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Fungal Pathogen Genomics

2-6 June 2024

course information

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WELLCOME CONNECTING SCIENCE

Fungal Pathogen Genomics

2-6 June 2024

A course held at the Wellcome Sanger Institute, Hinxton

Course Information Handbook

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Table of Contents

COURSE ORGANISATION AND DEVELOPMENT STAFF

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David Roos	FungiDB/VEuPathDB (University of Pennsylvania)
Nishadi De Silva	Ensembl Fungi (EBI)

Course Instructors

Jodi Lew-Smith	CGD/SGD Candida Genome Database, Stanford University
Steven Ahrendt	JGI/Mycocosm
Manuel Carbajo	Ensembl
Kathryn Crouch	FungiDB/VEuPathDB (University of Glasgow)
Stuart Brown	VEuPathDB
Louisee Mirabueno	Ensembl

Speakers

Jane Usher	Exeter University
Jason Rudd	Rothamsted Research

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COURSE TIMETABLE

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Time (BST)	**Sunday 2 June**	**Monday 3 June**	**Tuesday 4 June**	**Wednesday 5 June**	**Thursday 6 June**	**Friday 7 June**
07:30		*BREAKFAST*	*BREAKFAST*	*BREAKFAST*	*BREAKFAST*	*BREAKFAST*
09:00		**Evaluating gene model evidence**	**Enrichment analysis**	**Galaxy: SNP analysis (lecture)**	**Functional analysis:**	Group projects (cont.)
09:30				SNP analysis	**Pathways and metabolites**	
10:00						
10:30 - 11:00		*TEA BREAK*	*TEA BREAK*	*TEA BREAK*	*TEA BREAK*	*TEA BREAK*
11:00	REGISTRATION	**Comparative Genomics & Orthology**	Enrichment analysis, cont.	SNP analysis	**Manual curation in Apollo**	**Group presentations**
11:30	**WCS Orientation**					
12:00	***Instructor & Database Introductions***				**Group projects**	(12 min per team)
12:30			**Galaxy: RNA-Seq Result analysis (lecture)**	**Introduction to group projects**		
13:00-14:00	*LUNCH*	*LUNCH*	*LUNCH*	*LUNCH*	*LUNCH*	*LUNCH & DEPARTURE*
14:00	**Introduction to Database Queries**	Comparative Genomics & Orthology *(cont.)*	RNA-Seq Analysis Part 2	**Course photo & the Sanger Tour**	Group projects (cont.)	
14:30						
15:00						
15:30 - 16:00	*TEA BREAK*	*TEA BREAK*	*TEA BREAK*	*TEA BREAK*	*TEA BREAK*	
16:00	Introduction to Database Queries, cont.	**Galaxy NGS data analysis**	RNA-Seq Analysis Part 2	**SNPs & variants**	Group projects (cont.)	
16:30		**Introductory lecture**				
17:00	**Transcriptomics & Proteomics**					
17:30		Deploy RNA-Seq & SNP workflows				
18:00			**Research Seminar**	**Research Seminar**		
18:30			**Dr Jane Usher**	**Dr Jason Rudd**		
19:00-20:00	*WELCOME RECEPTION & DINNER*	*DINNER*	*DINNER*	*DINNER*	*DINNER*	
20:00-21:00	**Flash participant presentations**	*Adjourn (bar open)*	*Adjourn (bar open)*	*Adjourn (bar open)*	*Adjourn (bar open)*	
	Adjourn (bar open)					

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Fungal Pathogen Genomics

Programme Overview

Summary

The kingdom of Fungi encompasses a diverse range of organisms adapted to various environmental niches, playing crucial roles in ecosystems, human/animal/plant health, and global food security. Species like Fusarium, Pyricularia, Ustilago, Puccinia, and Zymoseptoria, etc. threaten agricultural ecosystems and food security worldwide. Fungal pathogens such as Aspergillus, Candida, Mucor, Cryptococcus, Histoplasma, Coccidioides, Batrachochytrium, and others are of great concern for medical and veterinary professionals due to their potential to cause allergies, illnesses, and life-threatening infections. Furthermore, fungi serve as essential model systems in basic and applied research and play pivotal roles in biotechnology, food production, biomedical and pharmaceutical research and the biofuel industry.

High-throughput 'omics' data technologies empower scientists to conduct extensive analyses of the genomes, transcriptomes, proteomes, genetic variance data of a wide array of fungal and oomycete organisms. These analyses are essential for inquiries regarding pathogenicity, host-pathogen interactions, and the discovery of novel drug targets. To enhance accessibility and re-use of data, and facilitate analysis of different types of data, several web-based bioinformatic resources have been developed.

This week-long course represents a collaborative teaching effort involving the following resources dedicated to supporting research on fungal and oomycete species:

- FungiDB/VEuPathDB
- Ensembl Fungi/EBI
- SGD/CGD
- MycoCosm/JGI

The Fungal Pathogen Genomics course offers hands-on training on how to use unique, web-based tools provided by individual resources and apply them to both public and private datasets. Participants will learn to formulate testable hypotheses and explore genomes, functional omics datasets (transcriptomics, SNP, proteomics, etc.) and other data types across multiple databases.

Target audience

This course is aimed at graduate students, postdocs, clinical scientists, clinicians/healthcare professionals or lab heads working on fungal pathogens.

Programme

Daily activities will encompass both individual and group training exercises, complemented by supplementary lectures focusing on bioinformatics techniques and tools utilized by various databases. For example, the learning materials will address the following topics:

- Navigating gene record pages and genome browsers.
- Identification of orthologs and orthology-based inference.
- Comparative genomics, gene trees, whole-genome alignment.
- Creating in-silico experiments in FungiDB by employing search queries and public omics datasets and performing enrichment analysis on search results.
- Omics data analysis and visualization in VEuPathDB Galaxy, including RNA-Seq and Variant calling analysis (with Ensembl Variant Effect Predictor tool).
- Identifying genetic interactions, virulence genes, secondary metabolites.
- Introduction to manual genome curation using Apollo, a web-based platform for structural and functional genome annotation.

The programme also features talks by globally renowned guest speakers and provides occasions for engaging in scientific discussions and networking within a friendly environment.

Learning outcomes:

After attending this course, participants will be able to:

- Navigate each database and answer research questions by creating custom queries across multiple bioinformatics resources.
- Utilize built-in web-based bioinformatics tools to mine omics data, including:
    - finding genes
    - identifying orthologs in other species via orthologous transformations,
    - determining trends through GO and metabolic pathway enrichment analysis,
    - visualizing genomics, proteomics, and transcriptomics data,
- Perform RNA-Seq and Variant calling analysis in VEuPathDB Galaxy, exporting and sharing analysis results, and more.
- Gain an understanding of the practical advantages and limitations of the tools used in bioinformatics, ensuring that the skills acquired are pertinent to their research and future endeavors.
- Develop skills and knowledge necessary to actively contribute to community genome curation and annotation efforts.
- Apply their newfound skills in web-based bioinformatic resources in their research projects.

Course timetable with content

Sunday, 2nd of June

Time (BST)	Content
10:00 – 10:30	Registration
10:30 – 12:30	**Welcome, and Instructor/Database introductions** Wellcome Genome Science MycoCosm/JGI FungiDB SGD/CD Ensembl Fungi
12:30 –	**1. Introduction to database queries** • FungiDB site search & advanced search strategies • SGD YeastMine • Ensembl Fungi – BioMart • Ensembl Fungi Molecular Interactions
13:00 – 14:00	Lunch
– 15:30	Introduction to database queries, Cont/…
15:30 –	**2. Transcriptomics & Proteomics** • Ensembl Fungi Track Hubs • SGD Expression tools - SPELL • FungiDB Transcriptomic & Proteomic analysis
16:00 – 16:30	Tea break
– 19:00	Transcriptomics & Proteomics, Cont/…
19:00– 20:00	**Welcome reception & Dinner**
20:00 – 21:00	Participant presentations (2 min flash talks)

Monday, 3rd of June

Time (BST)	Content
09:00	Breakfast
09:00 – 10:30	**3. Evaluating gene model evidence**
10:30 – 11:00	Tea break
11:00 – 13:00	**4. Comparative Genomics & Orthology and Evolutionary analysis & cross-species inference** • Ensembl Fungi – WGA • MycoCosm CAZy enzymes • MycoCosm Synteny • Exploring protein domains and clusters across Ensembl & MycoCosm • SGD predicting fungal biology • Ensembl Fungi Evolutionary analysis (gene trees) • FungiDB &OrthoMCL: Orthology and Phyletic Patterns
13:00 – 14:00	Lunch
14:00 –	Comparative Genomics & Orthology, Cont/…
15:30– 16:00	Tea break
16:00 – 19:00	**5. NGS data analysis I** Background & Intro to VEuPathDB Galaxy • Deploying RNA-Seq • Deploying SNP workflows
19:00– 20:00	**Dinner**

Tuesday, 4th of June

Time (BST)	Content
– 09:00	Breakfast
09:00 –	**6. Enrichment analysis** • SGD GO Slim mapper • CGD GO Term finder • FungiDB GO enrichment
10:30 – 11:00	Tea break
– 12:30	Enrichment analysis, Cont/…
12:30 –	**7. NGS Data analysis II -** RNA-Seq analysis Part 2 Exporting and analysing data from Galaxy
13:00 – 14:00	Lunch
15:00 –	**NGS Data analysis**, Cont/…
15:30– 16:00	Tea break
– 18:00	**NGS Data analysis**, Cont/…
18:00 – 19:00	**Research Seminar**
19:00– 20:00	**Dinner**

Wednesday 5th of June

Time (BST)	Content
– 09:00	Breakfast
09:00 –	**8. NGS Data analysis II -** SNP analysis Part 2 Exporting and analysing data from Galaxy
10:30 – 11:00	Tea break
– 12:00	**NGS Data analysis**, Cont/…
12:15 – 12:30	**Introduction to group projects.**
13:00 – 14:00	Lunch
14:00 – 15:00	**Sanger Tour**
15:30– 16:00	Tea break
16:00 – 18:00	**3. SNPs & Variants** • SGD Variant viewer • FungiDB SNP analysis & CNVs • Exploring variant in Ensembl Fungi
18:00 – 19:00	**Research Seminar**
19:00– 20:00	**Dinner**

Thursday 6th of June

Time (BST)	Content
– 09:00	Breakfast
09:00 –	**8. Functional analysis: Pathways & metabolites** • MycoCosm KEGG Browser & Secondary metabolism clusters • FungiDB pathways & metabolites
10:30 – 11:00	Tea break
11:30 – 12:00	Manual curation in Apollo
12:00 –	**Group Projects**
13:00 – 14:00	Lunch
14:00 – 15:00	Group Projects, Cont/…
15:30– 16:00	Tea break
– 19:00	Group Projects, Cont/…
19:00– 20:00	**Dinner**

Friday 7th of June

Time (BST)	Content
– 09:00	Breakfast
09:00 –	Group Projects, Cont/…
10:30 – 11:00	Tea break
11:00 – 13:00	**Group Projects Presentations (12 min per team max)**
13:00 – 14:00	Lunch & Departure

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FungiDB Site Search

Learning objectives:

- Use keywords in site search.
- Filter site search results by categories, organisms, and other categories.
- Export results to a search strategy.
- Find genes with gene IDs.

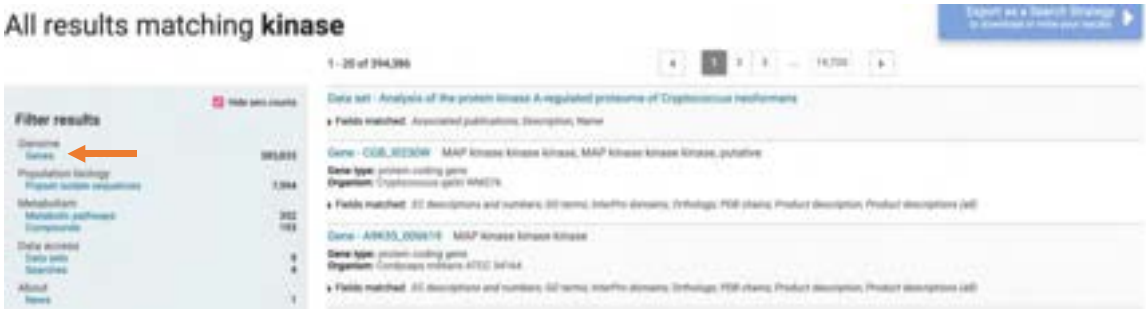
The site search can be accessed from the header of the site and is available from every page. The site search queries the database for a term (e.g., text) or a specific ID and returns a list of pages and documents that contain the query term.

Site search: text, term or gene id.

- Enter the word **kinase** in the site search window (at the top centre of the page). Click on the "enter" key on your keyboard or on the search icon as shown in the screenshot below.

- How many results with the word kinase did you get? Are all these records genes?

- Explore the filter panel on the left side of the page. Filter the results to view gene results only (hint: click on the word **Genes** in the “Filter results” section):



Notice that clicking on the “Genes” category reveals additional filtering options (on the left) and activates the “Export as a Search Strategy” button on the top right, which is now shown in dark blue color. This is because the search strategy can be deployed on a single category only (e.g. Genes or Data sets, but not both).

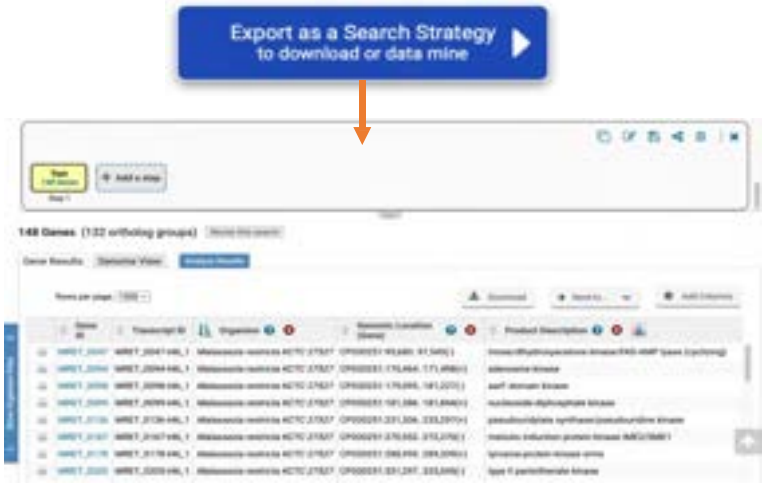
- Select and apply the “Product descriptions (all)” filter.

Note: The applied filter can be easily cleared by clicking on “Clear filter” option as shown in the screenshot below.

- In the “Filter organisms” section, select to filter gene results by ***Malassezia restricta*** **KCTC 27527**. How many genes contain “kinase” in the product description field in this organism?

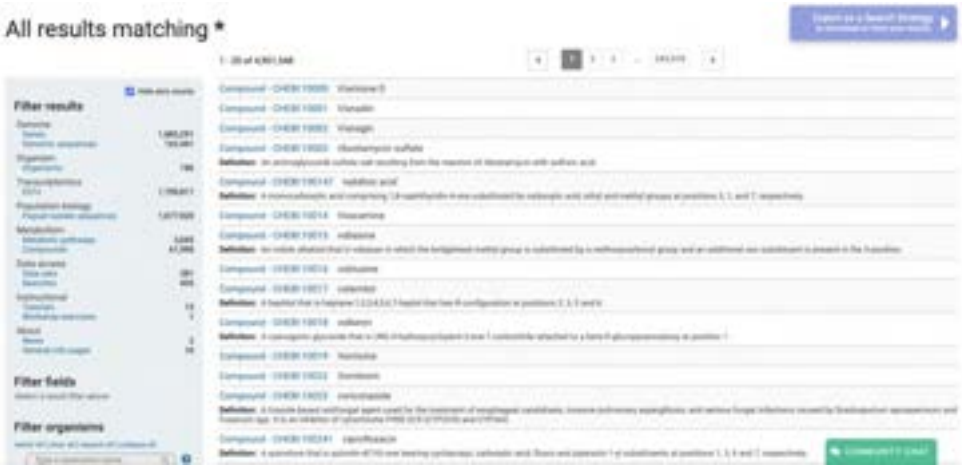
- Export the results to a search strategy.

To achieve this, click on the blue button called “Export as a search strategy…” at the top right-hand side of the results page.



- Try running the same search but this time use a wild card (*) (e.g., kinase*).

When the wild card is combined with a word (**kinase*** or ***kinase**), the search will retrieve compound words ending or beginning with the word kinase (e.g. *kinase - phosphofructo**kinase**). The wild card (*) can be used alone to retrieve all records available to the site search (see screenshot below).



- The site search also works with gene ids. Run a site search for the following gene id: Afu2g13260

The gene id search will return the gene record card for **Afu2g13260**.

Clicking on the gene link in blue within the card will bring up the gene record page for this gene.

Clicking on the "Export as a Search Strategy" button will create a search strategy with a single gene ID. This may be useful if you are interested in cross-referencing different types of data for one gene.

Search strategy links:
kinase - https://fungidb.org/fungidb/app/workspace/strategies/import/9c47e36cfa7790f6
kinase* - https://fungidb.org/fungidb/app/workspace/strategies/import/eee9e7d2dfb3e7c1
Afu2g13260 -
https://fungidb.org/fungidb/app/workspace/strategies/import/6fc6b7e52a15b76b

Advanced Search Strategies

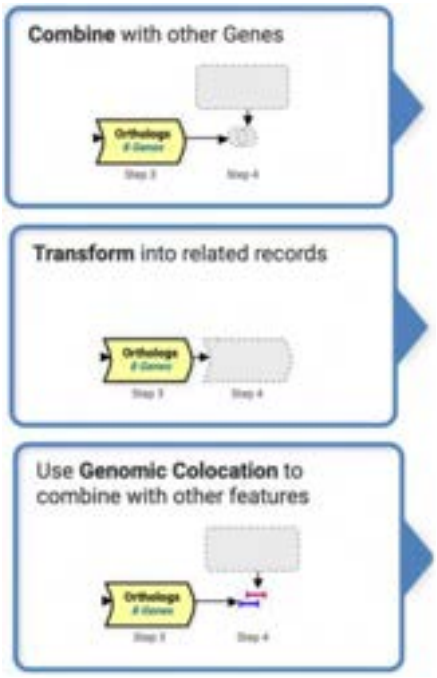
Learning objectives

- Deploy search for different types of data and create an advanced search strategy in FungiDB.

The strategy system offers a unique system of structured searches that can be combined to create multi-step *in-silico* experiments. As seen above, searches can be deployed from the site search, or the 'Search For…' menu on the home page, and from the 'Searches' dropdown menu in the header of every page.

Searches listed under the "Genes" category will return a list of gene IDs, while searches listed under the 'SNPs' or 'Metabolic Pathways' will return records relevant to SNPs data (e.g., sequences) and metabolic pathways, respectively.

When creating multi-step search strategy, the search strategy steps can be combined via three methods:



Combine with other Genes

Compare results that are gene lists

Transform into related records

Transform results into orthologs (e.g. Aspergillus > Candida), metabolic pathways or compounds.

Use Genomic Colocation

Combine different data types (e.g., cross-reference gene lists with metabolic pathway IDs).

Within the search strategy, each step is connected via the system of Boolean operators that can intersect, unite, or subtract similar records (e.g., gene lists) and cross-references different types of data via the genomic colocation option.

Steps within the strategy can also be concealed using "ignore step" Boolean operators, enabling rapid modifications to the strategy without necessitating step deletion.

Creating advanced search strategies in FungiDB.

In this exercise, we identify *Aspergillus fumigatus* Af293 genes that:

A. Are up-regulated when *Aspergillus* is exposed to human airway epithelial cells,
B. Have non-synonymous mutations identified by whole genome sequencing (WGS) of clinical isolates,
C. Are known to be immune-reactive.

Here is a step-by-step guide on how to create this in-silico experiment:

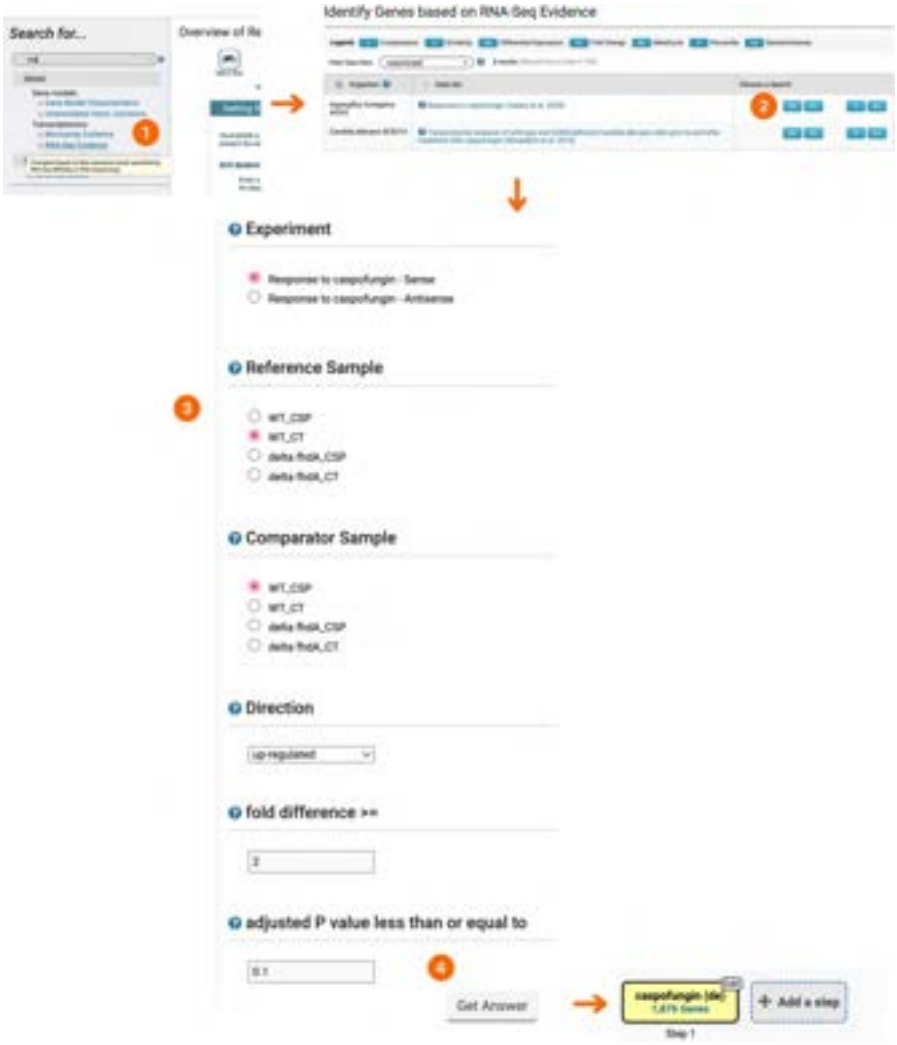
A. Deploy the "RNA-Seq Evidence" search to identify genes that are up-regulated when *Aspergillus* is exposed to human airway epithelial cells.

1. Select the search from the "Search for…" panel (shown below) or the "Searches" menu at the top of the page.

Tip: Utilize the filter box to promptly retrieve relevant search results.

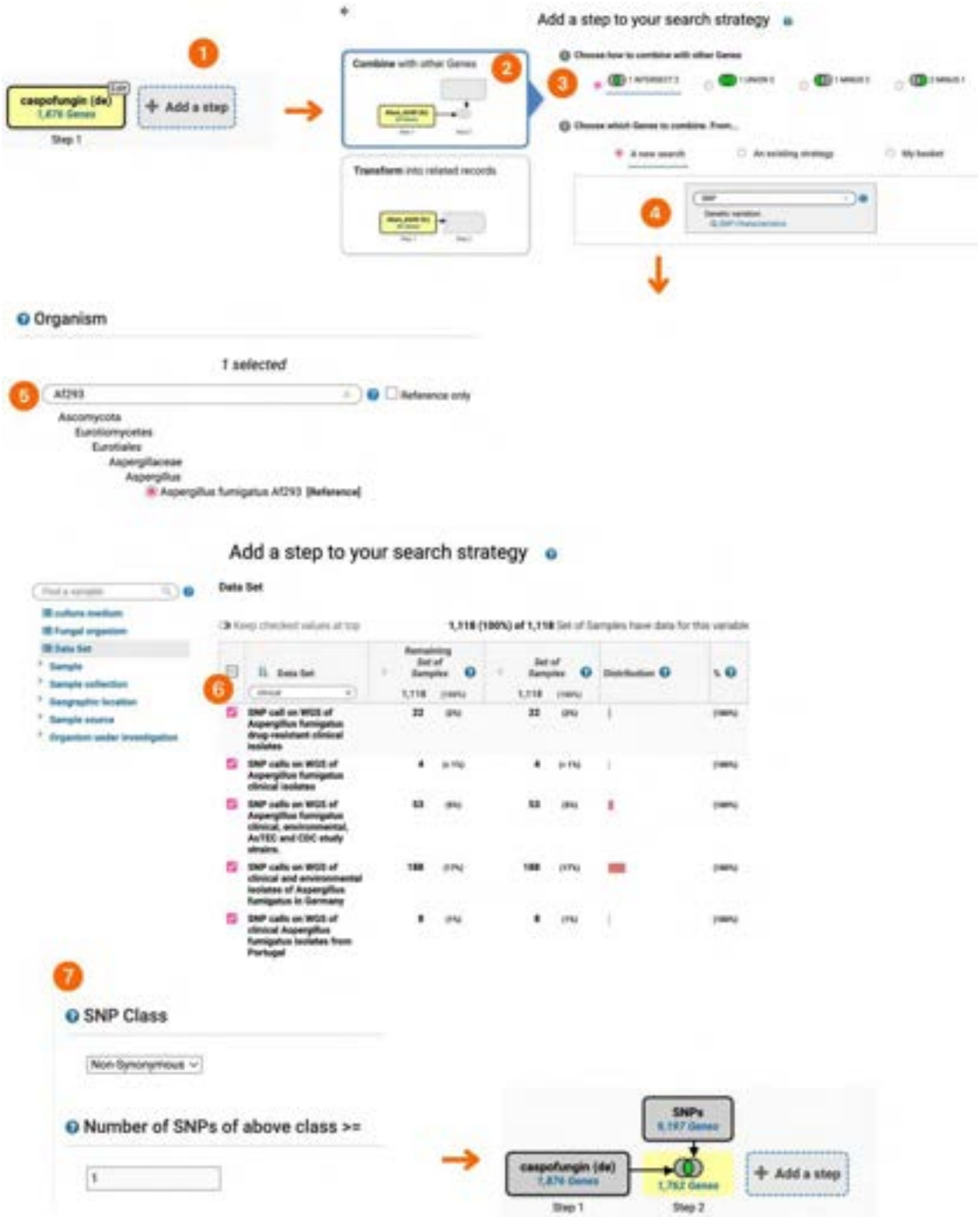
2. Identify the 'Response to caspofungin (Valero et al. 2020)' dataset and click on the DE (Differential expression) button.
3. Set up search parameters:
    i. Reference sample: WT_CT
    ii. Comparator sample: WT_CSP
    iii. Direction: up-regulated
    iv. Fold: 2

    Leave other parameters at default.
4. Click on the "Get Answer" button.



