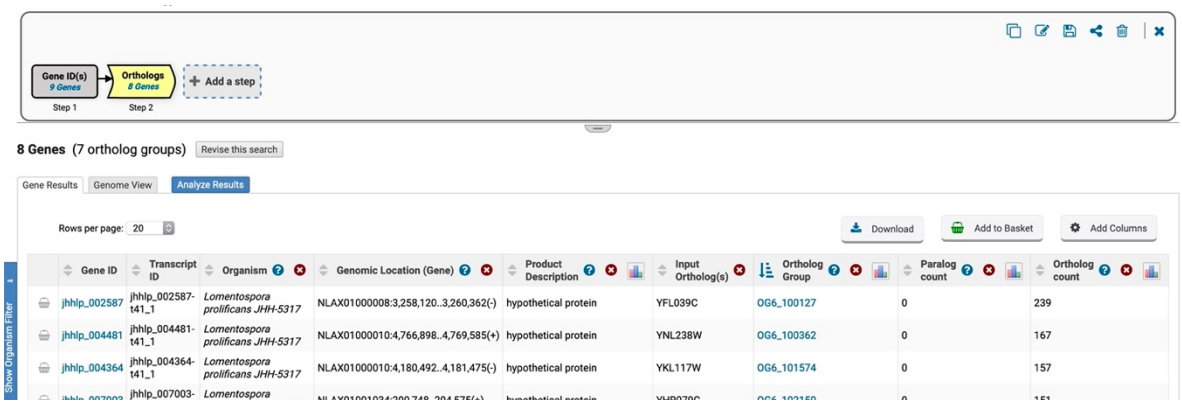




- Find orthologs in *Lomentospora prolificans*.

Click on the "Add a step" button to **Transform** the list **into related records.** Select the option to transform into **orthologs**, then use the search bar to filter on *Lomentospora prolificans* and **Run Step**.





How many of the interacting *S. cerevisiae* genes have a hypothetical protein ortholog in *Lomentospora prolificans*? Can you find jhhlp_004726 amongst these genes?

Strategy URL:
https://fungidb.org/fungidb/app/workspace/strategies/import/c0978bdb48a8392d

Glycosylphosphatidylinositol (GPI)-anchored proteins are involved in cell wall integrity and cell-cell interactions and perturbations in GPI biosynthesis lead to hypersensitivity to host defenses. Given the accumulated biological information we uncovered at SGD and FungiDB, summarize your predictions about the hypothetical *L. prolificans* protein jhhlp_004726.

- What is the likely jhhlp_004726 ortholog in *S. cerevisiae?*
  - Is this gene a GPI-protein in yeast?
- Do you have sufficient information to think the hypothetical gene in *L. prolificans* may be a putative GPI-anchor protein?
- How many "synthetic" genetic interactors exist in SGD for MKC7 in yeast?
  - What GO terms were enriched in biological processes associated with MKC7 interactors in *S. cerevisiae*?
  - How many orthologs of these genes are found in *L. prolificans?*
  - Why do you think the number of genes vary between *S. cerevisiae* and *L. prolificans*?

Additional resources:

More info on Fischer's exact test:
http://udel.edu/~mcdonald/statfishers.html

Some more info about Odds ratios:
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2938757/

False discovery rates and P value correction:
http://brainder.org/2011/09/05/fdr-corrected-fdr-adjusted-p-values/