B. Deploy SNP Characteristic search to identify genes with non-synonymous SNPs using WGS data of clinical isolates.

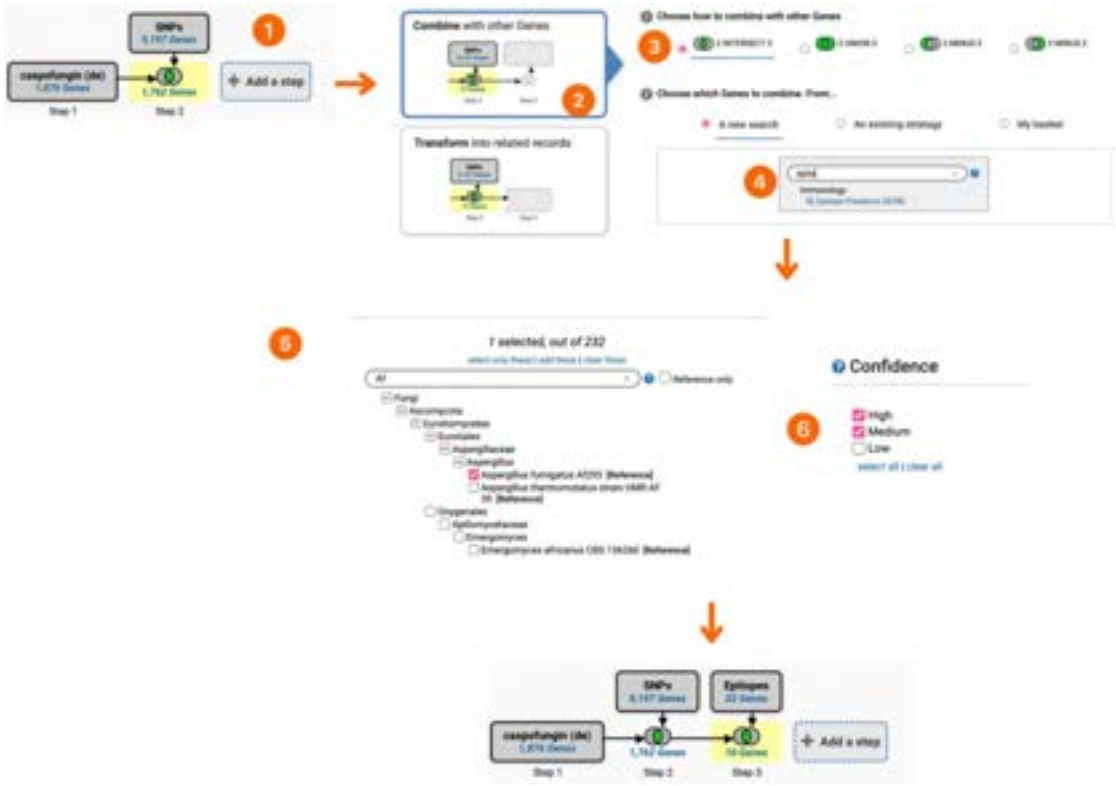
1. Within the search strategy, click on the “Add a step” button.
2. Make sure to use the “Combine with other Genes” option.
3. Select the “1 INTERSECT 2” Boolean operator (if not selected by default already)
4. Use filter to identify the new search to deploy – “SNP Characteristics”. Click on the “SNP Characteristics” link in blue to deploy the search.
5. Filter for ‘Af293’ and select “Aspergillus fumigatus Af293”
6. Filter datasets for “clinical” and select all 5 datasets
7. Specify specific SNP characteristics (SNP Class = Non-Synonymous; Number of SNPs of above class >=1) and run the analysis.



C. Identify genes with immune reactivity.

Epitopes are recognized by the immune system and can be used for vaccine development. Identify genes that have annotated epitope records.

1. Click on the “Add a step” button.
2. Make sure to select the “Combine with other Genes” option.
3. Select the “2 INTERSECT 3” Boolean operator (if not selected by default)
4. Filter available searches for “epitope” to identify and deploy the “Epitope Presence (IEDB)” search.
5. Set organism to Aspergillus fumigatus Af293.
6. Set Confidence to “high” and “Medium” and click on the Run Step button.



Well done! You have created an in-silico experiment using three different types of data – RNA-Seq, SNPs, and epitope data.

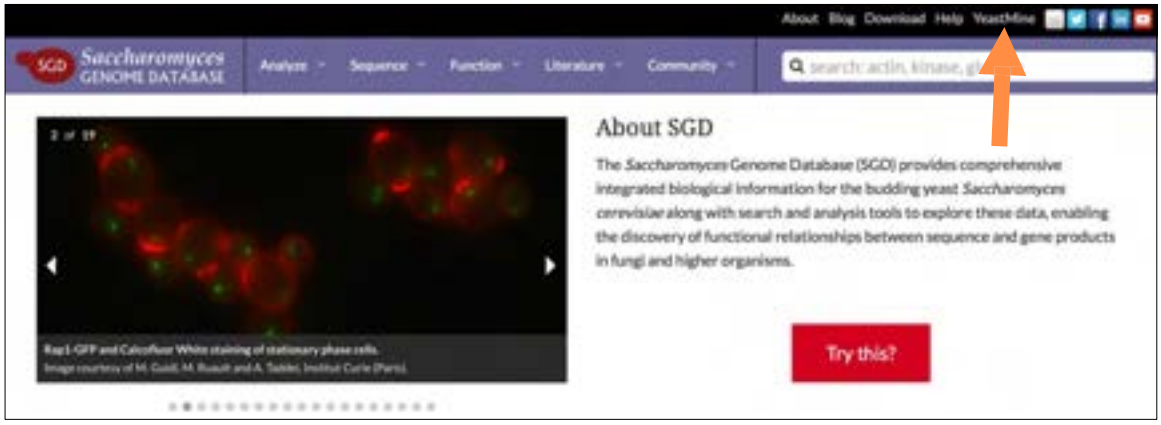
Search strategy link:
https://fungidb.org/fungidb/app/workspace/strategies/import/0e675d26ca9287cb

Search Strategies in SGD

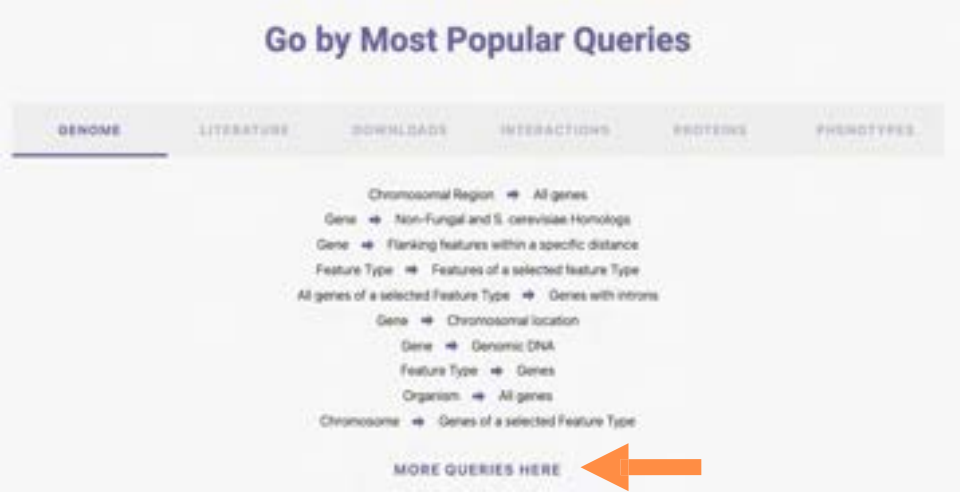
In addition to a faceted search tool, SGD provides **YeastMine** (https://yeastmine.yeastgenome.org/) as a means for users to conduct more advanced queries. YeastMine enables rapid retrieval and manipulation of curated biological data on *S. cerevisiae* genes and genomic features. By creating gene lists, users can retrieve data on multiple genes at once. Gene lists can then be continually modified, analyzed, and refined as desired, enabling you to answer complex biological questions such as, “How many plasma membrane proteins are required for viability?” or “Which kinases, if knocked out, increase chronological lifespan?”

In this exercise, we will use YeastMine to search for as-yet undiscovered mitochondrial ribosomal proteins in yeast.

- Access YeastMine from SGD home page (http://www.yeastgenome.org); click on YeastMine in the upper right corner above the search box.



1. **Create a list of proteins that are known subunits of the mitochondrial ribosome (MTR):**

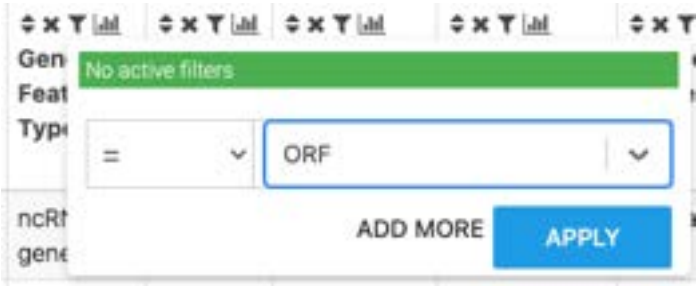


- Click on "More queries here"

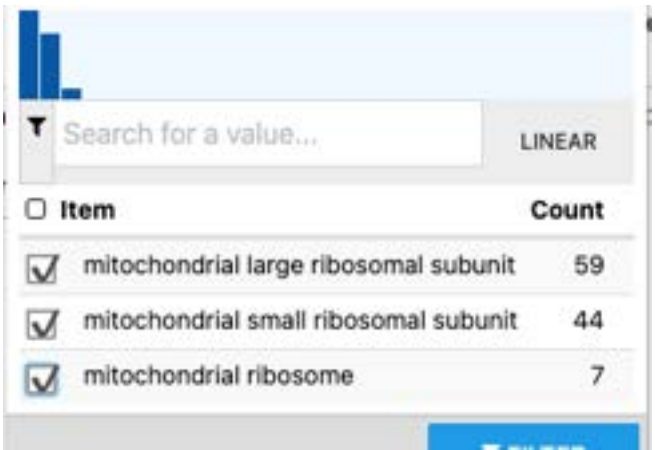
- And then select the **FUNCTION** tab and then **GO Slim Term => Gene**.

Enter "mitochondrion" as your GO slim term. This will return many bottom of the results and click "**View 3494 rows**."

- In the Query Results, first go to the Gene Feature Type column, click the filter icon and then select "**ORF**" from the drop-down menu and "Apply."



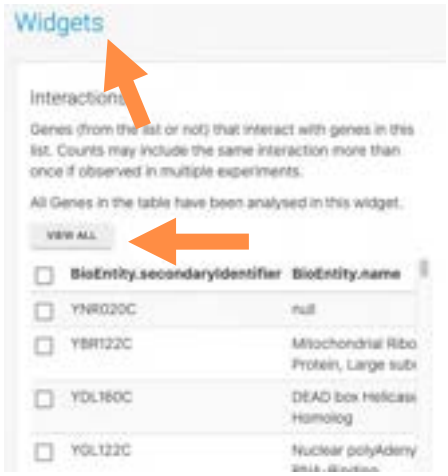
- Next go to the "Ontology Term Name" column, hit the graph icon, and select the boxes for "**mitochondrial large ribosomal subunit**," "**mitochondrial small ribosomal subunit,"** and "**mitochondrial ribosome**." Hit FILTER and you'll get 108 results.



- Save this list by clicking the **Save List** button on the upper right of the table and selecting **"Genes (89)"** at the top of the pull-down. Give it the name "List 1 MTR genes" and save.

2. Find proteins that genetically interact with MTR proteins:

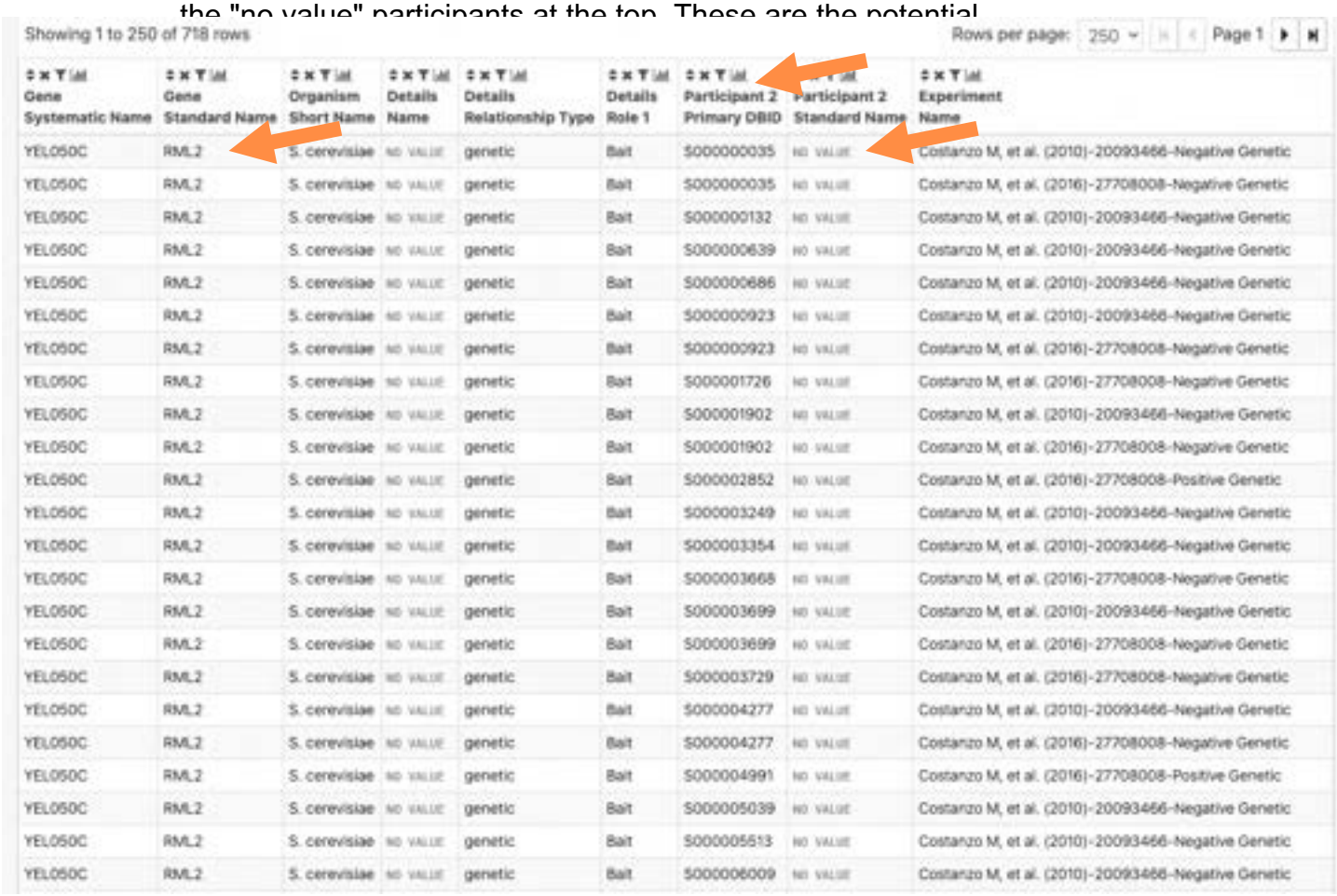
- Scroll down to below the table and find the "Widgets" section, click "View All."



- The results table shows all genes/proteins with genetic or physical interactions with the MTR genes. There are over 17K of them.
- Go to the column for "**Details Relationship Type**" and filter for "**Genetic**." Hit Apply and you'll get a list of 8970 rows.

3. **Find MTR interactors that are uncharacterized:**

- Go to the "**Gene Standard Name**" column and click the filter icon. For the purposes of this exercise, filter to include ONLY the gene **RML2,** which yields 718 rows.
- Go to the column **"Participant 2 Standard Name"** (these are the genes that interact with RML2) and SORT this column by descending order, which puts the "no value" participants at the top. These are the potential



Showing 1 to 250 of 718 rows — Rows per page: 250 — Page 1

Gene Systematic Name	Gene Standard Name	Organism Short Name	Details Name	Details Relationship Type	Details Role 1	Participant 2 Primary DBID	Participant 2 Standard Name	Experiment Name
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000000035	NO VALUE	Costanzo M, et al. (2010)-20093466-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000000035	NO VALUE	Costanzo M, et al. (2016)-27708008-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000000132	NO VALUE	Costanzo M, et al. (2010)-20093466-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000000639	NO VALUE	Costanzo M, et al. (2010)-20093466-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000000686	NO VALUE	Costanzo M, et al. (2010)-20093466-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000000923	NO VALUE	Costanzo M, et al. (2010)-20093466-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000000923	NO VALUE	Costanzo M, et al. (2016)-27708008-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000001726	NO VALUE	Costanzo M, et al. (2016)-27708008-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000001902	NO VALUE	Costanzo M, et al. (2010)-20093466-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000001902	NO VALUE	Costanzo M, et al. (2016)-27708008-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000002852	NO VALUE	Costanzo M, et al. (2016)-27708008-Positive Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000003249	NO VALUE	Costanzo M, et al. (2010)-20093466-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000003354	NO VALUE	Costanzo M, et al. (2010)-20093466-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000003668	NO VALUE	Costanzo M, et al. (2016)-27708008-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000003699	NO VALUE	Costanzo M, et al. (2010)-20093466-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000003699	NO VALUE	Costanzo M, et al. (2016)-27708008-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000003729	NO VALUE	Costanzo M, et al. (2016)-27708008-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000004277	NO VALUE	Costanzo M, et al. (2010)-20093466-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000004277	NO VALUE	Costanzo M, et al. (2016)-27708008-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000004991	NO VALUE	Costanzo M, et al. (2016)-27708008-Positive Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000005039	NO VALUE	Costanzo M, et al. (2010)-20093466-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000005513	NO VALUE	Costanzo M, et al. (2010)-20093466-Negative Genetic
YEL050C	RML2	S. cerevisiae	NO VALUE	genetic	Bait	S000006009	NO VALUE	Costanzo M, et al. (2010)-20093466-Negative Genetic

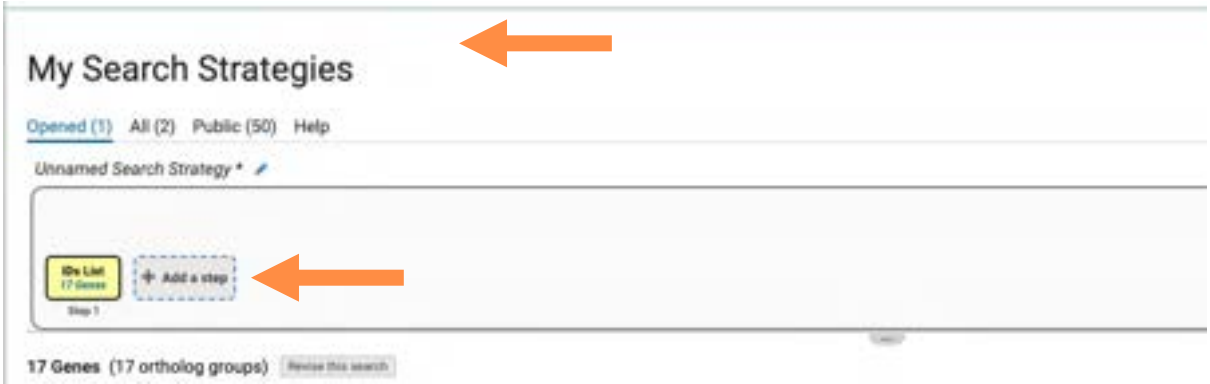
- Given the current state of bugginess in this tool (it's undergoing major revision), you'll do best to copy and paste the section of the list that includes just the rows that say "no value" in the Participant 2 column. Copy this section and paste it into a blank worksheet.
- In the worksheet, sort by **column G** (the "Participant 2 Primary DBID") and then do a quick de-dupe of the list to leave 17 potential uncharacterized interactors. Save this worksheet as "**List 2: Uncharacterized MTR interactors**"
- Copy this list of 17 database IDS.

4. Analyze the results in FungiDB

- The results of the above YeastMine analysis suggest 17 genes that potentially encode undiscovered subunits of the mitochondrial ribosome. Although these genes are uncharacterized, more data may exist on their orthologs in other organisms. Use FungiDB to survey the function of orthologs in other Fungi.
- Open the FungiDB homepage (http://fungidb.org/). In the "**Search for…"** section on the left, click "Genes" and then open the "**Annotation, curation and identifiers**" section and click on "**List of ID(s)**".

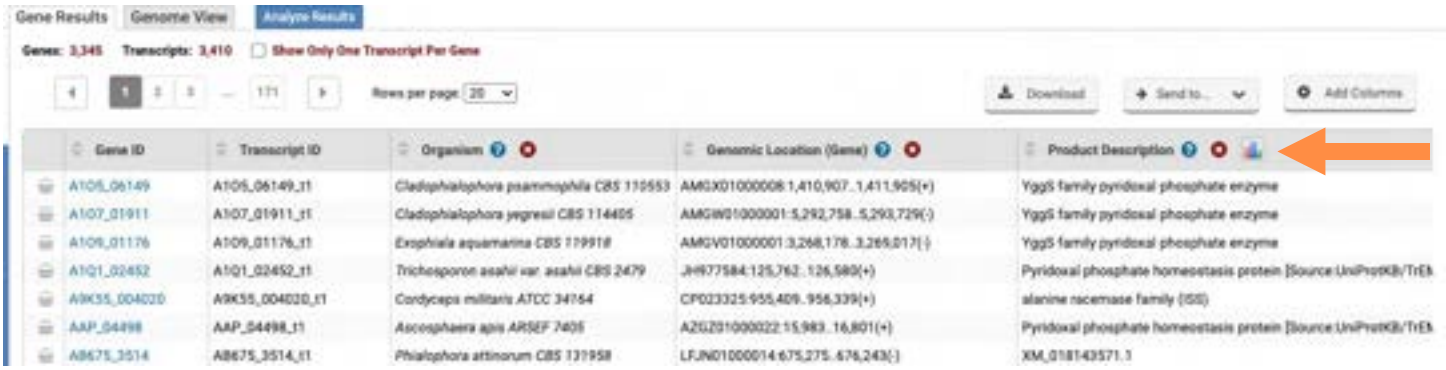
- Using your list of DBIDs from YeastMine, copy and paste the systematic names of your results into the "**Enter a list of IDs**" box. Click on "**Get Answer**"

- Click on the "**Add a step**" button.



- In the resulting pop-up window, click on **Transform into Related Records**. Select **Orthologs** and then **Fungi** and click on **Run Step**.

- Orthologs from multiple species will be shown in the results table. Peruse the "**Product Description**" column. Do the descriptions of these orthologs support the prediction that the 17 yeast genes encode subunits of the mitochondrial ribosome? Click on the bar graph icon by the Product Description column to see a word cloud of entries in this column.

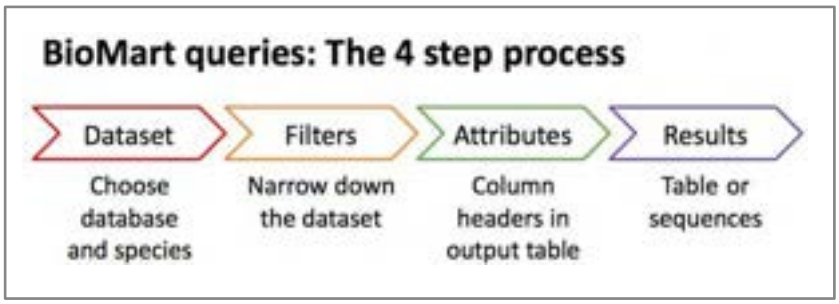


Exercise: Ensembl Fungi BioMart

Links to be clicked shown in blue, text to be entered shown in red.

Follow these instructions to guide you through BioMart to answer the following query:

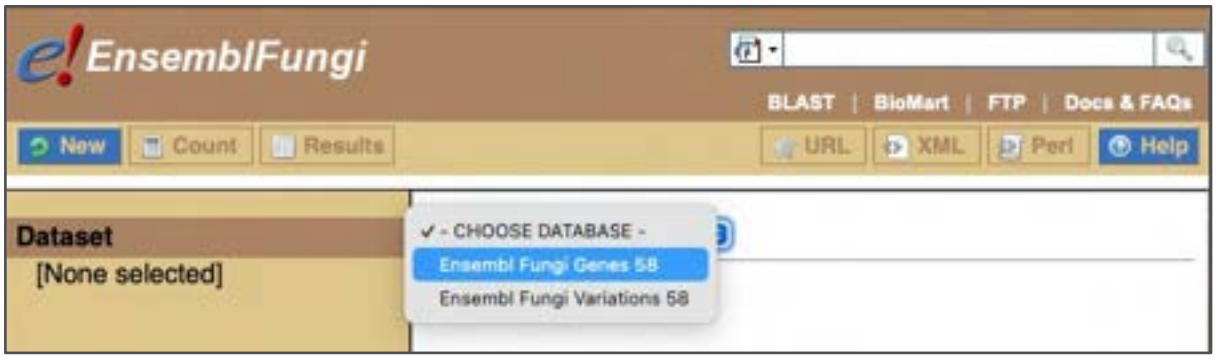
(a) How many genes within the 14:1128520-1142558 region are found in *Fusarium solani* that do not have an orthologue in *Fusarium verticillioides*?
(b) Export the gene name, locations and GO terms associated with these genes
(c) Export their cDNA sequences



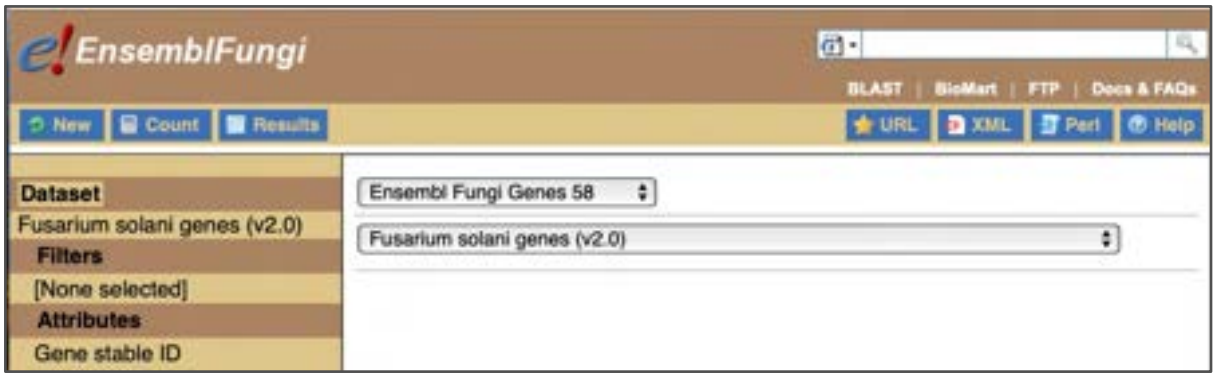
Click on BioMart in the top header of any fungi.ensembl.org page or enter https://fungi.ensembl.org/biomart/martview/ into your browser.

NOTE: These answers were determined using BioMart Ensembl Fungi 58

Step 1a: Choose Ensembl Fungi Genes 58 as the database

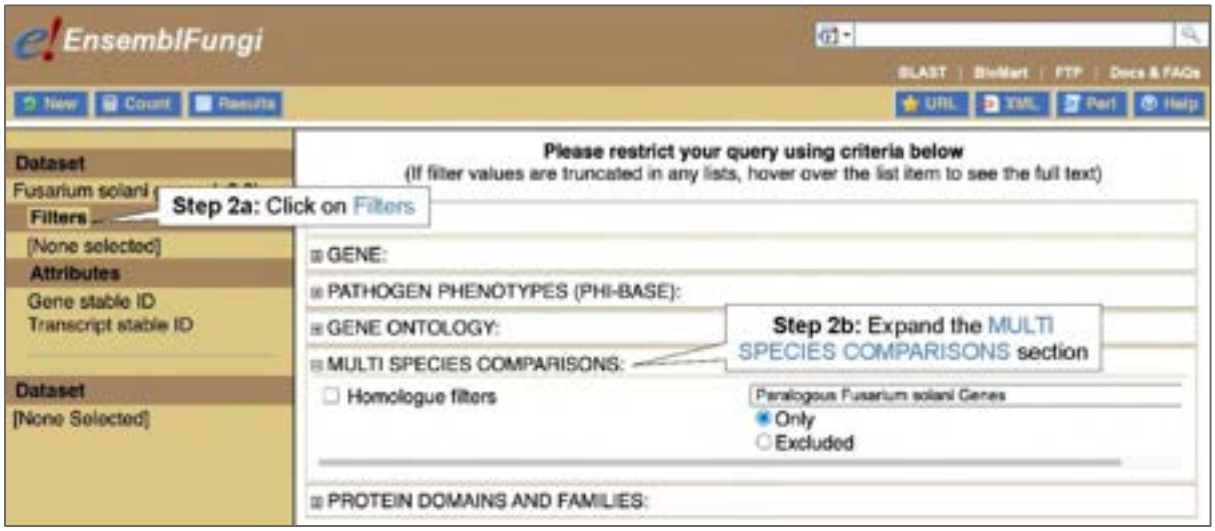


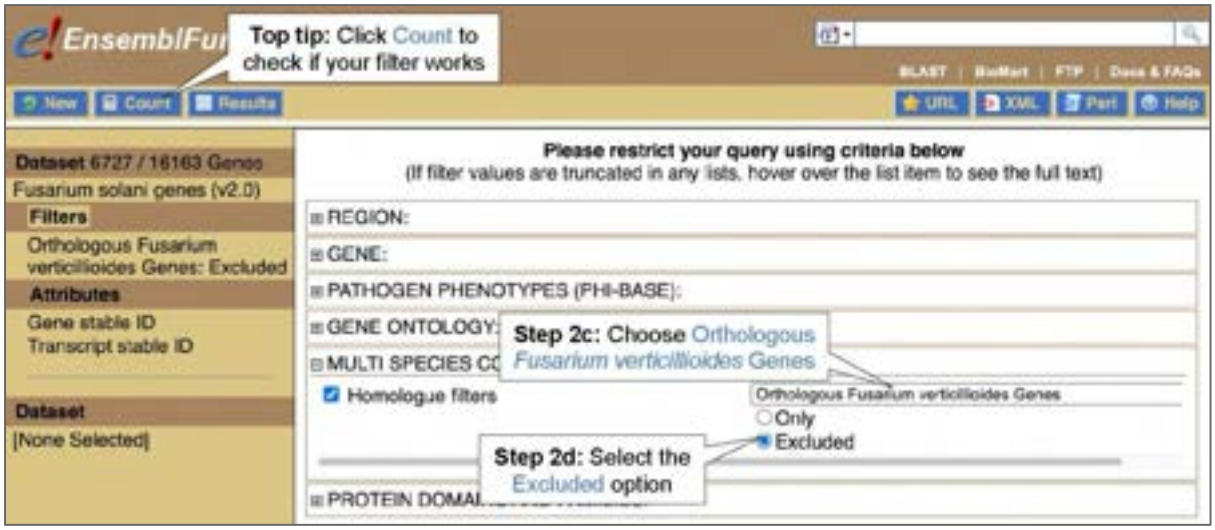
Step 1b: Choose *Fusarium solani* genes (v2.0) as the dataset

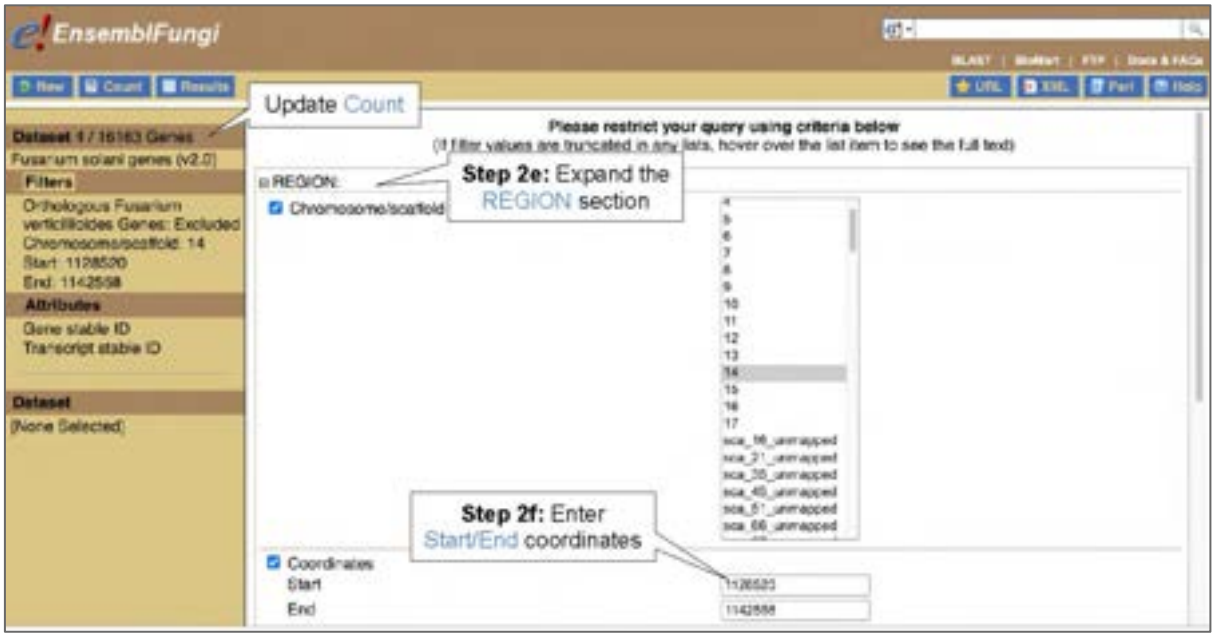


Step 2: Choose appropriate filters

We want to narrow down the dataset of all *F. solani* genes to a subset of genes matching our filters. We are interested in *F. solani* genes that **do not** have an orthologue with *F. verticillioides*. We need to filter the dataset to find these genes.







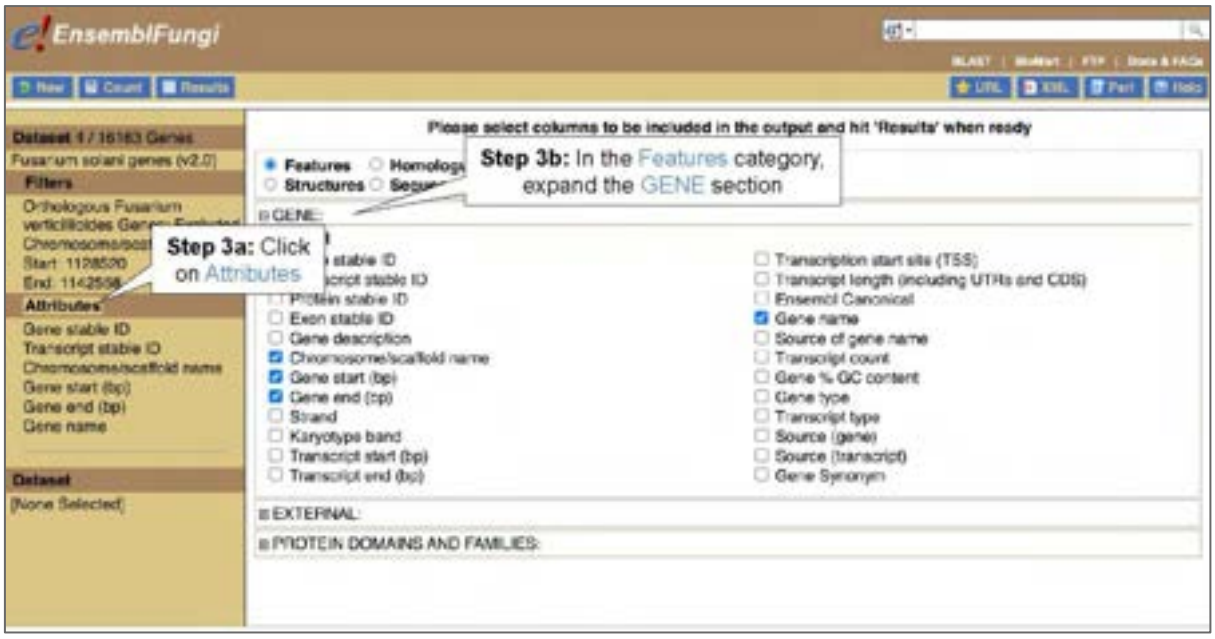
Using the Count function we can see that there are 4 *F. solani* genes (out of a total of 16,163) in the 14:1128520-1142558 region that do not have an orthologue in *F. verticillioides*.

Step 3: Select Attributes

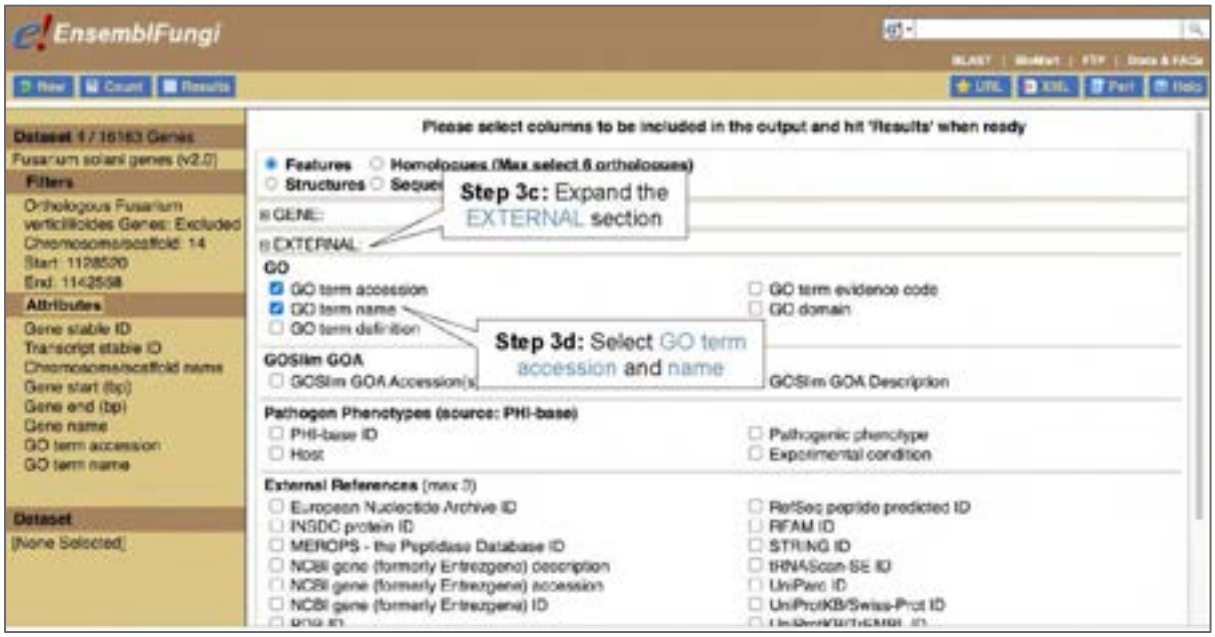
Attributes (our desired output) are defined by what we would like to learn about the data. We want to find out more information about these genes, including:

1. Gene name
2. Locations
3. Associated GO terms
4. cDNA sequences

There are four main attribute types: Features, Structures, Homologues and Sequences. BioMart allows querying only one type at a time. We can answer points 1-3 in a single query as they can all be found under Features, but we will need to build a second query to answer point 4 (Sequence type).



Make sure that Features is selected at the top of the page. Expand the GENE section, select Chromosome/scaffold name, Gene start and Gene end, and Gene name.



Expand the EXTERNAL section. This section contains lots of identifiers from databases outside of Ensembl. Select GO term accession and GO term name.

Step 4: Get results!

You will retrieve your BioMart results in tabular format. Notice the order of the columns - these are in the same order in which you selected your Attributes.

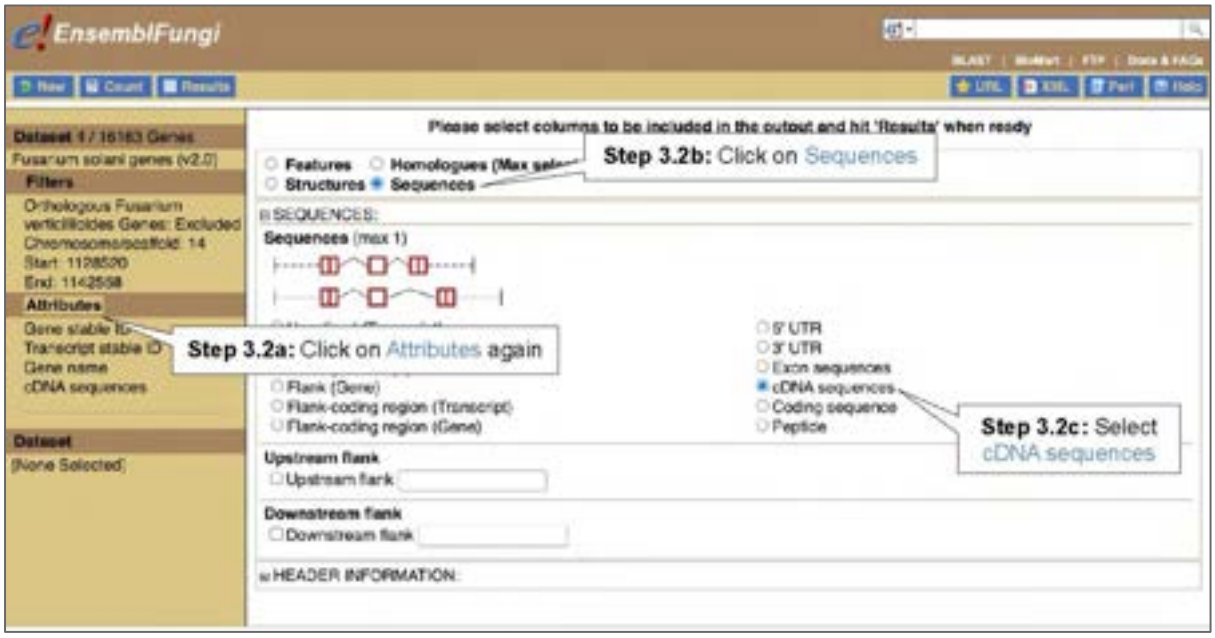
You can download the data if you like. The output table shows only 10 first rows by default.

You can click on the location links and explore the synteny between the two species on the Ensembl Fungi browser.

What about the last point? 'Export their cDNA sequences?'
In the Attributes section there are some 'radio buttons'. If you'd like to export Sequence data, you need to build a separate query.

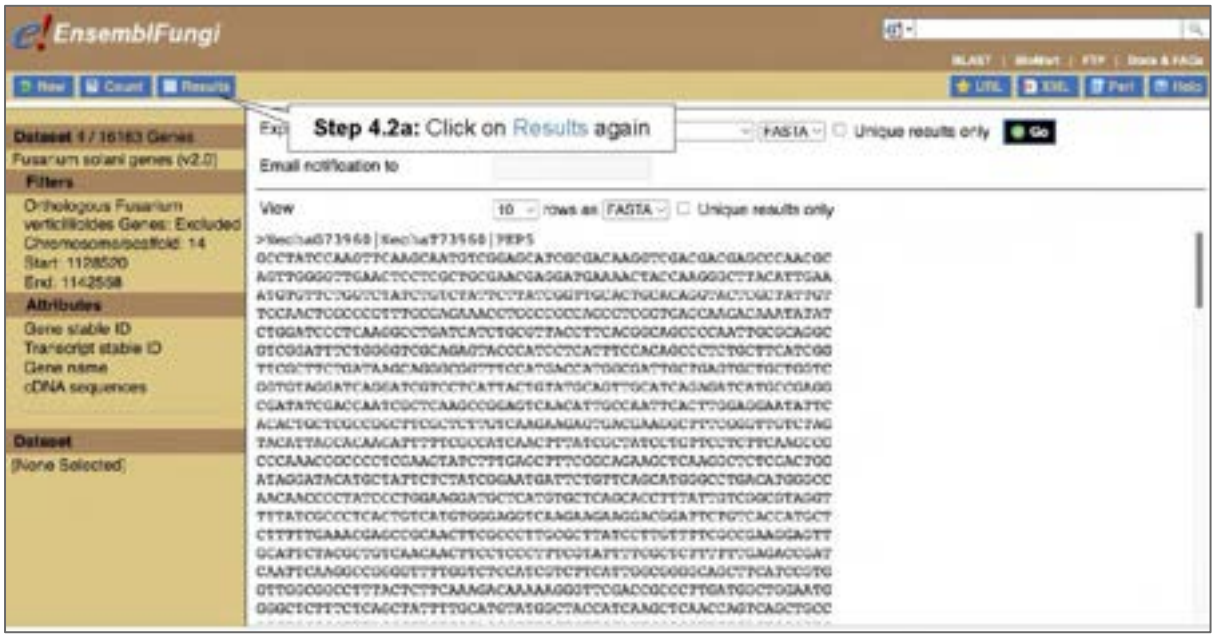
Step 3.2: Let's go back to step 3: Selecting attributes

From the results page, click back to Attributes in the left-hand navigation panel – there's no need to start from scratch.



Also expand the HEADER INFORMATION section and select Gene name.

Step 4.2: View results for the sequences



What did you learn about these genes in this exercise?

Could you learn these things from the Ensembl browser? Would it take longer?

For more details on BioMart, have a look at this publication:

Kinsella RJ, Kähäri A, Haider S, et al. Ensembl BioMarts: a hub for data retrieval across taxonomic space. Database : the Journal of Biological Databases and Curation. 2011;2011:bar030. DOI: 10.1093/database/bar030. PMID: 21785142; PMCID: PMC3170168.

Additional BioMart Exercise 1 – Export orthologues

Use Ensembl Fungi BioMart to retrieve all *Zymoseptoria tritici* genes associated with the GO term 'detoxification' located on chromosome 1. Export the gene IDs, names, homology type and confidence of their orthologues in *Blumeria graminis*, *Botrytis cinerea*, *Cryptococcus neoformans* and *Saccharomyces cerevisiae*.

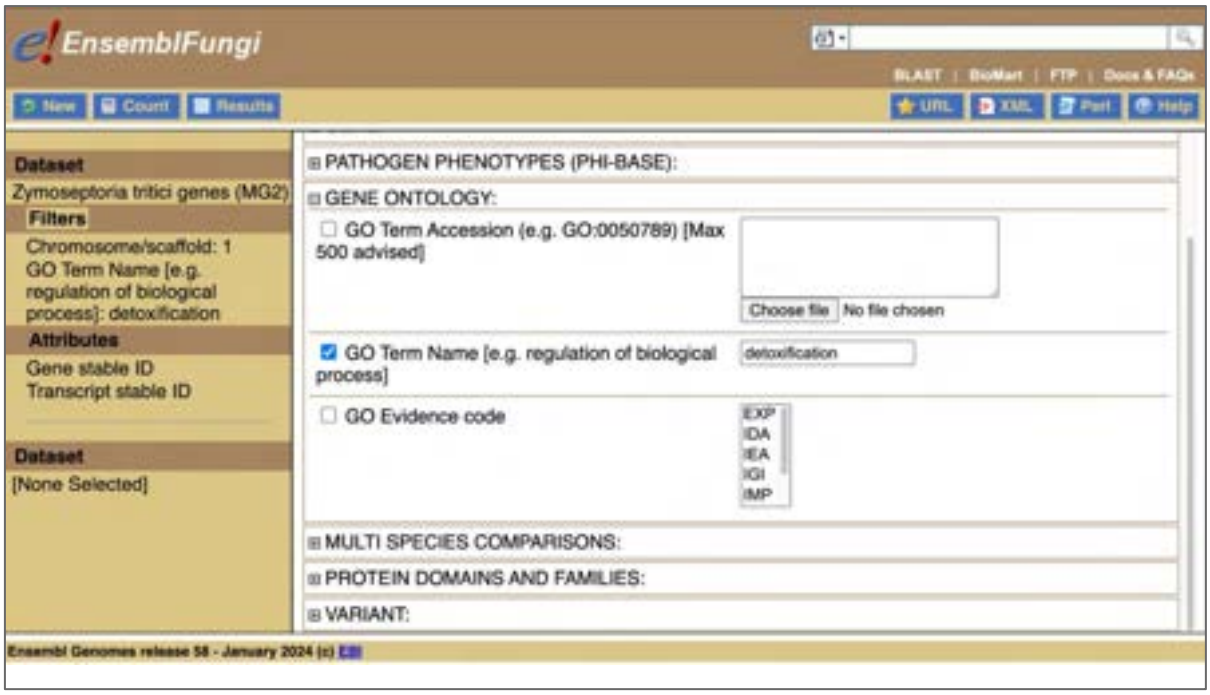
(a) Do all of these *Z. tritici* genes have an orthologue in the other species? Which of these species are pathogenic? Do you see a correlation?
(b) Can you find an orthologue in *Cryptococcus neoformans* with high orthology confidence? What is the Gene ID? We will explore more about this orthologue in the exercise section for the Evolutionary Analysis module.

Exercise 1 answers

You can open BioMart by clicking BioMart in the navigation bar at the top of any Ensembl Fungi page, or by entering the URL https://fungi.ensembl.org/biomart/martview/ in your browser. Using the four-step process described above, we need to select the following in BioMart options in the left-hand panel:

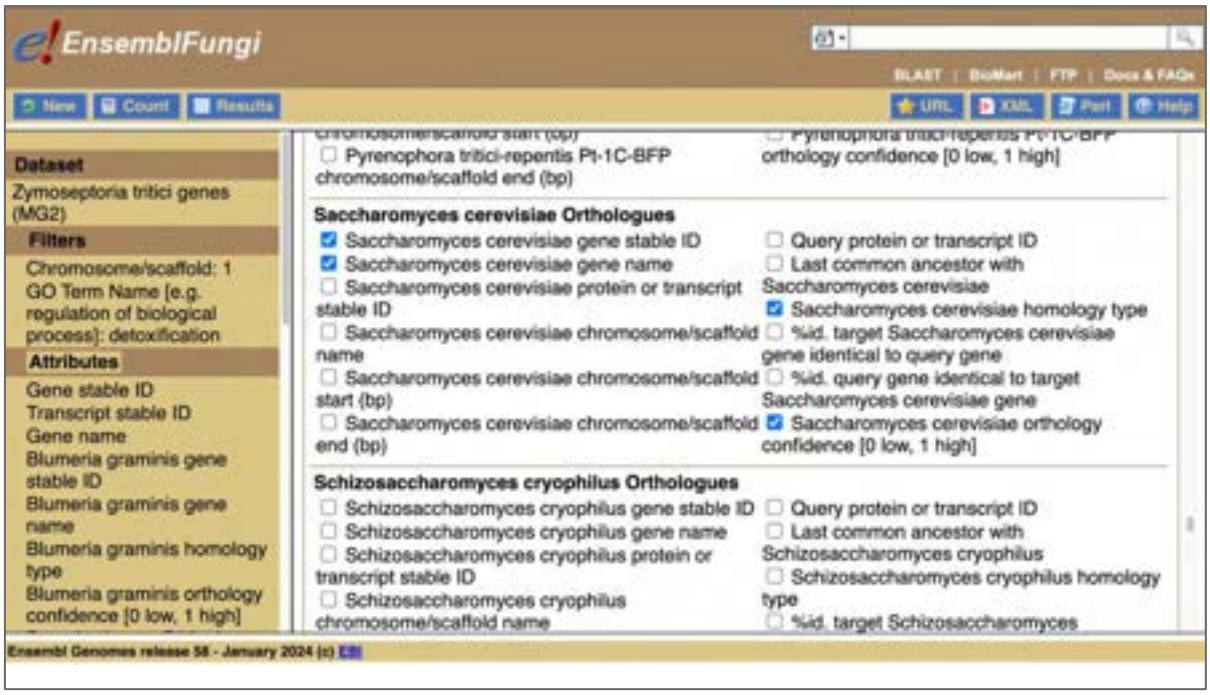
1. Dataset: Click on CHOOSE DATABASE and select Ensembl Fungi Genes 58 from the drop-down menu. Click on CHOOSE DATASET and select *Zymoseptoria tritici* genes (MG2) from the drop-down menu.

2. Filters: Open the REGION tab and select 1 under Chromosome/scaffold. Open the GENE ONTOLOGY tab and enter detoxification under GO Term Name. Click on the Count button in the top left-hand corner. Your filter should apply to 19/11,091 genes.

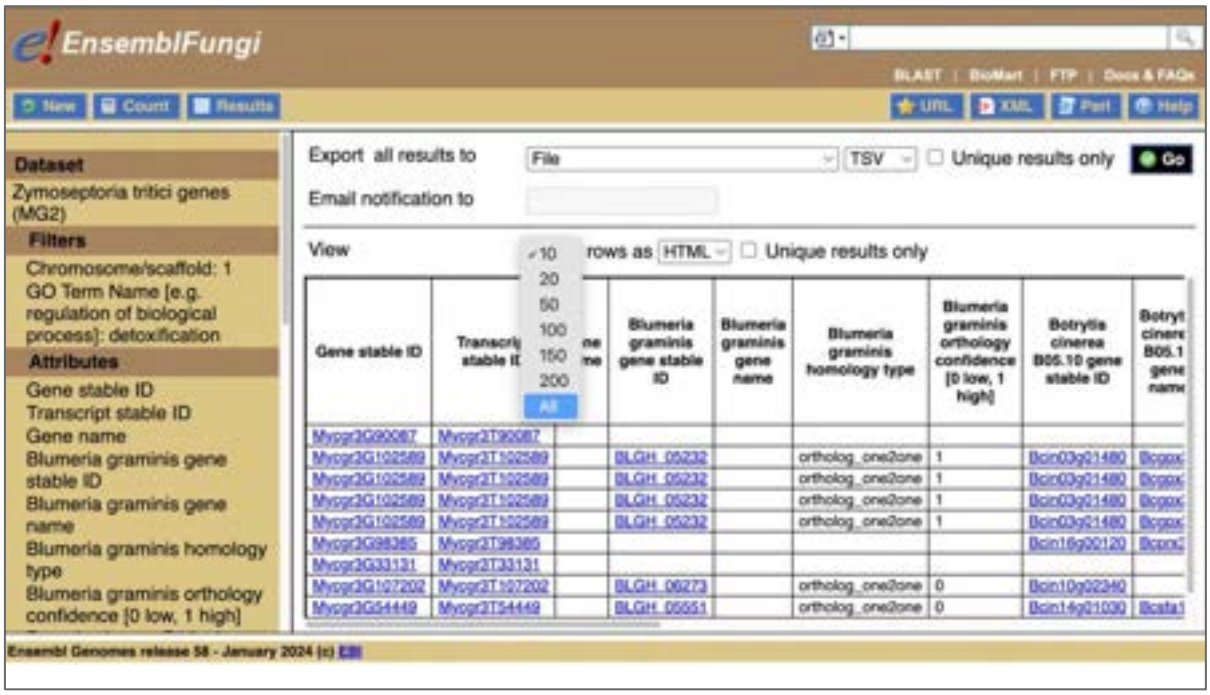


3. Attributes: Select Homologues from the options on the top. Open the GENE tab and select Gene name. Open the ORTHOLOGUES [A-E] tab and select the following options:
    - *Blumeria graminis* gene stable ID
    - *Blumeria graminis* gene name
    - *Blumeria graminis* homology type
    - *Blumeria graminis* orthology confidence [0 low, 1 high]
    - *Botrytis cinerea B05.10* gene stable ID
    - *Botrytis cinerea B05.10* gene name
    - *Botrytis cinerea B05.10* homology type
    - *Botrytis cinerea B05.10* orthology confidence [0 low, 1 high]
    - *Cryptococcus neoformans* var. *neoformans* JEC21 gene stable ID
    - *Cryptococcus neoformans* var. *neoformans* JEC21 gene name
    - *Cryptococcus neoformans* var. *neoformans* JEC21 homology type
    - *Cryptococcus neoformans* var. *neoformans* JEC21 orthology confidence [0 low, 1 high]

    Open the ORTHOLOGUES [P-T] tab and select the following options:
    - *Saccharomyces cerevisiae* gene stable ID
    - *Saccharomyces cerevisiae* gene name
    - *Saccharomyces cerevisiae* homology type
    - *Saccharomyces cerevisiae* orthology confidence [0 low, 1 high]



4. Results: Click on the Results button in the top left-hand corner to view your output table. Select All from the drop-down menu to open the full table in a new tab.



(a) No, not all *Z. tritici* genes located on chromosome 1 with the associated GO term
'detoxification' have an orthologue in the other species. *B. graminis* causes powdery mildew on grasses (e.g. cereals), *B. cinerea* is known to cause botrytis bunch rot in grape and *C. neoformans* is the causative agent of cryptococcosis and cryptococcal meningitis. Do you see a correlation?

(b) CNM01690 in *C. neoformans* has high orthology confidence.

Additional BioMart Exercise 2 – Finding genes by protein domain

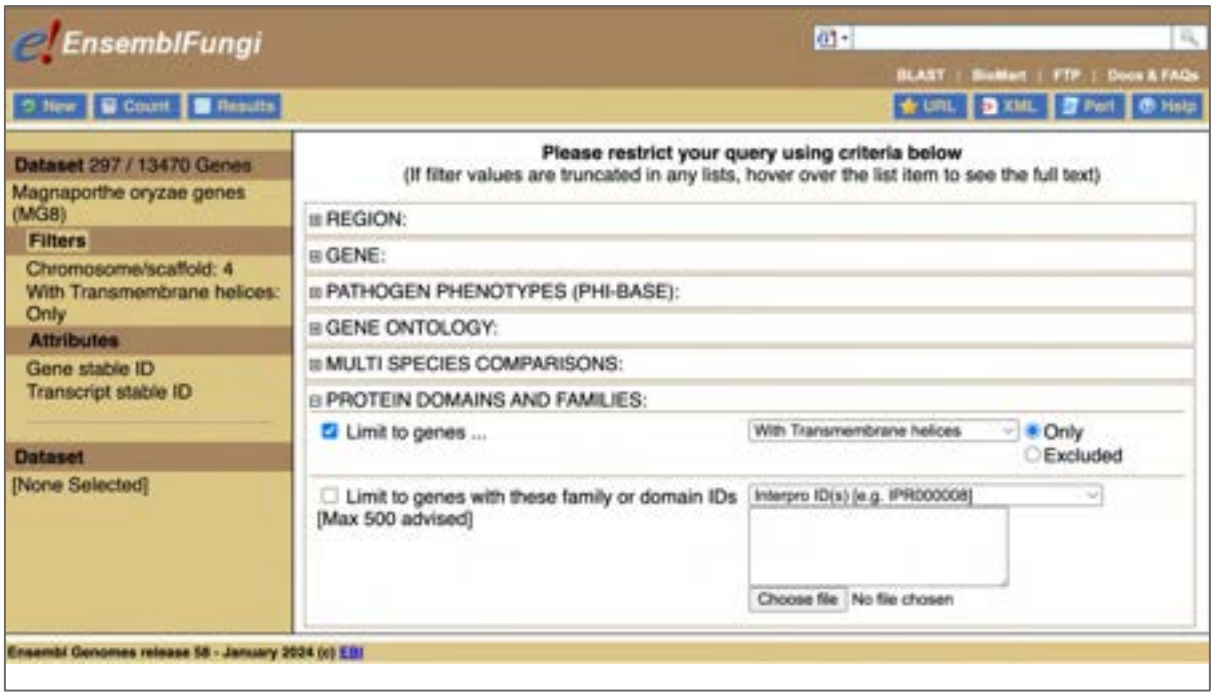
Generate a list of all *Magnaporthe oryzae* (MG8) genes on chromosome 4 that are annotated to contain Transmembrane domains/helices. Include the Ensembl gene stable ID and description.

Exercise 2 answers

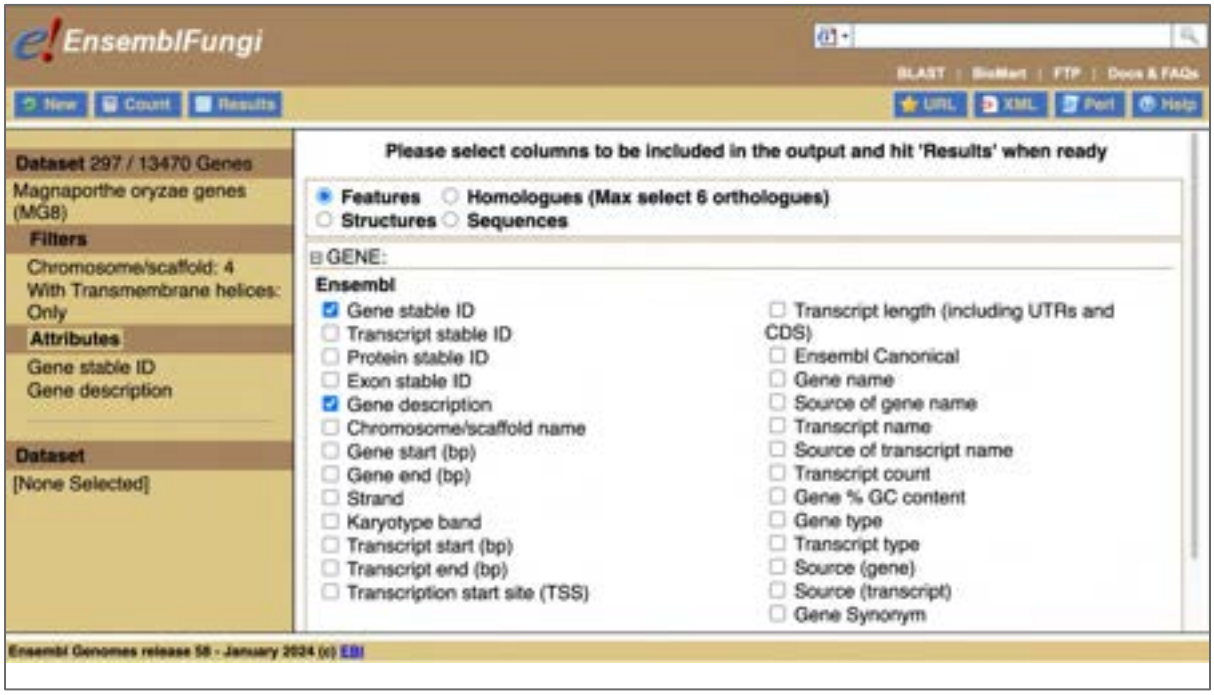
Click on the New button in the top left-hand corner to start a new BioMart query. Using the four-step process described above, we need to select the following in BioMart options in the left-hand panel:

1. Dataset: Click on CHOOSE DATABASE and select Ensembl Fungi Genes 58 from the drop-down menu. Click on CHOOSE DATASET and select *Magnaporthe oryzae* genes (MG8) from the drop-down menu.

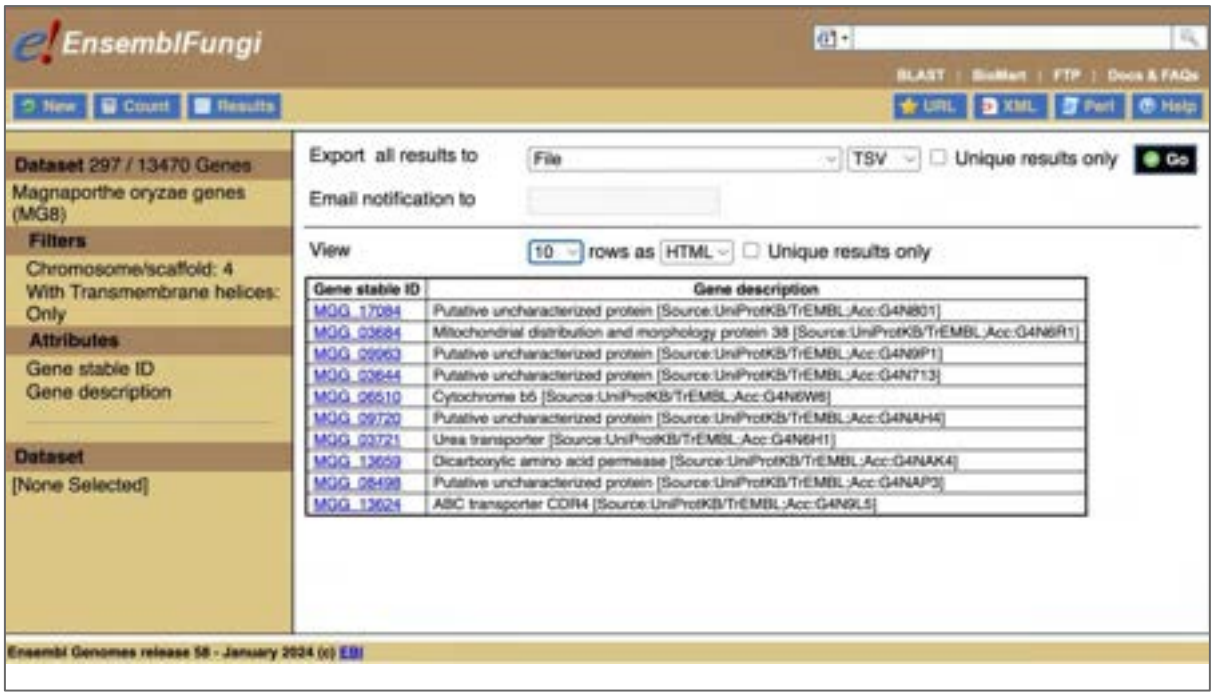
2. Filters: Open the REGION tab and select 4 under Chromosome/scaffold. Open the PROTEIN DOMAINS AND FAMILIES tab and select With transmembrane helices - Only under Limit to genes…. Click on the Count button in the top left-hand corner. Your filter should apply to 297/13,470 genes.



3. Attributes: Select Features from the options on the top. Open the GENE tab, unselect Transcript stable ID and select Gene description.



4. Results: Click on the Results button in the top left-hand corner to view your output table. Select All from the drop-down menu to open the full table in a new tab.


EnsemblFungi
BLAST | BioMart | FTP | Docs & FAQs
New
Count
Results
URL
XML
Perl
Help
Dataset 297 / 13470 Genes
Magnaporthe oryzae genes (MG8)
Filters
Chromosome/scaffold: 4
With Transmembrane helices: Only
Attributes
Gene stable ID
Gene description
Dataset
[None Selected]
Export all results to
File
TSV
Unique results only
Go
Email notification to
View
10
rows as
HTML
Unique results only
Gene stable ID
Gene description
MGG_17084
Putative uncharacterized protein [Source:UniProtKB/TrEMBL;Acc:G4N801]
MGG_03684
Mitochondrial distribution and morphology protein 38 [Source:UniProtKB/TrEMBL;Acc:G4N6R1]
MGG_09963
Putative uncharacterized protein [Source:UniProtKB/TrEMBL;Acc:G4N9P1]
MGG_03644
Putative uncharacterized protein [Source:UniProtKB/TrEMBL;Acc:G4N713]
MGG_06510
Cytochrome b5 [Source:UniProtKB/TrEMBL;Acc:G4N6W8]
MGG_09720
Putative uncharacterized protein [Source:UniProtKB/TrEMBL;Acc:G4NAH4]
MGG_03721
Urea transporter [Source:UniProtKB/TrEMBL;Acc:G4N6H1]
MGG_13659
Dicarboxylic amino acid permease [Source:UniProtKB/TrEMBL;Acc:G4NAK4]
MGG_08498
Putative uncharacterized protein [Source:UniProtKB/TrEMBL;Acc:G4NAP3]
MGG_13624
ABC transporter CDR4 [Source:UniProtKB/TrEMBL;Acc:G4N9L5]
Ensembl Genomes release 58 - January 2024 (c) EBI

Additional BioMart Exercise 3 – Convert IDs

For a list of *Schizosaccharomyces pombe* UniProt (UniProtKB/Swiss-Prot) IDs, export the Gene name and description, as well as the PomBase IDs.

(a) Do these 36 protein IDs correspond to 36 genes?

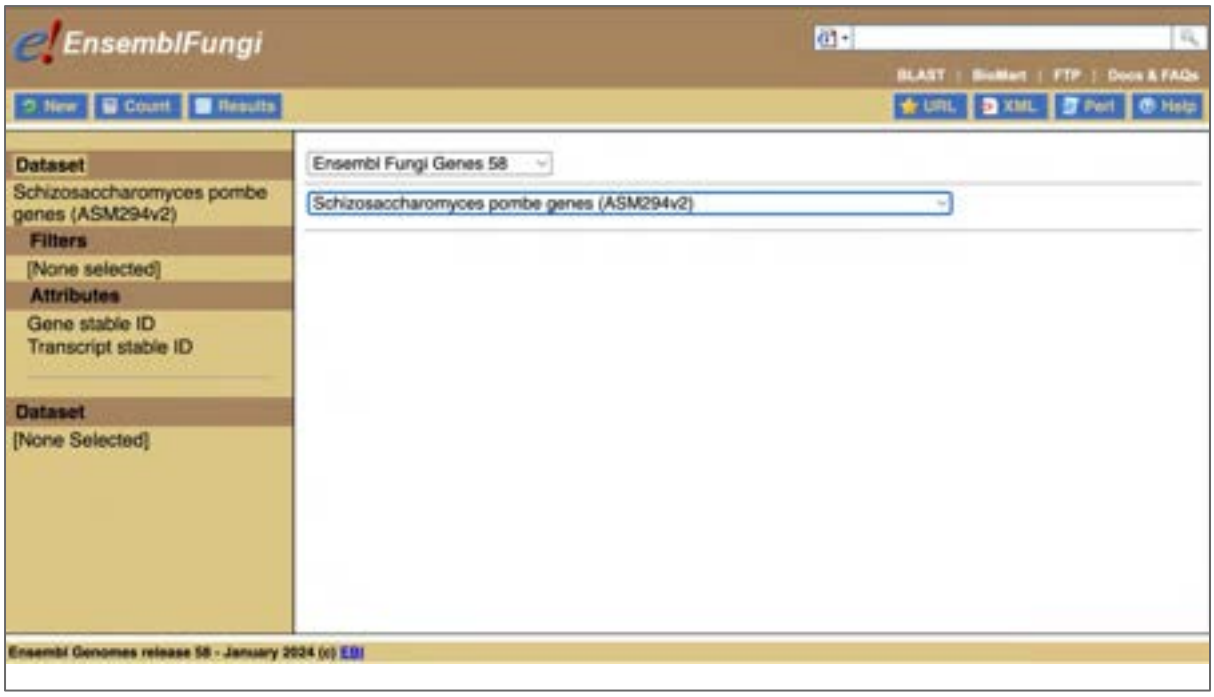
Input list of IDs:

Q92338	Q9US55	P78847	O74964
O13728	O14075	O94418	O14026
P49776	O94574	O94526	O74630
O74769	O94380	Q9UTG2	O14356
Q09170	P87172	O14326	O13339
Q9USK4	Q9USP5	Q9URZ3	P31411
O14040	Q9P7Y8	P42657	O13742
Q9Y804	Q9Y7Z8	P08647	O60159
O94552	Q10331	O74335	O9428

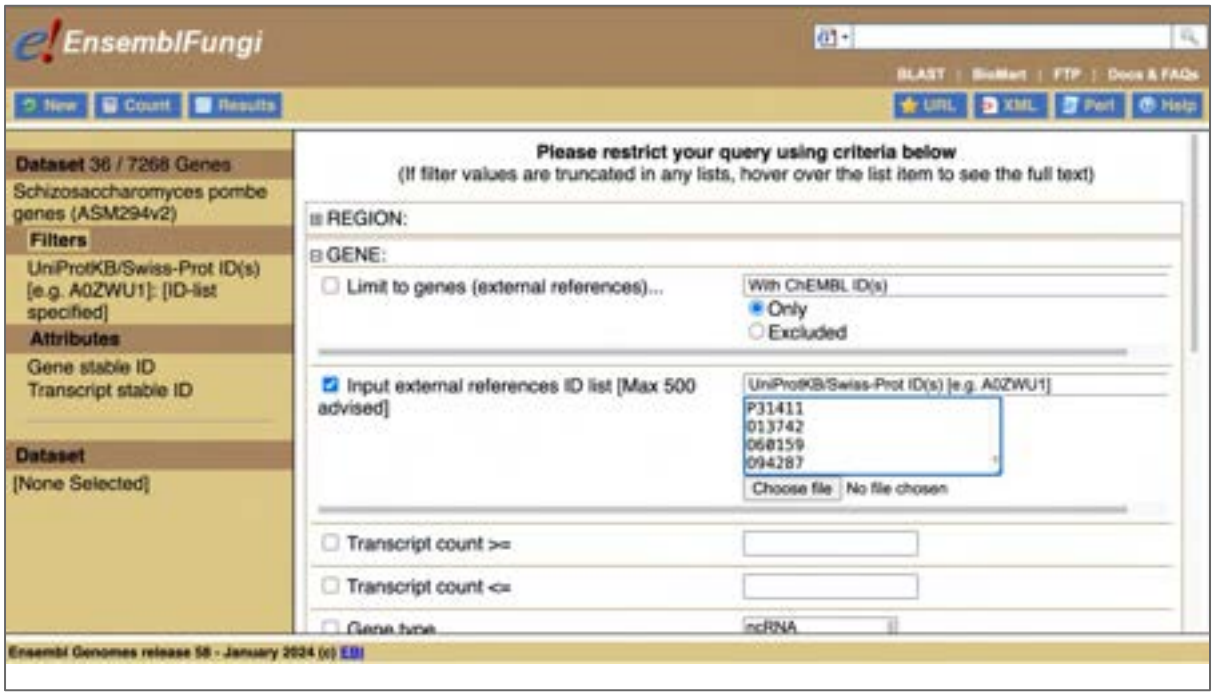
Exercise 3 answers

Click on the New button in the top left-hand corner to start a new BioMart query. Using the four-step process described above, we need to select the following in BioMart options in the left-hand panel:

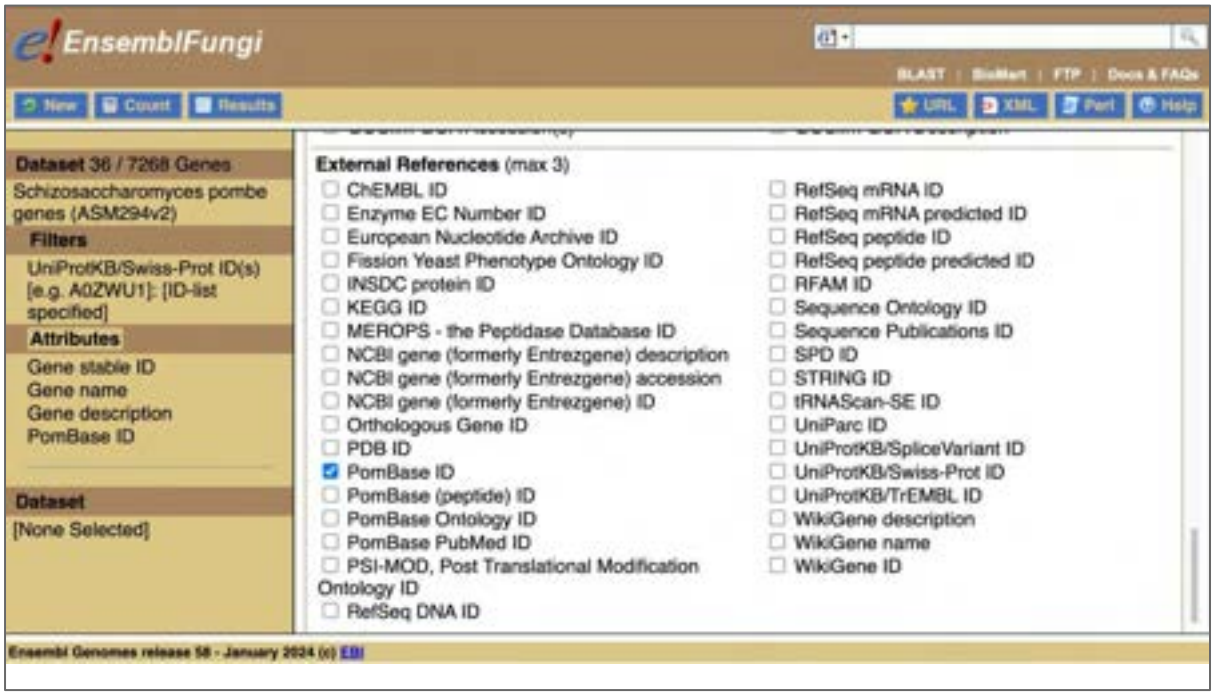
1. Dataset: Click on CHOOSE DATABASE and select Ensembl Fungi Genes 58 from the drop-down menu. Click on CHOOSE DATASET and select *Schizosaccharomyces pombe* genes (ASM294v2) from the drop-down menu.



2. Filters: Open the GENE tab and paste your list of IDs into the text box under Input external references ID list. Select UniProtKB/Swiss-Prot ID(s) [e.g. A0ZWU1] from the drop-down menu above to specify the type of IDs you are giving. Click on the Count button in the top left-hand corner. Your filter should apply to 36/7,268 genes.



3. Attributes: Select Features from the options on the top. Open the GENE tab, unselect Transcript stable ID and select Gene name and Gene description. Open the EXTERNAL tab, scroll down to External References and select PomBase ID.



4. Results: Click on the Results button in the top left-hand corner to view your output table. Select All from the drop-down menu to open the full table in a new tab.

Gene stable ID	Gene name	Gene description	PomBase ID
SPBC29A3.14c	trt1	telomerase reverse transcriptase 1 protein Trt1 [Source:PomBase;Acc:SPBC29A3.14c]	SPBC29A3.14c.1
SPAC15A10.08	ain1	alpha-actinin [Source:PomBase;Acc:SPAC15A10.08]	SPAC15A10.08.1
SPAC16E8.07c	vph1	V-type ATPase V0 subunit a (predicted) [Source:PomBase;Acc:SPAC16E8.07c]	SPAC16E8.07c.1
SPAC29B12.02c	set2	histone lysine methyltransferase Set2 [Source:PomBase;Acc:SPAC29B12.02c]	SPAC29B12.02c.1
SPAC2C4.07c	dis32	3'-5'-exoribonuclease activity Dis3L2 [Source:PomBase;Acc:SPAC2C4.07c]	SPAC2C4.07c.1
SPACUNK4.10		glyoxylate reductase (predicted) [Source:PomBase;Acc:SPACUNK4.10]	SPACUNK4.10.1
SPBC16E9.11c	pub3	HECT-type ubiquitin-protein ligase E3 Pub3 (predicted) [Source:PomBase;Acc:SPBC16E9.11c]	SPBC16E9.11c.1
SPBC30D10.10c	tor1	phosphatidylinositol kinase Tor1 [Source:PomBase;Acc:SPBC30D10.10c]	SPBC30D10.10c.1
SPBC19C7.11		ClC chloride channel (predicted) [Source:PomBase;Acc:SPBC19C7.11]	SPBC19C7.11.1
SPBC17F3.01c	rga5	Rho-type GTPase activating protein Rga5 [Source:PomBase;Acc:SPBC17F3.01c]	SPBC17F3.01c.1
SPCC23B6.03c	tel1	ATM checkpoint kinase [Source:PomBase;Acc:SPCC23B6.03c]	SPCC23B6.03c.1
SPBC24C6.08c	bhd1	folliculin/Birt-Hogg-Dube syndrome ortholog Bhd1 [Source:PomBase;Acc:SPBC24C6.08c]	SPBC24C6.08c.1
SPBC4B4.03	rsc1	RSC complex subunit Rsc1 [Source:PomBase;Acc:SPBC4B4.03]	SPBC4B4.03.1
SPBC887.02		ClC chloride channel (predicted) [Source:PomBase;Acc:SPBC887.02]	SPBC887.02.1
SPBC1604.15	gpi16	pig-T, Gpi16 (predicted) [Source:PomBase;Acc:SPBC1604.15]	SPBC1604.15.1
SPCC1620.11	nup97	nucleoporin Nic96 homolog [Source:PomBase;Acc:SPCC1620.11]	SPCC1620.11.1
SPBC609.02	ptn1	phosphatidylinositol-3,4,5-trisphosphate3-phosphatase Ptn1 [Source:PomBase;Acc:SPBC609.02]	SPBC609.02.1
SPCC18.18c	fum1	fumarate hydratase (predicted) [Source:PomBase;Acc:SPCC18.18c]	SPCC18.18c.1
SPBC1773.17c		glyoxylate reductase (predicted) [Source:PomBase;Acc:SPBC1773.17c]	SPBC1773.17c.1
SPAC17H9.09c	ras1	GTPase Ras1 [Source:PomBase;Acc:SPAC17H9.09c]	SPAC17H9.09c.1
SPAC637.05c	vma2	V-type ATPase V1 subunit B [Source:PomBase;Acc:SPAC637.05c]	SPAC637.05c.1
SPAC17A2.13c	rad25	14-3-3 protein Rad25 [Source:PomBase;Acc:SPAC17A2.13c]	SPAC17A2.13c.1
SPCC4G3.02	aph1	bis(5'-nucleosidyl)-tetraphosphatase [Source:PomBase;Acc:SPCC4G3.02]	SPCC4G3.02.1
SPCC290.03c	nup186	nucleoporin Nup186 [Source:PomBase;Acc:SPCC290.03c]	SPCC290.03c.1
SPBC3D6.07	gpi3	pig-A, phosphatidylinositol N-acetylglucosaminyltransferase subunit Gpi3 (predicted) [Source:PomBase;Acc:SPBC3D6.07]	SPBC3D6.07.1
SPCC18B5.11c	cds1	replication checkpoint kinase Cds1 [Source:PomBase;Acc:SPCC18B5.11c]	SPCC18B5.11c.1
SPBC428.01c	nup107	nucleoporin Nup107 [Source:PomBase;Acc:SPBC428.01c]	SPBC428.01c.1
SPBC2D10.18	abc1	ABC1 kinase family ubiquinone biosynthesis protein Abc1/Coq8 [Source:PomBase;Acc:SPBC2D10.18]	SPBC2D10.18.1
SPAPYUG7.03c	mid2	medial ring protein Mid2 [Source:PomBase;Acc:SPAPYUG7.03c]	SPAPYUG7.03c.1
SPAC869.10c	put4	proline specific plasma membrane permease Put4 (predicted) [Source:PomBase;Acc:SPAC869.10c]	SPAC869.10c.1
SPAC1002.03c	gls2	glucosidase II alpha subunit Gls2 [Source:PomBase;Acc:SPAC1002.03c]	SPAC1002.03c.1
SPCC4B3.14	cwf20	complexed with Cdc5 protein Cwf20 [Source:PomBase;Acc:SPCC4B3.14]	SPCC4B3.14.1
SPCC11E10.02c	gpi8	pig-K [Source:PomBase;Acc:SPCC11E10.02c]	SPCC11E10.02c.1
SPAC1805.15c	pub2	HECT-type ubiquitin-protein ligase E3 Pub2 [Source:PomBase;Acc:SPAC1805.15c]	SPAC1805.15c.1
SPBC146.13c	myo1	myosin type I [Source:PomBase;Acc:SPBC146.13c]	SPBC146.13c.1
SPBC146.06c	fan1	Fanconi-associated nuclease Fan1 [Source:PomBase;Acc:SPBC146.06c]	SPBC146.06c.1

(a) Yes, the 36 UniProt IDs correspond to 36 genes. However, not all of them have a gene name assigned to them (e.g. SPACUNK4.10).

Exercise: Exploring host-pathogen interactions in Ensembl Fungi

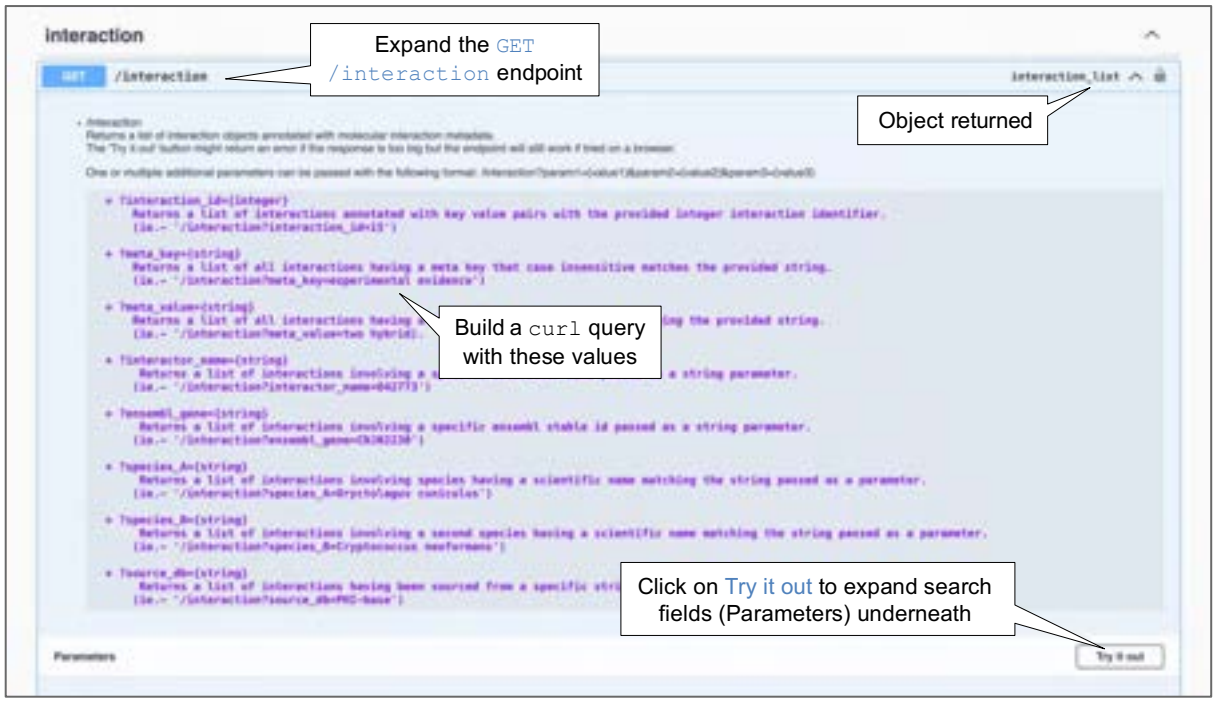
Links to be clicked shown in blue, text to be entered shown in red.

Zymoseptoria tritici (also known as *Septoria tritici* and *Mycosphaerella graminicola*) is a fungal pathogen that causes septoria leaf blotch disease in *Triticum aestivum* (wheat). This fungus is considered a major threat to wheat production worldwide, and its ability to rapidly adapt to fungicides and host plants makes it a significant challenge for disease management.

You can explore molecular interactions of genes in Ensembl Fungi, ranging from pathogen-host interactions to symbiotic relationships across microbes and other Ensembl species.

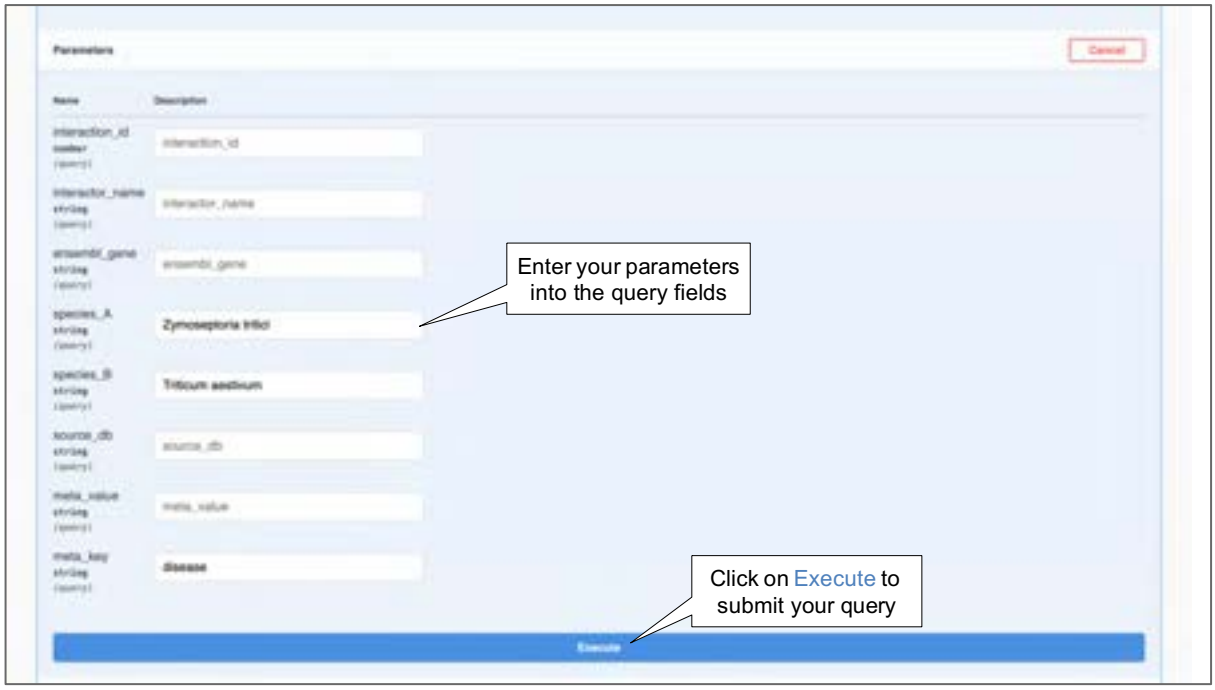
Step 1: Find all genes involved in molecular interactions for *Zymoseptoria tritici*. From the Ensembl Interactions REST API page https://interactions.rest.ensembl.org, search for all *Zymoseptoria tritici* genes that have a pathogenic interaction with *Triticum aestivum* (wheat).

Enter https://interactions.rest.ensembl.org/interactions_by_prodname/ into your browser and expand the `GET /interaction` documentation by clicking on interaction_list. This opens a description and all available parameters for the endpoint. Click on Try it out to start your REST API request.

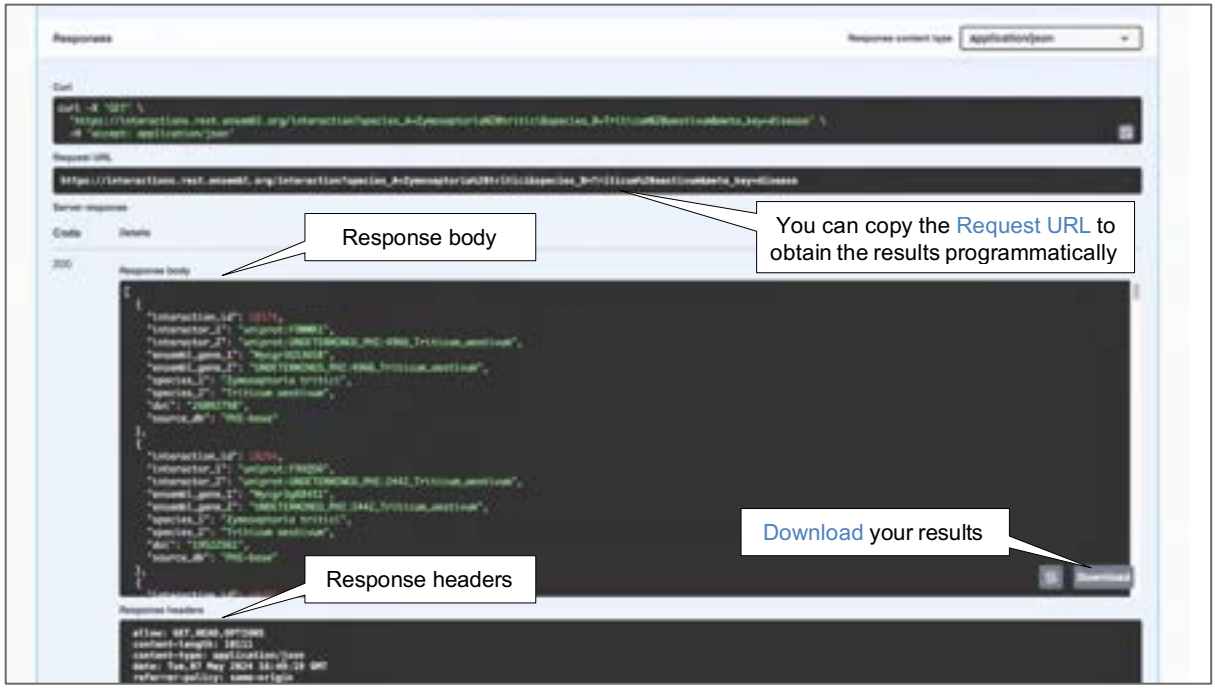


Scroll down to the 'Parameters' section and fill in the query fields as follows:
species_A: *Zymoseptoria tritici*
species_B: *Triticum aestivum*
meta_key: disease

Click on Execute to submit your request.



Scroll down to ‘Responses’ to view your output.

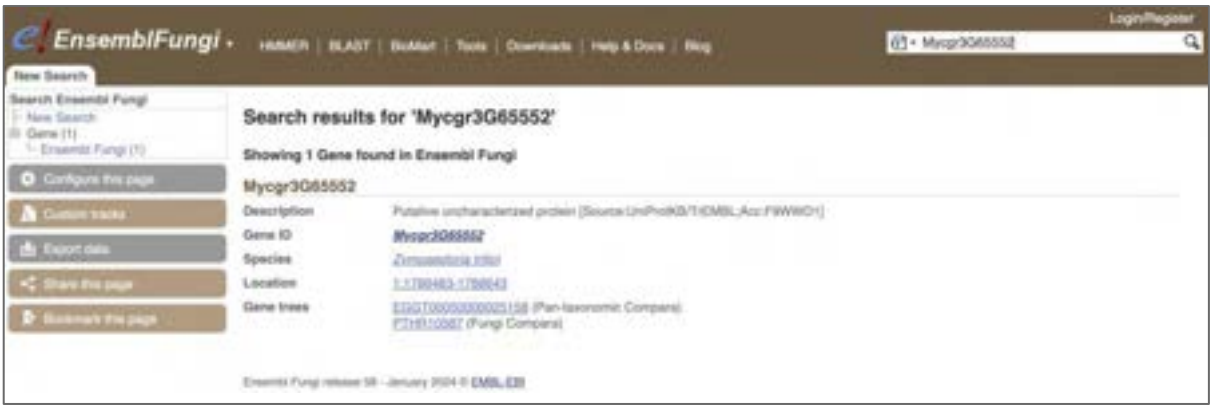


Here, you can obtain the Curl script and request URL to access the same results programmatically.

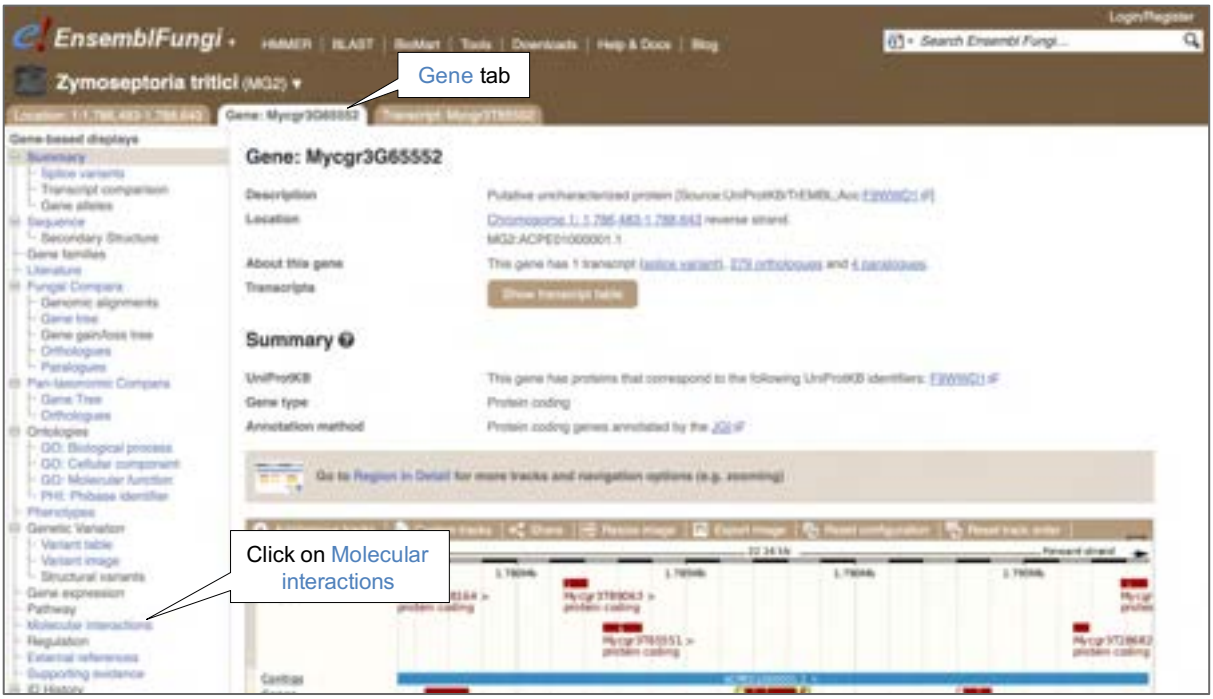
Under ‘Server response’, you should get the following output: `zymoseptoria_tritici": ["Mycgr3G53658", "Mycgr3g88451", "Mycgr3G85040", "Mycgr3G40048", "Mycgr3G111221", "Mycgr3G103264", "Mycgr3G89160", "Mycgr3G80707",`

```
"Mycgr3G65552", "Mycgr3g105487", "Mycgr3G70181", "Mycgr3G46840",
"Mycgr3G93828", "Mycgr3G31676", "Mycgr3G51018", "Mycgr3G36951",
"Mycgr3G77528", "Mycgr3G39611", "Mycgr3G96592", "Mycgr3G86705",
"Mycgr3G107320", "Mycgr3G74194", "Mycgr3G87000", "Mycgr3G100355",
"Mycgr3G92404", "Mycgr3G69942"]
```

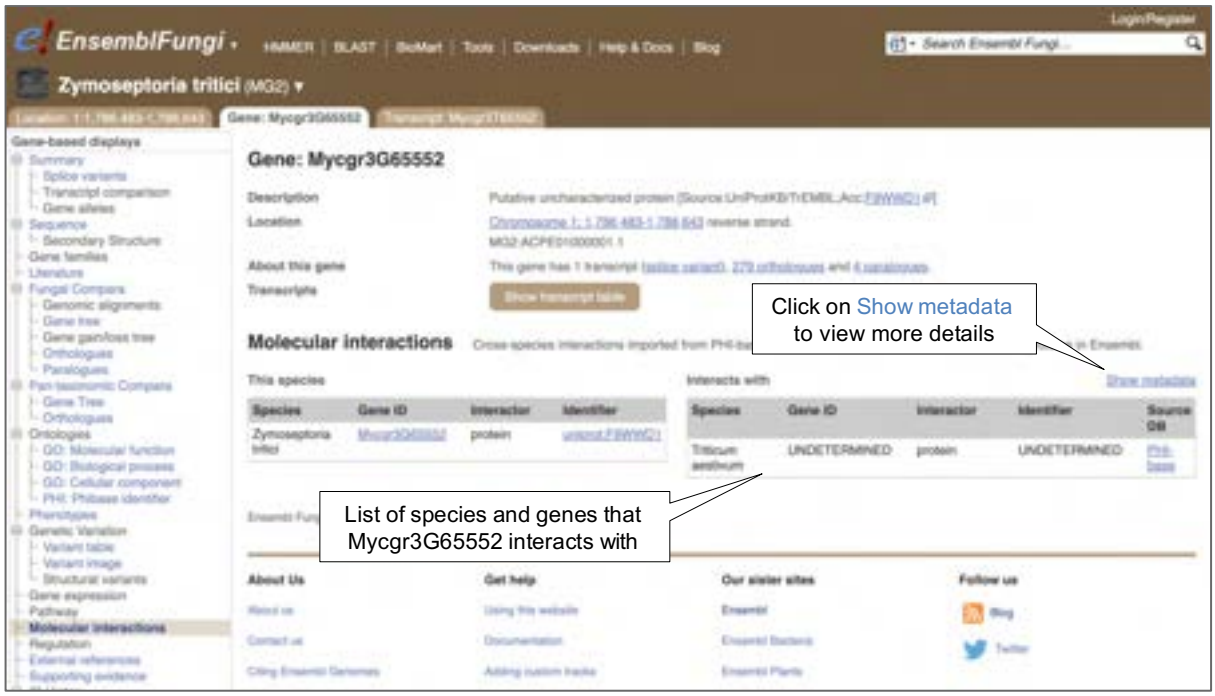
Step 2: Let's find out more about the gene Mycgr3G65552 in the Ensembl Fungi browser. On the the Ensembl Fungi homepage, enter the gene ID Mycgr3G65552 in the top right-hand corner and hit Search. Click on the gene ID Mycgr3G65552 to open the 'Gene' tab.



To find a list of species with which this particular *Z. tritici* gene has molecular interactions with, click on Molecular interactions in the left-hand panel.



From this page, we can see that *Z. tritici* is known to interact with *T. aestivum*.

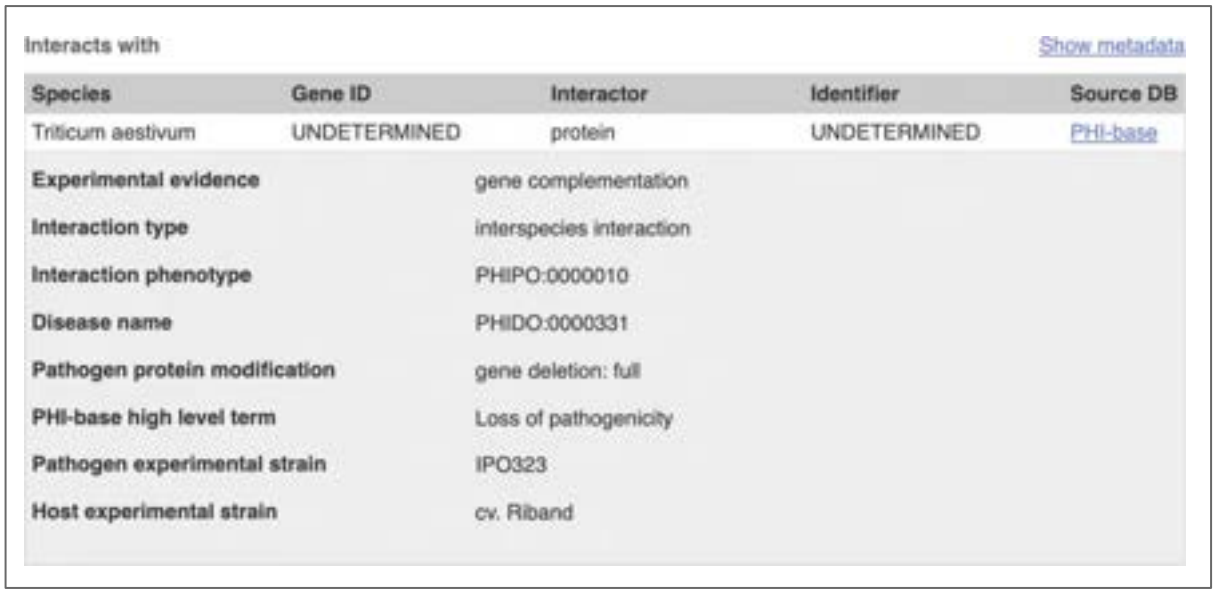


Can you find the wheat gene ID that Mycgr3G65552 interacts with? Look at the Interacts with table. The gene ID is 'UNDETERMINED'. This means a molecular interaction has been experimentally verified between Mycgr3G65552 and wheat, but the former gene has not been identified yet.

Interacts with — Show metadata

Species	Gene ID	Interactor	Identifier	Source DB
Triticum aestivum	UNDETERMINED	protein	UNDETERMINED	PHI-base

Can you find out what the phenotype for this interaction is? Click on Show metadata at the top right-hand corner of the 'Interacts with' table. Based on PHI-base, the interaction is associated with 'loss of pathogenicity'.

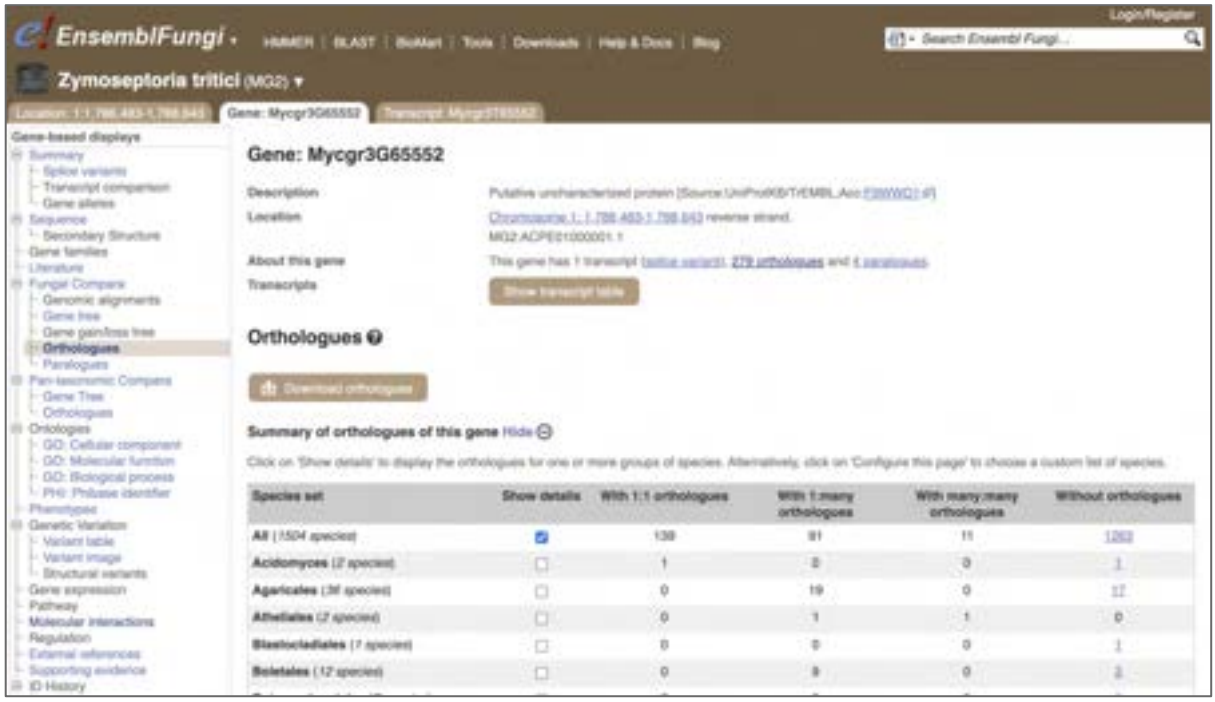


Interacts with — Show metadata

Species	Gene ID	Interactor	Identifier	Source DB
Triticum aestivum	UNDETERMINED	protein	UNDETERMINED	PHI-base

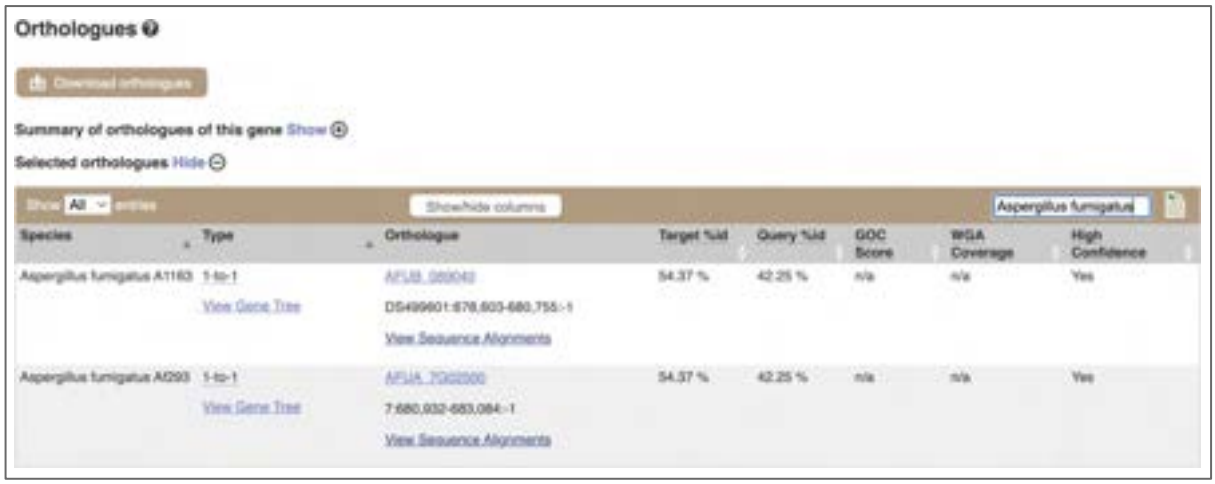
Experimental evidence	gene complementation
Interaction type	interspecies interaction
Interaction phenotype	PHIPO:0000010
Disease name	PHIDO:0000331
Pathogen protein modification	gene deletion: full
PHI-base high level term	Loss of pathogenicity
Pathogen experimental strain	IPO323
Host experimental strain	cv. Riband

Step 3: Next, let's find all fungal orthologues. There are several ways of doing this. One way is to go to Fungal Compara: Orthologues in the left-hand panel.

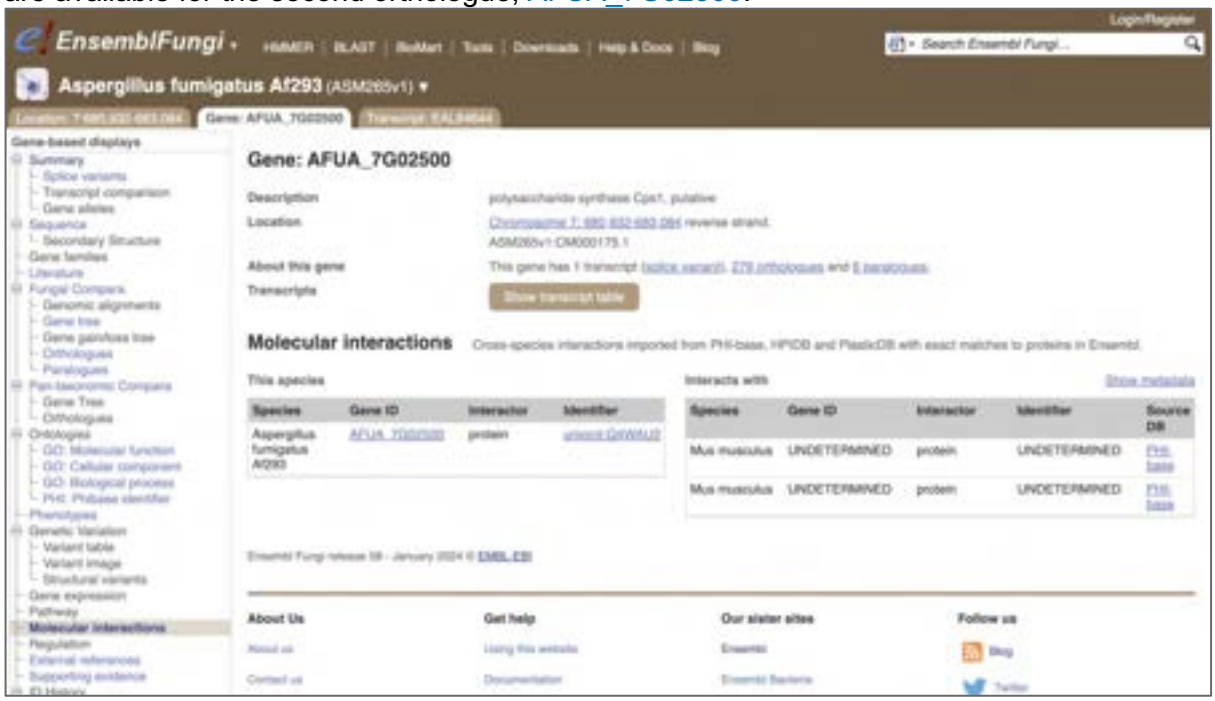


Can you find out if there are any orthologues in *Aspergillus fumigatus* with molecular interaction entries?

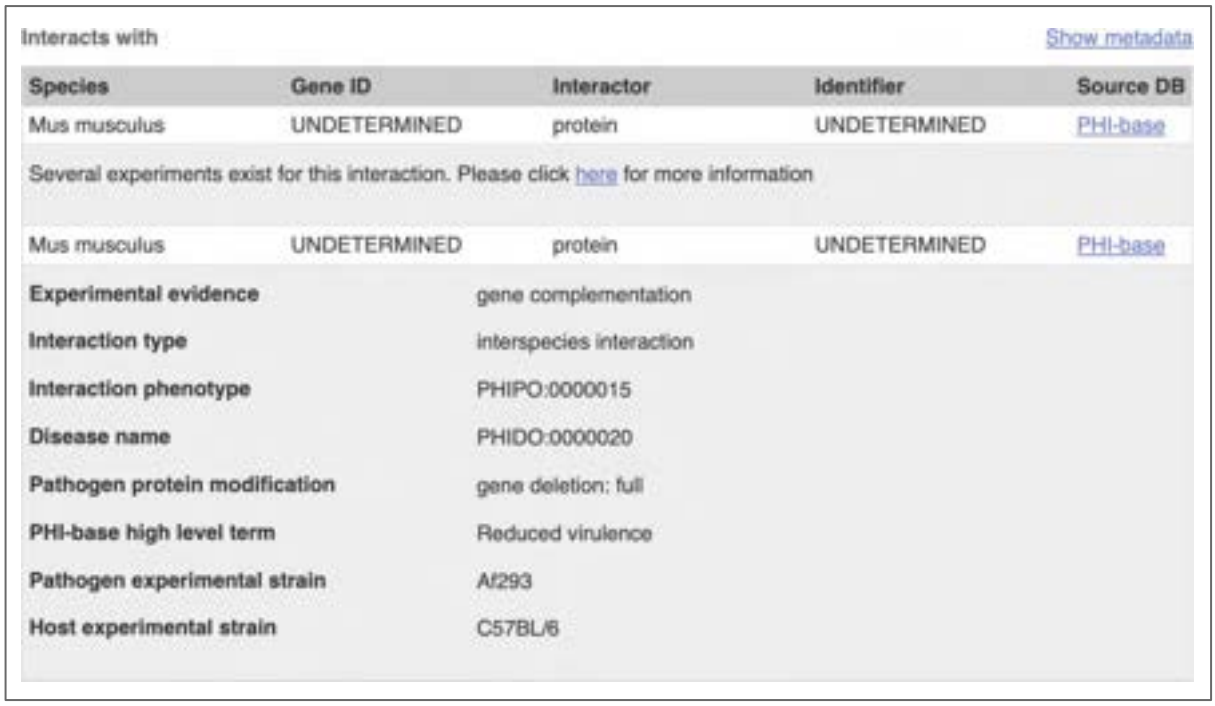
Step 4: You can hide the 'Summary of orthologues of this gene' table by clicking the Hide button. Enter *Aspergillus fumigatus* in the filter box on the top right-hand corner of the Orthologues table.



There are two orthologues in *A. fumigatus*. Click each of the gene IDs to find out which one has an entry under the Molecular interactions 'Gene-based' display. Molecular interactions are available for the second orthologue, AFUA_7G02500.



What is the phenotype of the interaction for this orthologue with mice?



Interacts with — Show metadata

Species	Gene ID	Interactor	Identifier	Source DB
Mus musculus	UNDETERMINED	protein	UNDETERMINED	PHI-base
Several experiments exist for this interaction. Please click here for more information				
Mus musculus	UNDETERMINED	protein	UNDETERMINED	PHI-base

Experimental evidence	gene complementation
Interaction type	interspecies interaction
Interaction phenotype	PHIPO:0000015
Disease name	PHIDO:0000020
Pathogen protein modification	gene deletion: full
PHI-base high level term	Reduced virulence
Pathogen experimental strain	Af293
Host experimental strain	C57BL/6

The phenotype for the orthologue in mice is ‘reduced virulence’.

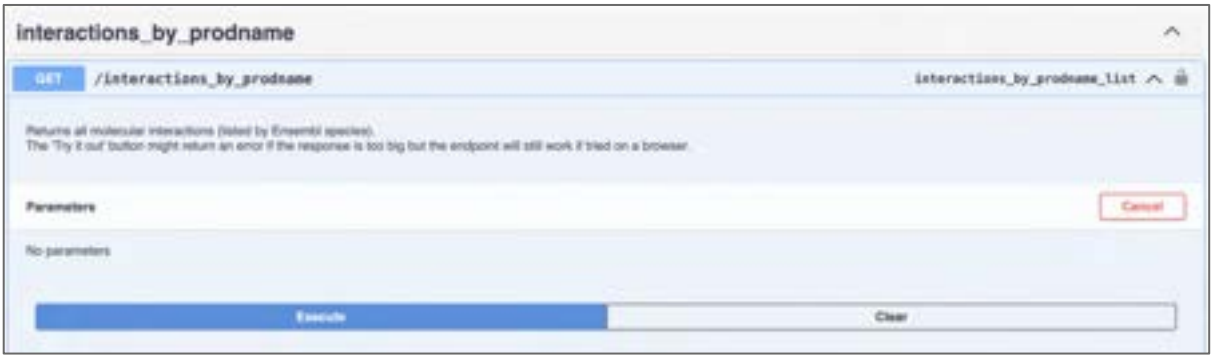
Additional host-pathogen exercise 1 – Exploring GO terms and phenotypes

Botrytis cinerea is a necrotrophic fungus that infects a wide range of crops and ornamental plants, causing significant economic losses in agriculture and horticulture industries. It is known to cause botrytis bunch rot in various species. Use Ensembl Fungi to find out more information about molecular interactions in the species and answer the following questions:

(a) Using the Ensembl Interactions REST API, can you retrieve all genes with molecular interaction information for *B. cinerea*?
(b) Open the 'Molecular interactions' page for the Bcin07g00720 gene in *B. cinerea*. What plant species does the gene interact with?
(c) Can you find the phenotype that is reported for each of the species the gene interacts with?
(d) Find all fungal orthologues. Is there any orthologue in *Magnaporthe oryzae* for Bcin07g00720? For which orthologue is molecular interaction information available?
(e) Which species does the *M. oryzae* orthologue interact with?
(f) Compare the molecular interaction phenotypes between the *B. cinerea* and *M. oryzae* orthologues. Can you find any common molecular functions that may explain this phenotype?

Exercise 1 answers:

(a) Go to the Ensembl Interactions REST API and expand the `GET /interactions_by_prodname` endpoint documentation. Click on Try it out and then Execute.

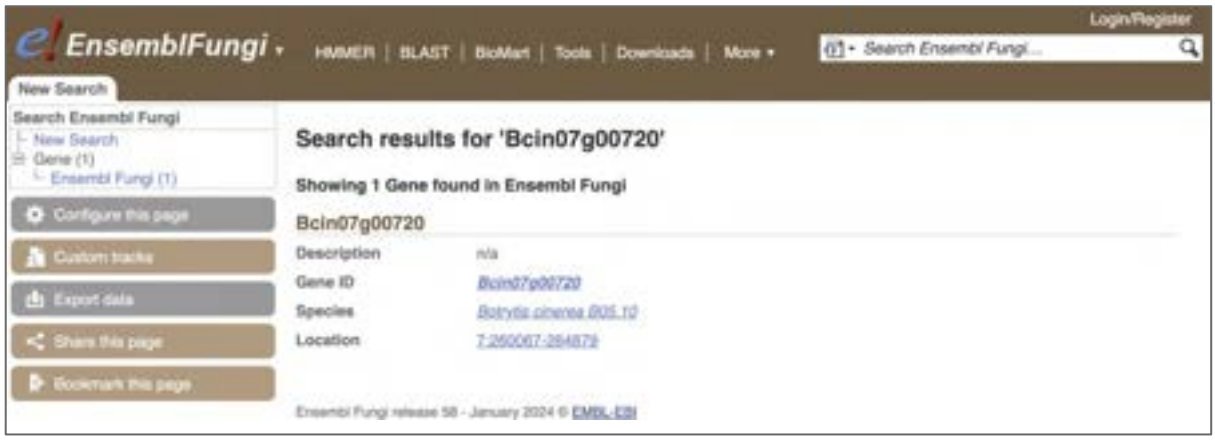


In the 'Response body', search for botrytis_cynerea. Alternatively, you can open the request URL https://interactions.rest.ensembl.org/interactions_by_prodname in your browser. You should get the following output:

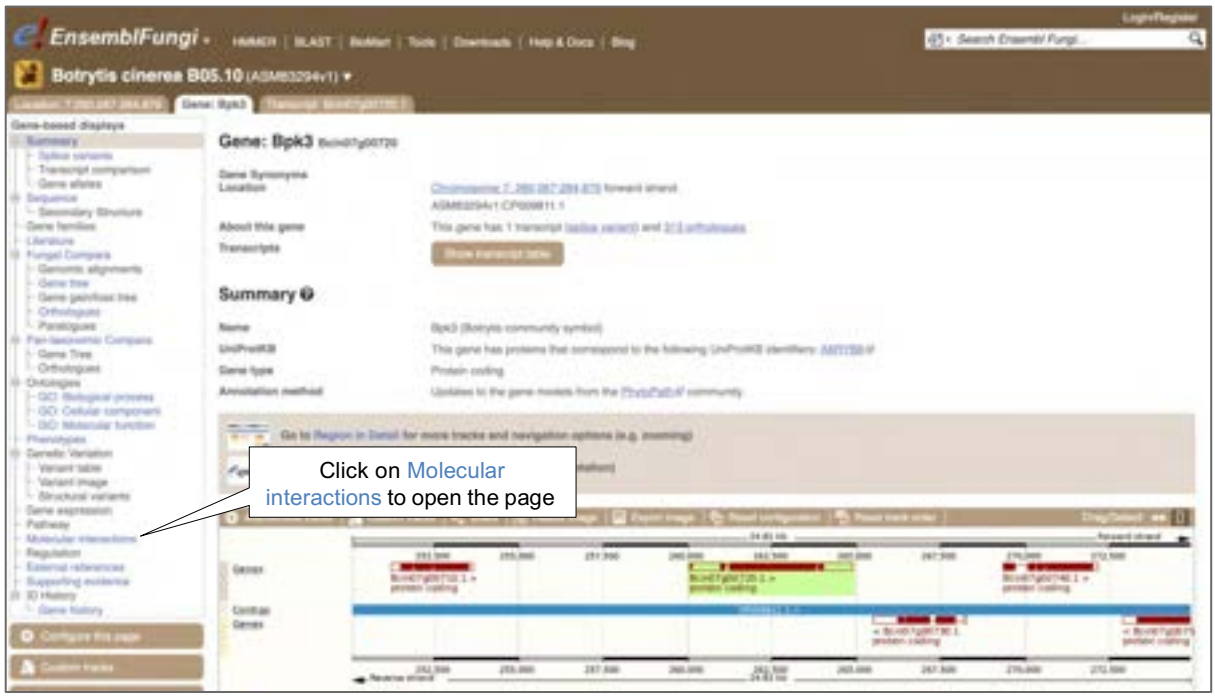
```
"botrytis_cinerea": ["Bcin07g00720", "Bcin02g02570",
"Bcin12g04900", "Bcin16g00630", "Bcin02g06770", "Bcin03g07190",
"Bcin09g02390", "Bcin09g01800", "Bcin07g03050", "Bcin08g05150",
"Bcin10g01250", "Bcin14g01870", "Bcin06g04870", "Bcin06g00240",
"Bcin06g03440", "Bcin03g07900", "Bcin03g06840",
```

```
"Bcin10g02530",    "Bcin08g02990",    "Bcin07g02610",
"Bcin03g08710",    "Bcin10g05590",    "Bcin16g01820",
"Bcin03g01540",    "Bcin14g00650",    "Bcin09g05460",
"Bcin10g02650",    "Bcin02g02780",    "Bcin05g03080",
"Bcin08g00160",    "Bcin01g06010",    "Bcin01g11360",
"Bcin15g00450",    "Bcin03g04600",    "Bcin09g01910",
"Bcin09g05050", "Bcin15g03580", "Bcin05g02590"]
```

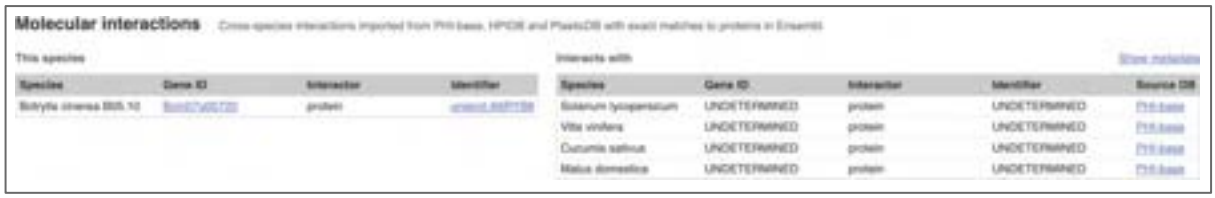
(b) Go to the Ensembl Fungi homepage and search for Bcin07g00720. In the results, click on the Gene ID to open the Gene tab.



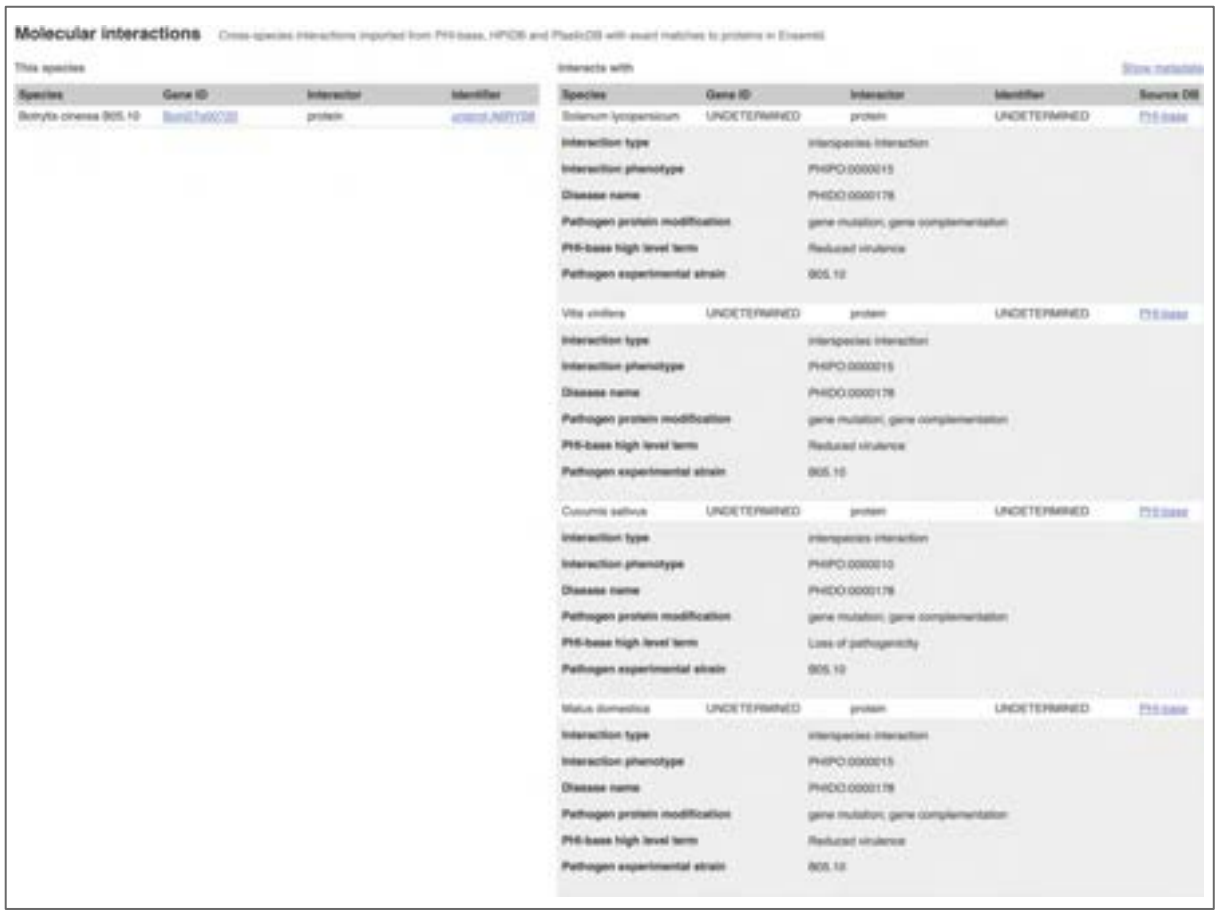
In the left-hand panel, click on Molecular interactions to open the page.



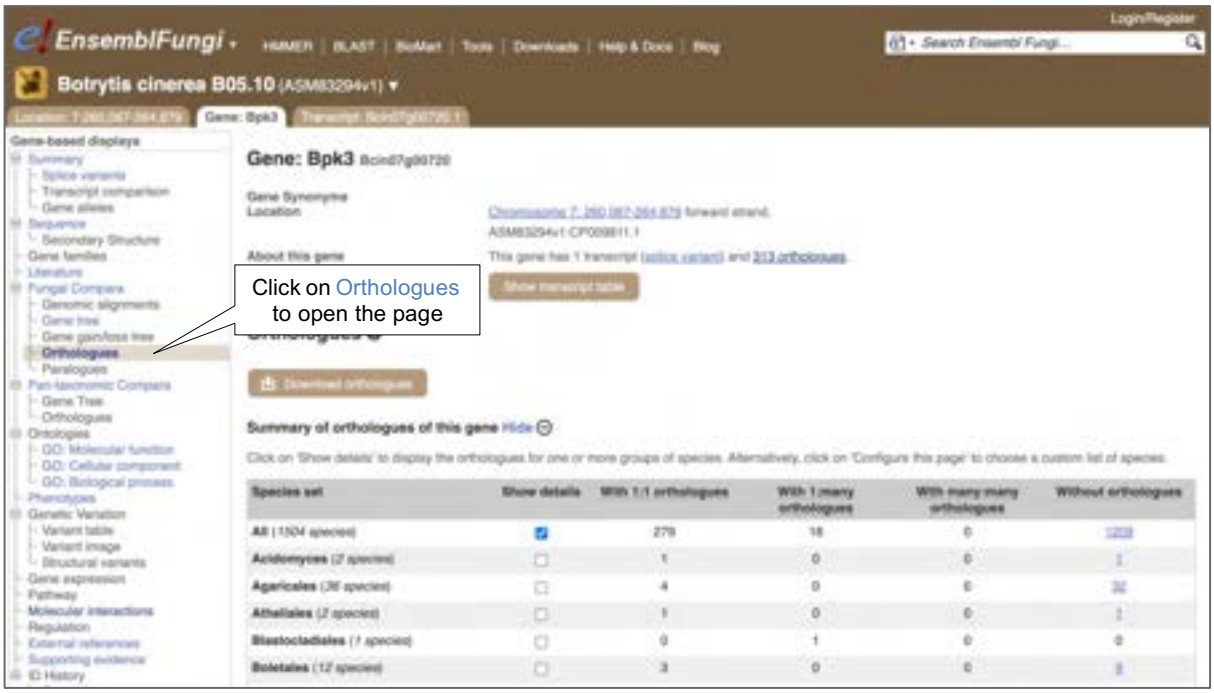
In the 'Molecular interactions' page, you can find all species the gene interacts with in the right-hand table. These include *Solanum lycopersicum* (tomato), *Vitis vinifera* (grape), *Cucumis sativus* (cucumber) and *Malus domestica* (apple).



(c) Click on Show metadata in the right-hand corner of the Interacts with table. You can find associated phenotypes under PHI-base high level term. The gene is associated with ‘Reduced virulence’ and ‘Loss of pathogenicity’.

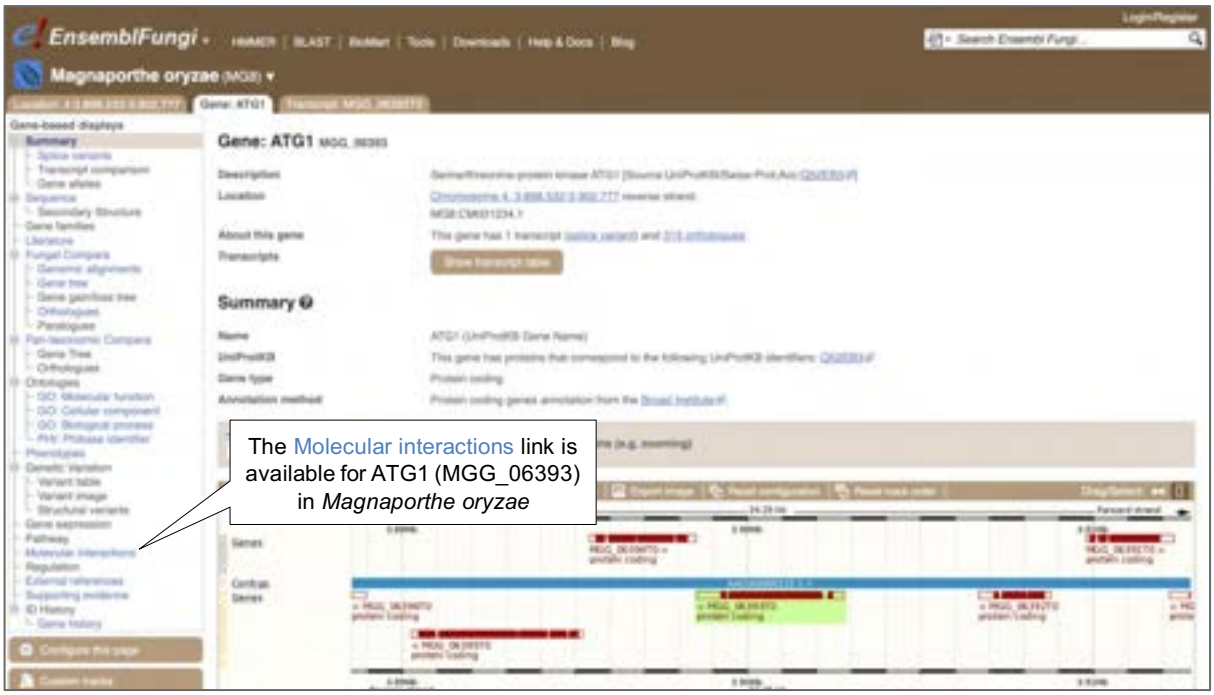


(d) To retrieve all fungal orthologues, go to Fungal Compara: Orthologues in the left- hand panel.



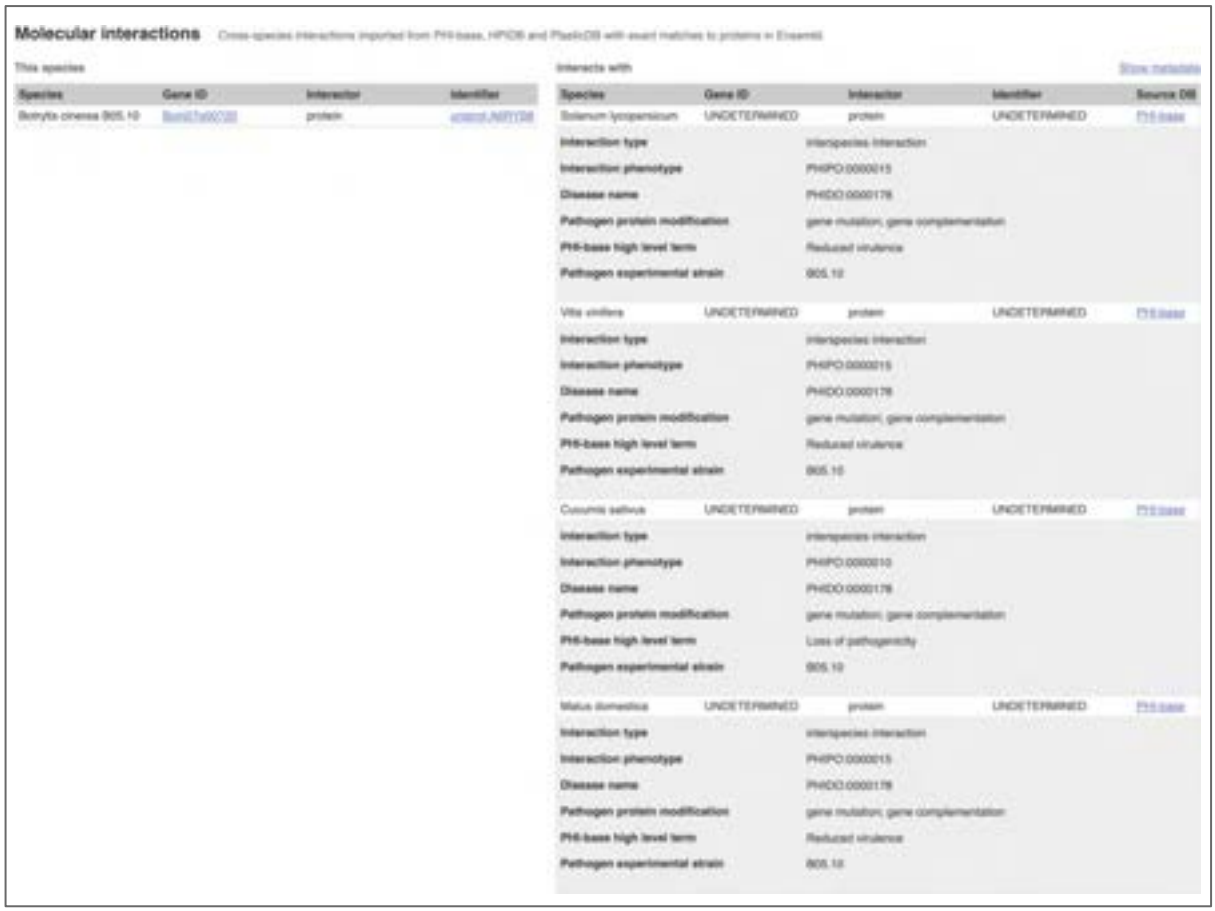
Scroll down to the Orthologues table and use the filter box in the top right-hand corner to search for *Magnaporthe oryzae*.

Click on each of the orthologue gene IDs to open their respective gene tab and find out if the Molecular interactions Gene-based display is available. Molecular interaction information is available for the orthologue ATG1 (MGG_06393).

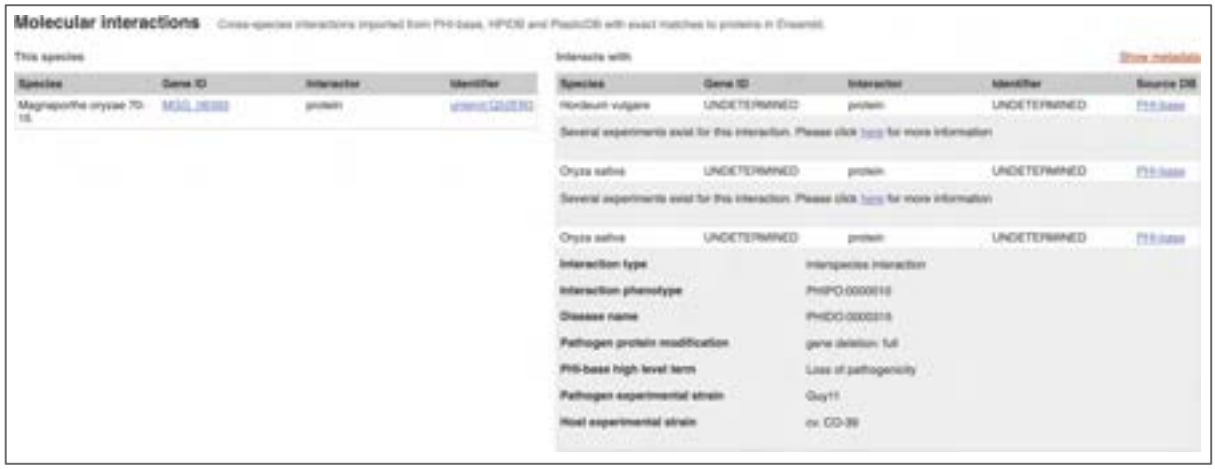


(e) Click on Molecular interactions in the left-hand panel. The ATG1 protein interacts with *Hordeum vulgare* (barley) and *Oryza sativa* (rice).

(f) Click on Show metadata to view the phenotypes associated with the molecular interactions. In *B. cinerea*, the phenotype is 'Loss of pathogenicity' and 'Reduced virulence'.



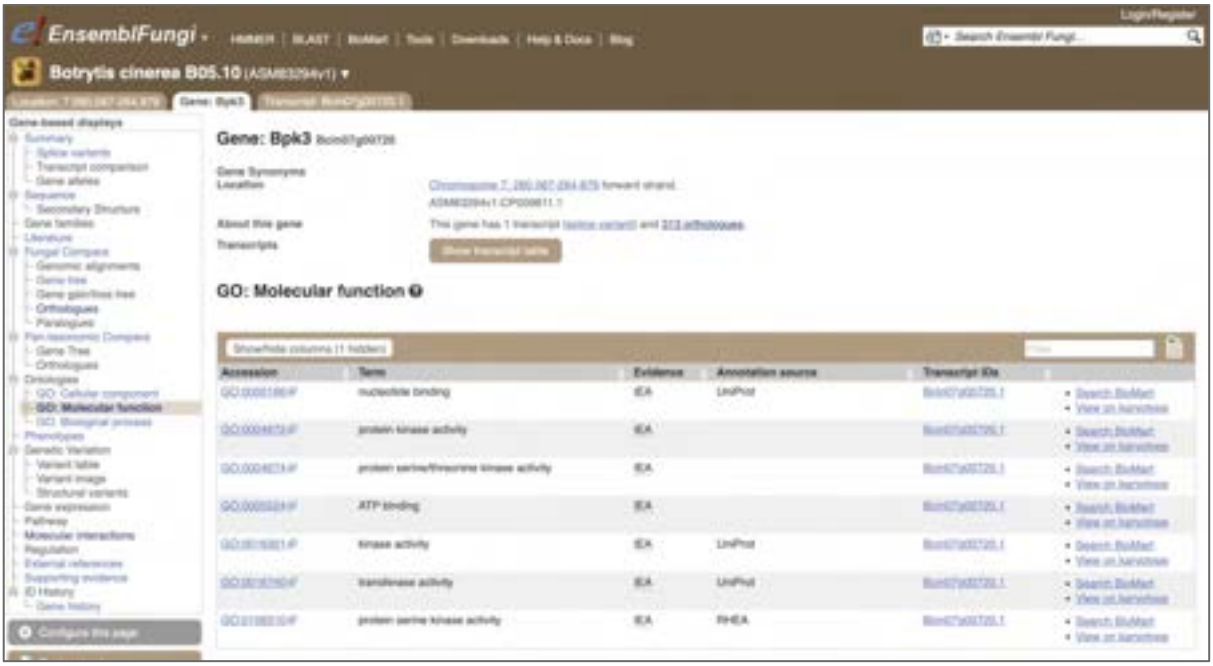
In *M. oryzae* the phenotype is ‘Loss of pathogenicity’ only.

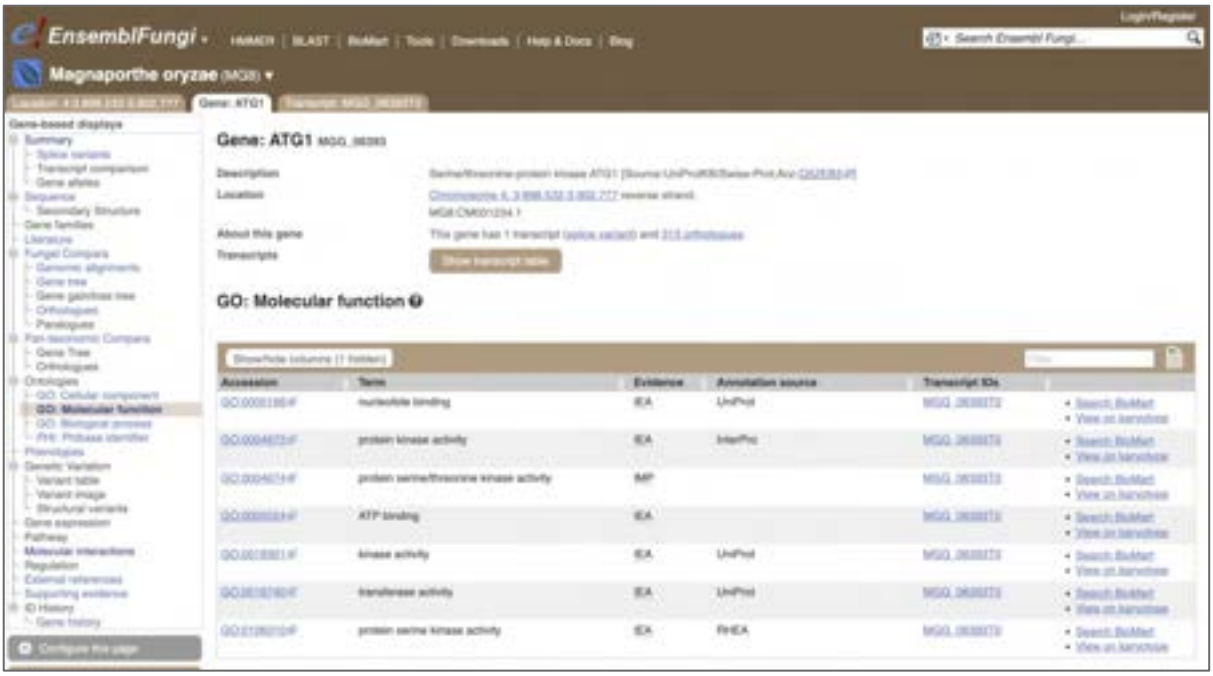


(g) Go to Ontologies: GO: Molecular function for both *B. cinerea* and *M. oryzae*. Comparing the GO terms for the two orthologues we can see that they have identical GO annotations:

- nucleotide binding
- protein kinase activity
- protein serine/threonine kinase activity

- ATP binding
- kinase activity
- transferase activity
- protein serine kinase activity





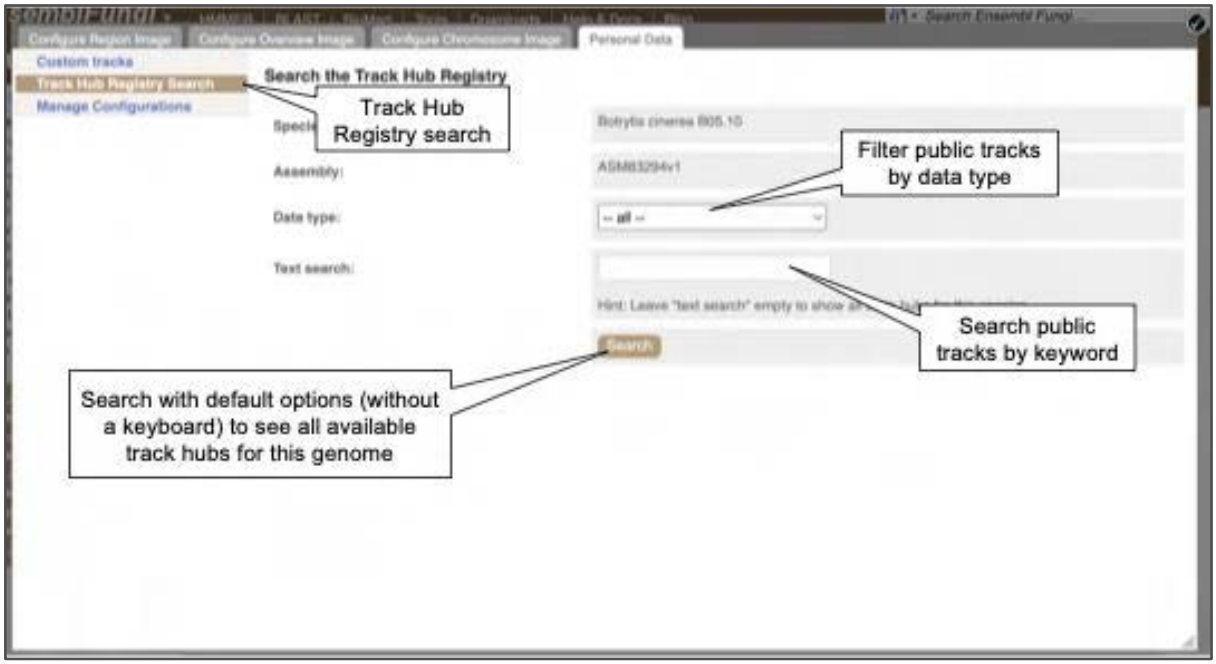
Exercise: Attaching Track Hubs to Ensembl Fungi

Links to be clicked shown in blue, text to be entered shown in red.

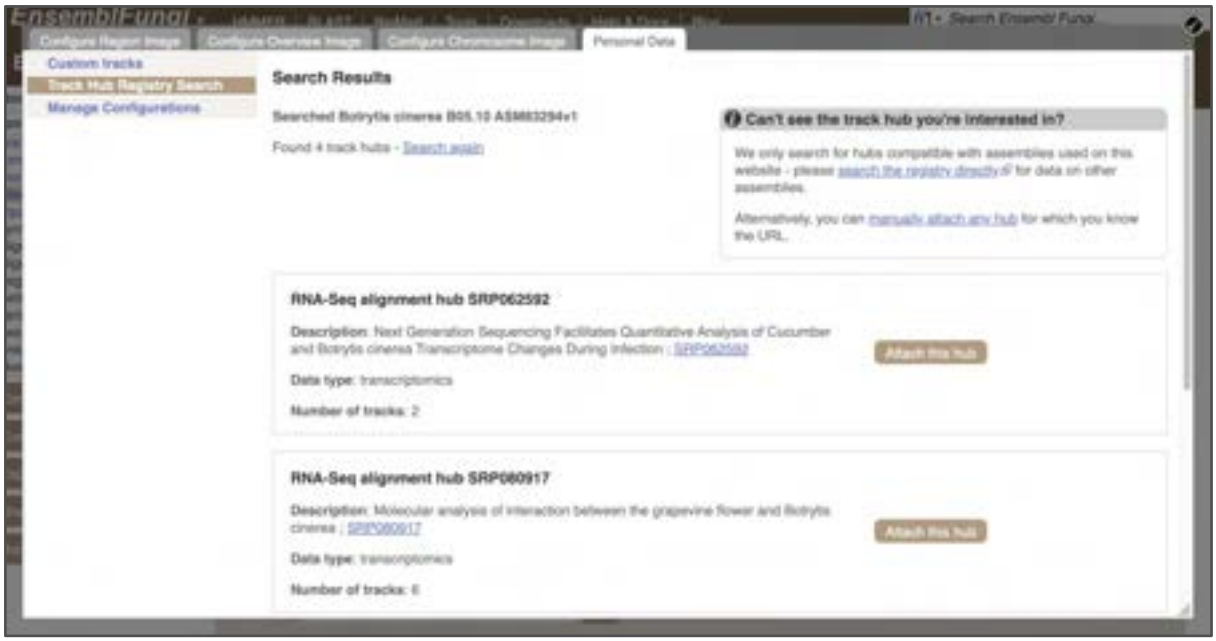
There are a number of publicly available datasets that are available to add to views in Ensembl. You can find full lists of these at https://trackhubregistry.org/. We're going to search and add these files from within Ensembl.

Go to fungi.ensembl.org on your browser and search for the region 6:1854110-1894000 in the species *Botrytis cinerea* B05.10.

This will take you directly to the Region in Detail page in the location tab. Click on the Custom tracks button  found just below the 'Configure this page' button on the left. In the pop-up menu, click on Track Hub Registry Search on the left-hand navigation panel.



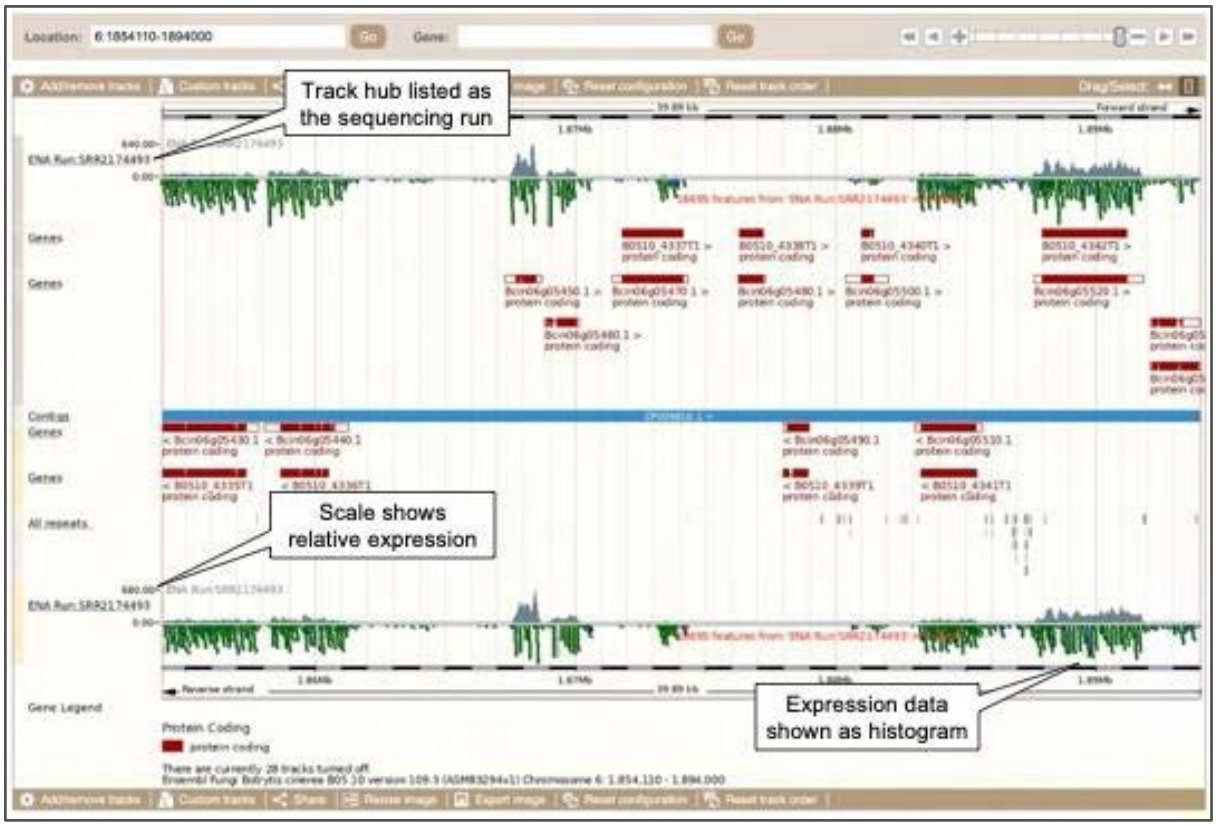
Just click Search with no options selected.



There are four available track hubs for this assembly.

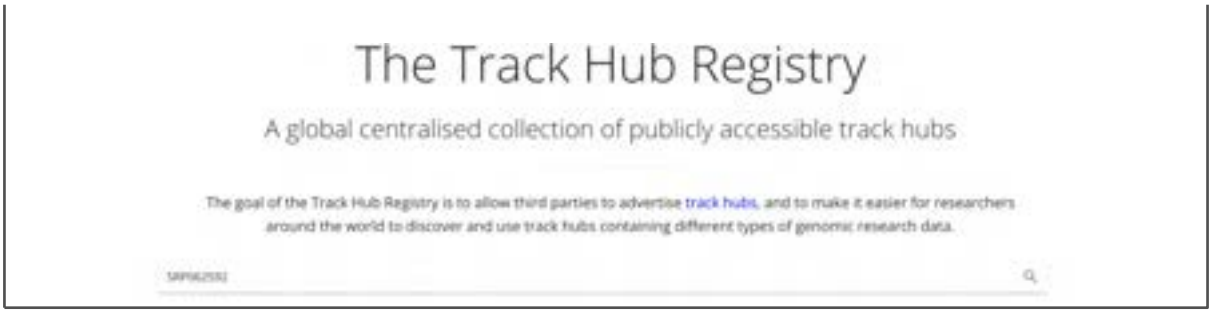
Choose the ‘RNA-Seq alignment hub SRP062592’ by clicking on the Attach this hub button on the right. It is a next-generation sequencing (NGS) quantitative analysis of cucumber and *B. cinerea* transcriptome changes during infection. Close the pop-up window.

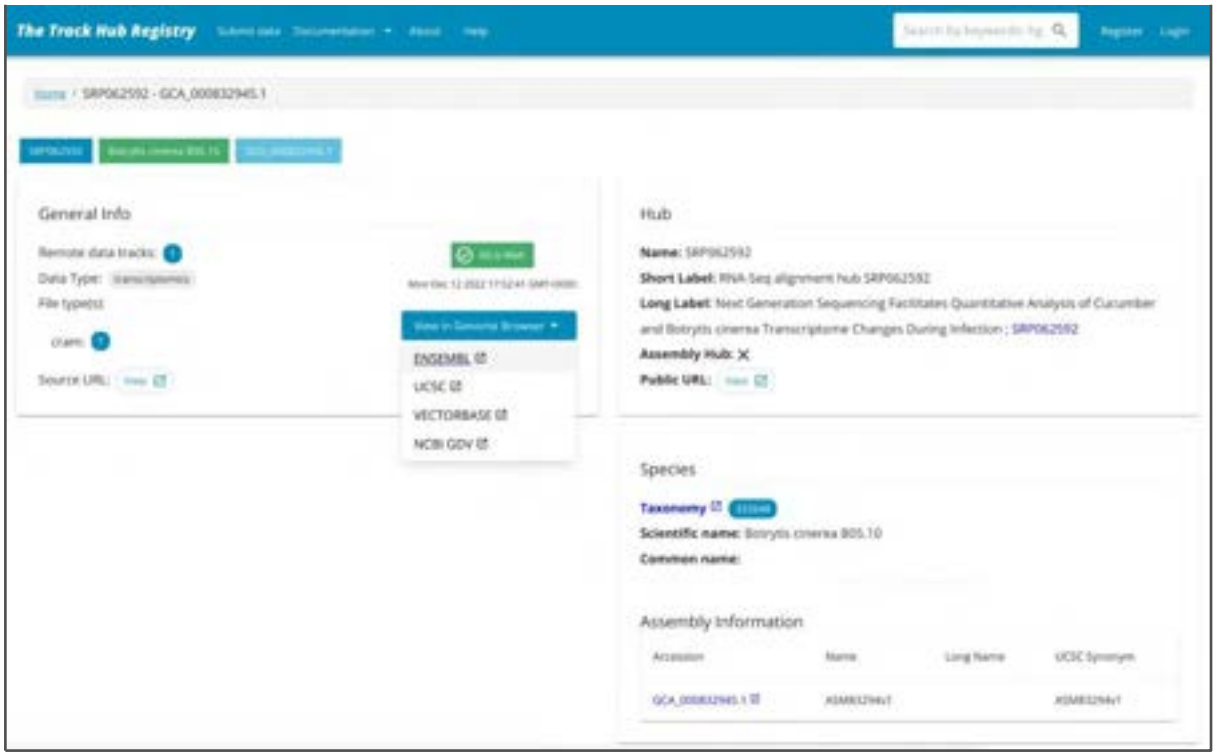
The track hub should now load and appear on the most-detailed image at the bottom of the ‘Region in detail’ page.



If you zoom in further, you can see a more detailed representation of the data:

(a) Go to www.trackhubregistry.org on your browser and search for SRP062592. Can you jump to Ensembl Fungi directly from the Track Hub Registry page?



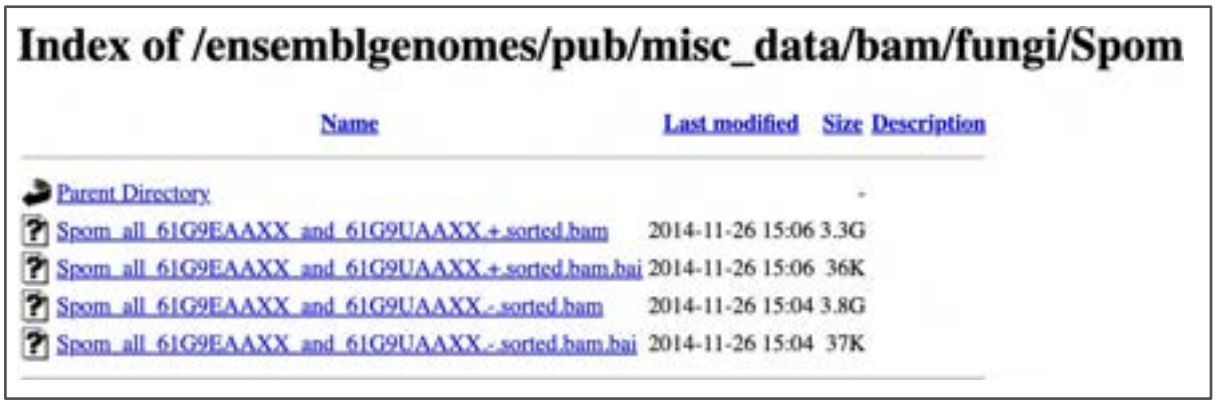


If you have your own files, or know a file you want to attach that is not present on the TrackHub registry, you can also attach these. There are two ways to do this, either by URL or by file upload.

Larger files, such as BAM files generated by NGS, need to be attached as remote files by URL. There are some BAM files for Schizosaccharomyces pombe available at: ftp://ftp.ensemblgenomes.org/pub/misc_data/bam/fungi/Spom/

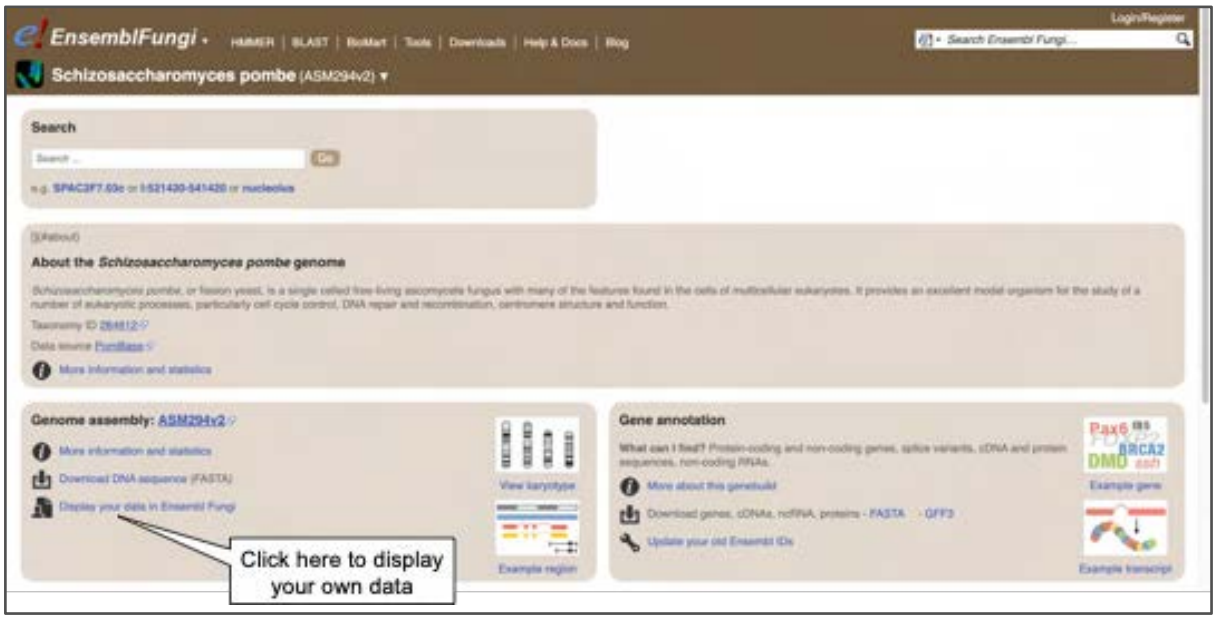
Let's take a look at that URL.

NOTE: Many internet browsers have recently dropped support for FTP, including the latest Firefox and Google Chrome versions. Firefox v87.0 still contains built-in FTP implementation. If you struggle to open the FTP site, try the HTTP version: https://ftp.ebi.ac.uk/ensemblgenomes/pub/misc_data/bam/fungi/Spom/

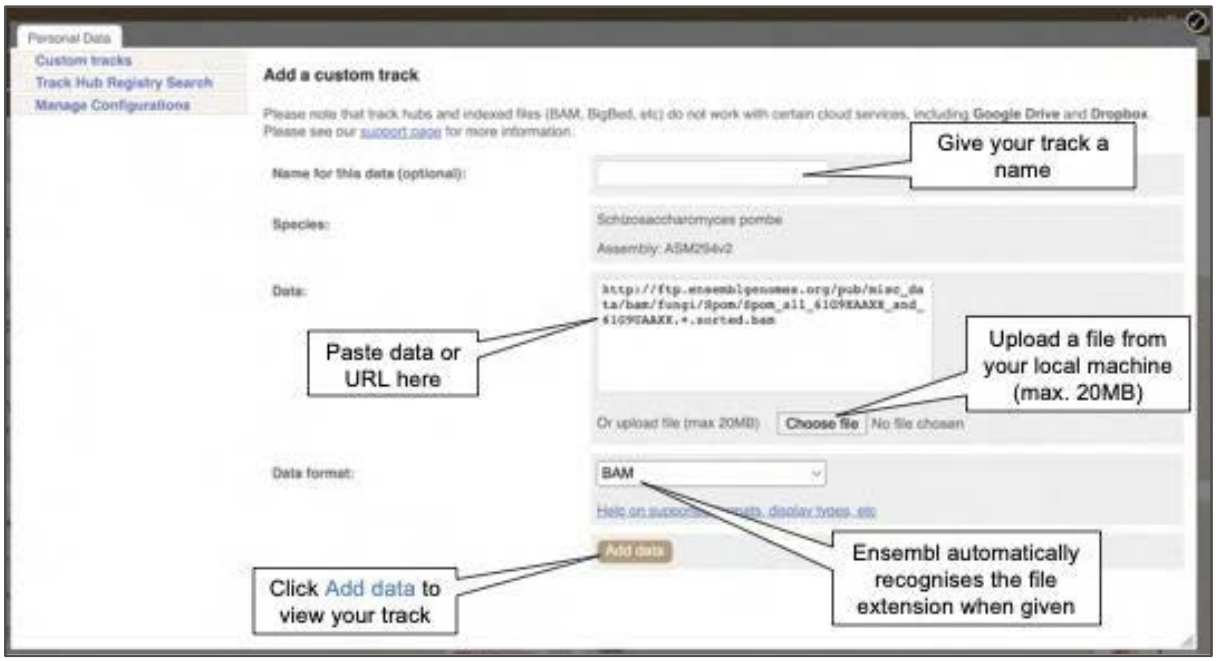


Here you can see two BAM files (file names ending in '.bam') with corresponding index files (file names ending in '.bam.bai'). We're interested in the files Spom_all_61G9EAAXX_and_61G9UAAXX.+.sorted.bam and Spom_all_61G9EAAXX_and_61G9UAAXX.+.sorted.bam.bai. These files are the BAM file and the index file respectively. When attaching a BAM file to Ensembl, there must be an index file in the same folder.

From the Ensembl Fungi homepage, click on *Schizosaccharomyces pombe* (ASM294v2), then on Display your data in Ensembl Fungi.



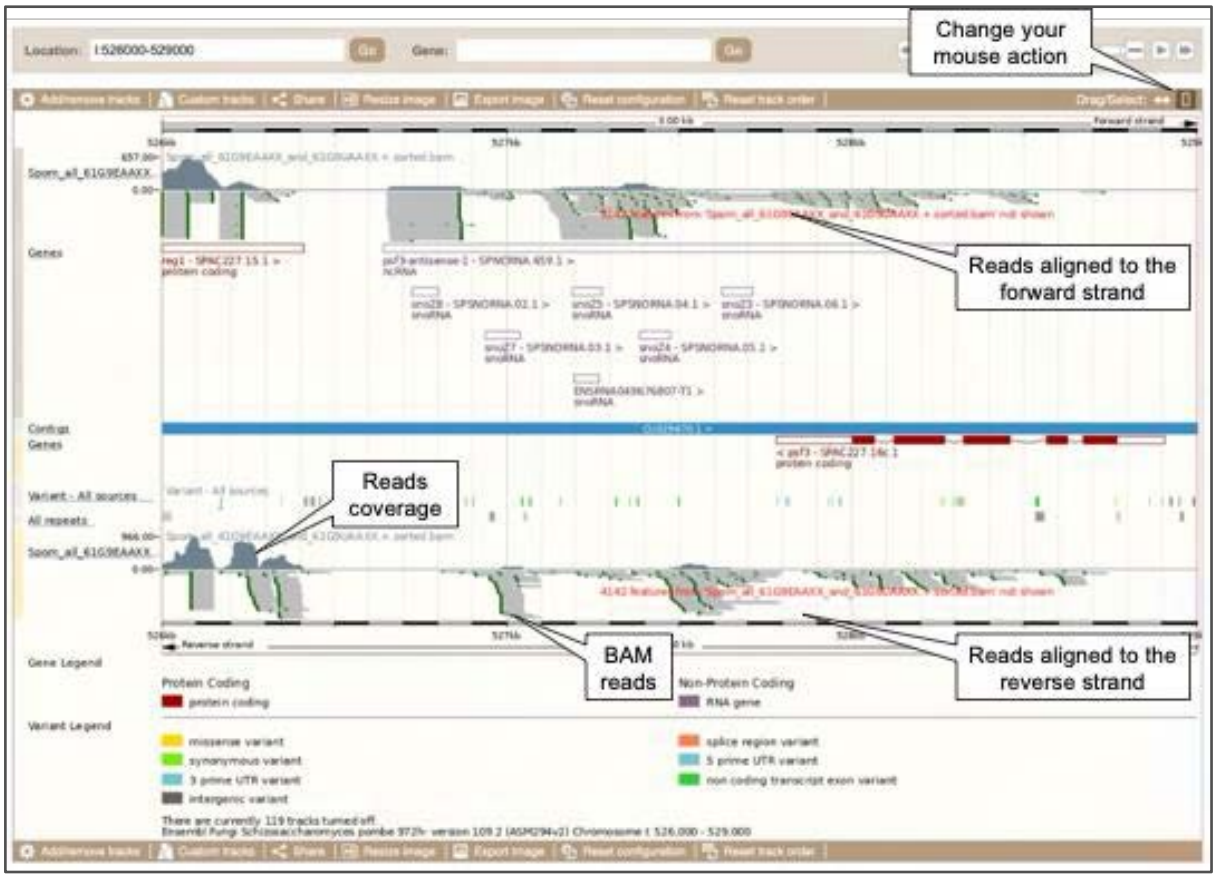
A menu will appear:



The interface detects file extensions if you upload or attach a file. If you want to upload a file, just click on Choose file, select the file from your local machine and it should automatically detect the file type you have submitted.

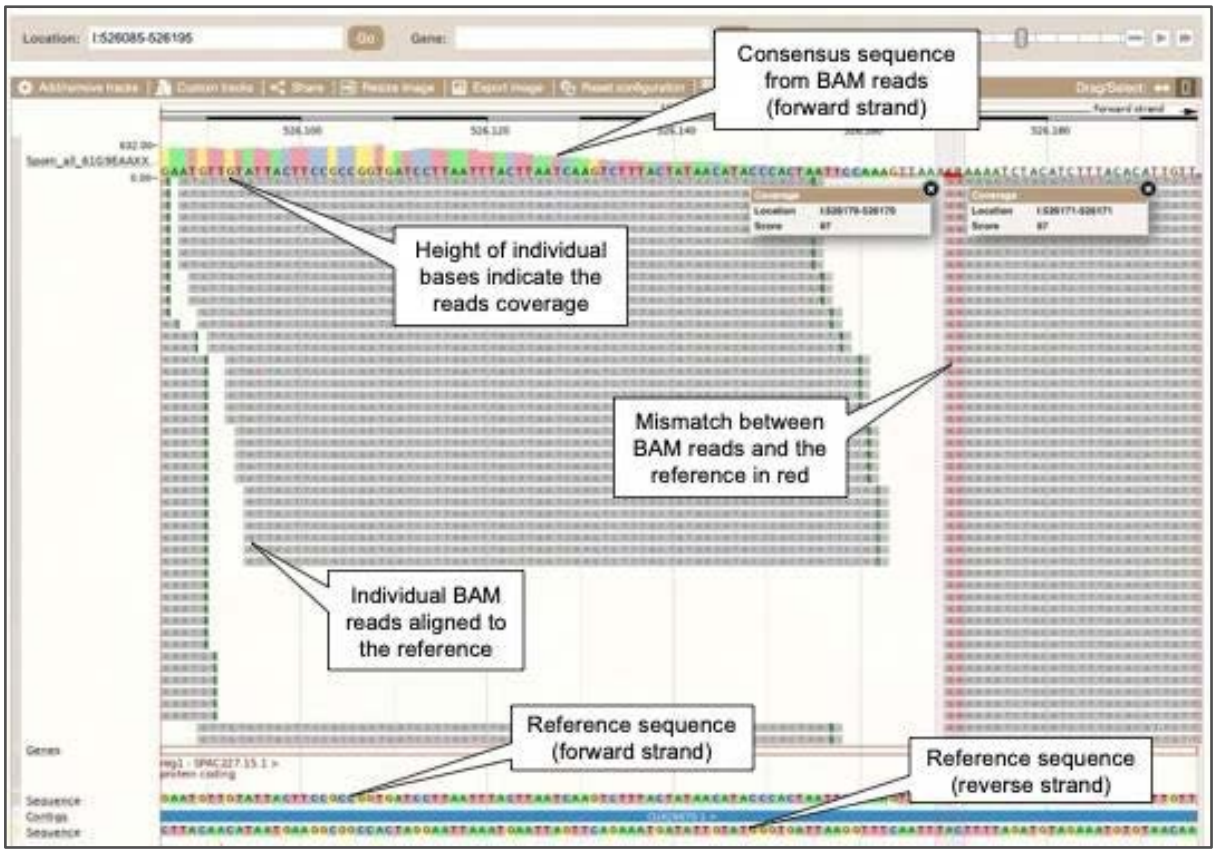
If you have a URL, like the one we located earlier, paste the URL of the BAM file itself into the 'Data' field (ftp://ftp.ensemblgenomes.org/pub/misc_data/bam/fungi/Spom/Spom_all_61G9EAAXX_and_61G9UAAXX.+.sorted.bam).

Since this is a file, the interface is able to detect the '.bam' file extension and automatically labels the format as BAM. Click on Add data and close the menu. It may take a while to load as there is a lot of data (Firefox tends to be fast). Once the data has been uploaded, you'll get a thank you message. Close the window and jump to a Location tab to see this data. Let's go to I:526000-529000.



Newly added BAM file track split into forward and reverse stranded reads. You can zoom in to see the sequence itself. Drag out boxes in the view to zoom in, until you see a sequence of individual reads, or jump to a 110 bp region: I:526085-526195.

(b) Change the track style of the newly added track to Unlimited (showing all reads). Can you spot a site called differently from the reference in our sample? What is its genomic position? What is the read coverage at this position on the forward strand? Would you consider it a real variant or an artefact?


Consensus sequence from BAM reads (forward strand)
Height of individual bases indicate the reads coverage
Mismatch between BAM reads and the reference in red
Individual BAM reads aligned to the reference
Reference sequence (forward strand)
Reference sequence (reverse strand)

Using SPELL to Analyze Expression Datasets & Coexpressed Genes at SGD

SPELL (**S**erial **P**attern of **E**xpression **L**evels **L**ocator) is a query-driven search engine for large gene expression microarray compendia. Given a small set of query genes, SPELL identifies which datasets are most informative for these genes, then within those datasets additional genes are identified with expression profiles most similar to the query set.

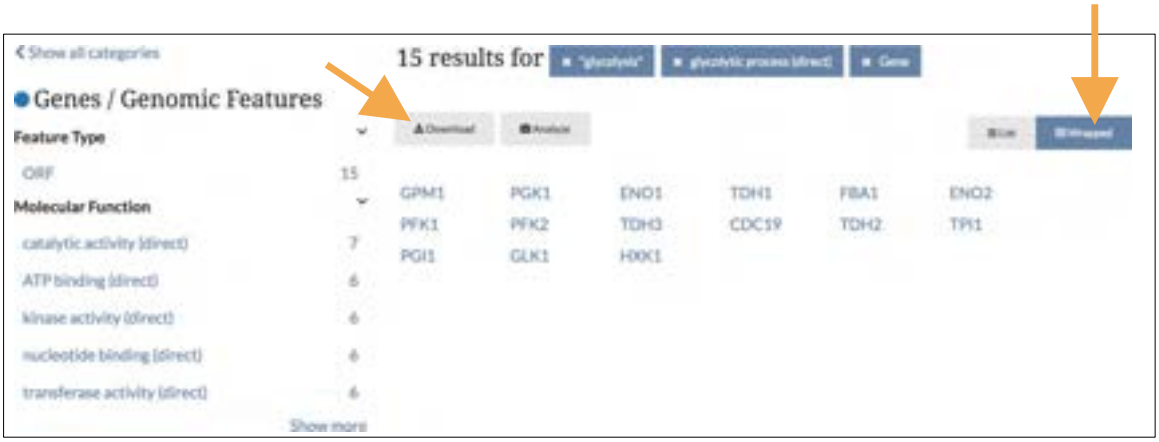
Use SPELL to find out which genes are coexpressed with genes involved in glycolysis.

Compile a list of genes involved in glycolysis.

- On the SGD home page (www.yeastgenome.org), enter glycolysis into the search box and hit Enter.

- On the Results page, click on the **Genes** category.

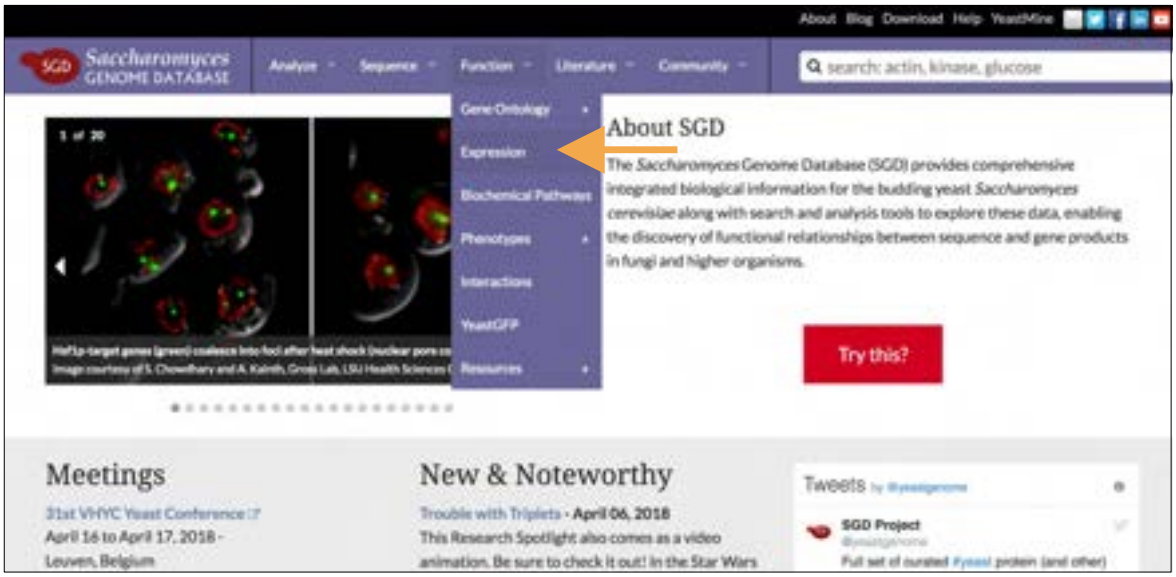
- Scroll down the page and find the **Biological Process** category on the left hand menu. Hit Show more and select **glycolytic process (direct).**
- To download the list of genes, click on **Wrapped** and then on **Download**.



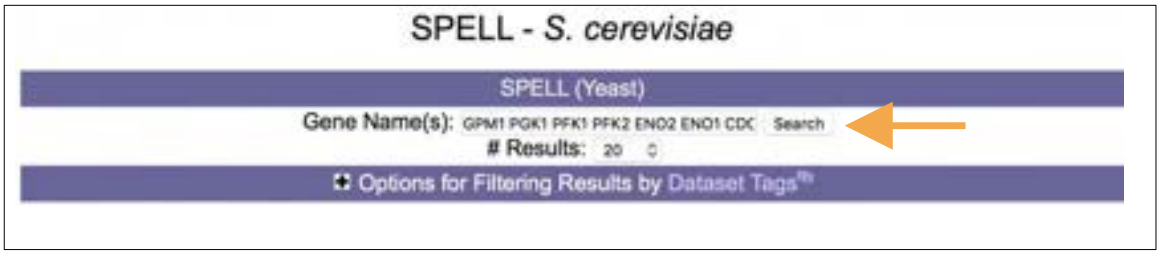
- The **Analyze** button, directly to the right of Download, enables you to import your search results directly into SPELL (among other tools at SGD). However, for the sake of demonstration, in this exercise we are instead going to enter our gene list into SPELL manually.

Import your gene list into SPELL and run a query:

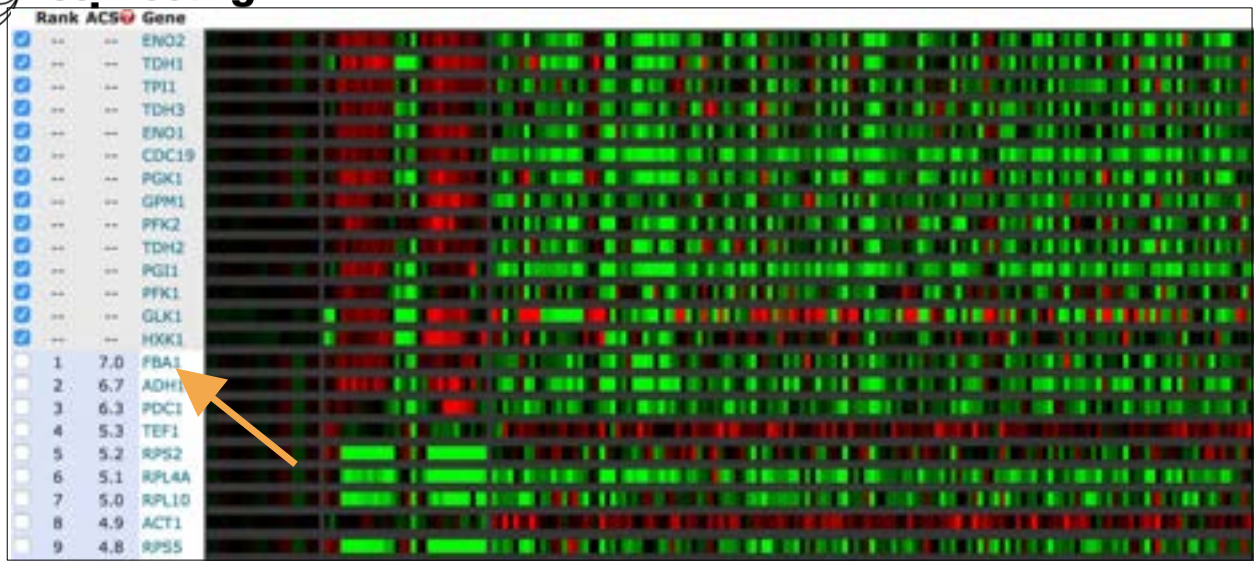
- To access SPELL, go to the SGD home page at www.yeastgenome.org, open the **Function** tab on top of the page and click on **Expression**. Or, if you are already on a Locus Summary page, open the Expression tab and click on the SPELL link under the histogram.



- On the SPELL page, copy and paste the list of glycolysis genes you downloaded in step 1 into the Gene Name(s) box. For the sake of demonstration, remove **FBA1** from your list before hitting Search. This is to test if SPELL can properly identify missing members of glycolysis based on coexpression.



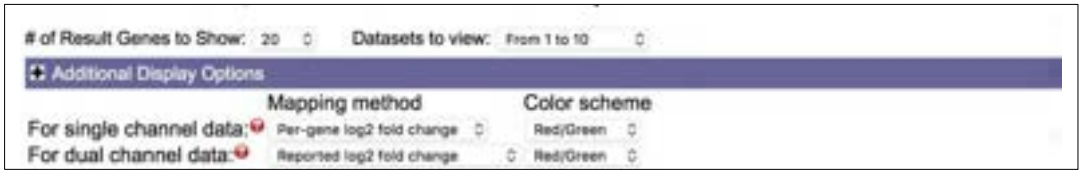
- Scroll down the list of genes on the left. Genes with checked boxes are from our query; the remaining genes are "hits", ordered from top to bottom according to their ranks. The rank reflects the correlation of expression of that gene with the query gene(s), given the relevance weight of that expression dataset. Thus, genes that show the highest degree of coexpression with the query genes in the most relevant datasets receive the highest rank.



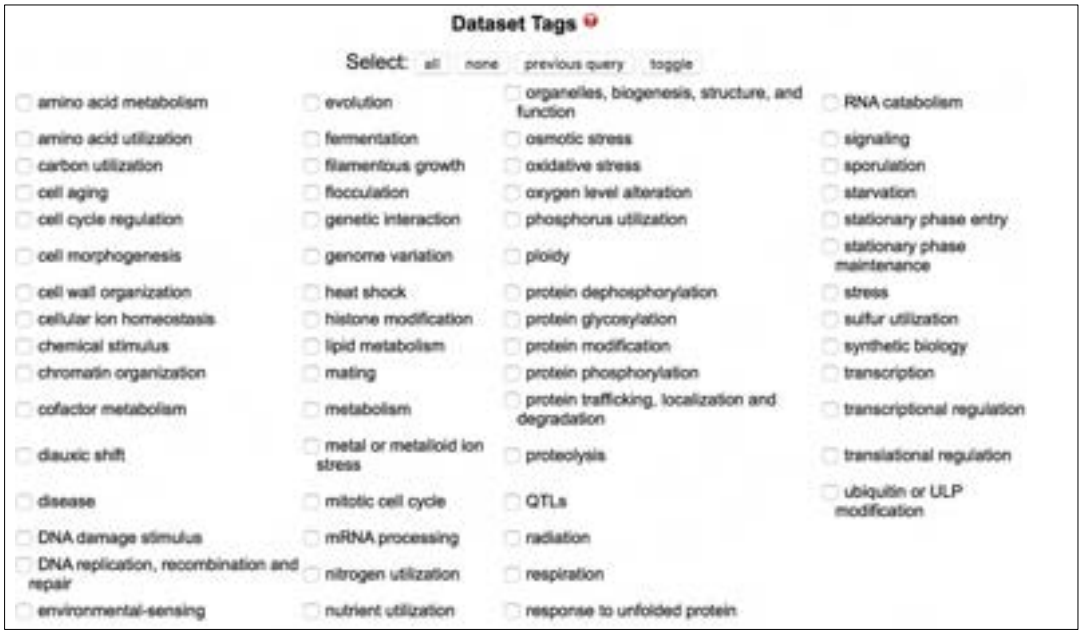
- Notice that the glycolysis gene we deleted earlier, FBA1, is indeed the highest-ranking gene!

- Examine other genes enriched for this query set. You can click on their names to be taken to their respective summary pages at SGD. Does it make sense for any of these genes to be highly coexpressed with members of glycolysis?

- Click on **+ Additional Display Options** to change the default mapping method and color scheme to blue/yellow. Directly above this section are options to change the number of genes and datasets shown in your results.



- To select only datasets with particular tags, click on **+ Options** for Filtering Results.



- Click on any patch in the heat map to open a page with information about its parent dataset.

- SPELL also runs a **Gene Ontology (GO) enrichment** for the results of your query. GO enrichments can tell you which gene ontology terms (in this case, biological process terms) are significantly associated with your set of genes. You can scroll down to the bottom of the page to view it.


GO Term Enrichment

GOTerm	P-val	% query	% genome	Annotated Genes
glucose catabolic process (biological_process)	1.33e-29	19 of 35	52 of 6381	FBA1, TDH3, ENO1, HXK1, HXK2, PFK2, GLK1, GPM1, PFK1, TPI1, TDH1, PGI1, ENO2, PDC1, ADH1, PDC5, CDC19, PGK1, TDH2
hexose catabolic process (biological_process)	2.39e-29	19 of 35	59 of 6381	FBA1, TDH3, ENO1, HXK1, HXK2, PFK2, GLK1, GPM1, PFK1, TPI1, TDH1, PGI1, ENO2, PDC1, ADH1, PDC5, CDC19, PGK1, TDH2
monosaccharide catabolic process (biological_process)	2.91e-27	19 of 35	66 of 6381	FBA1, TDH3, ENO1, HXK1, HXK2, PFK2, GLK1, GPM1, PFK1, TPI1, TDH1, PGI1, ENO2, PDC1, ADH1, PDC5, CDC19, PGK1, TDH2
glycolysis (biological_process)	4.79e-27	16 of 35	32 of 6381	FBA1, TDH3, ENO1, HXK1, HXK2, PFK2, GLK1, GPM1, PFK1, TPI1, TDH1, PGI1, ENO2, CDC19, PGK1, TDH2
glucose metabolic process (biological_process)	1.66e-23	19 of 35	99 of 6381	FBA1, TDH3, ENO1, HXK1, HXK2, PFK2, GLK1, GPM1, PFK1, TPI1, TDH1, PGI1, ENO2, PDC1, ADH1, PDC5, CDC19, PGK1, TDH2
single-organism carbohydrate catabolic process (biological_process)	3.62e-22	19 of 35	115 of 6381	FBA1, TDH3, ENO1, HXK1, HXK2, PFK2, GLK1, GPM1, PFK1, TPI1, TDH1, PGI1, ENO2, PDC1, ADH1, PDC5, CDC19, PGK1, TDH2
hexose metabolic process (biological_process)	4.32e-22	19 of 35	116 of 6381	FBA1, TDH3, ENO1, HXK1, HXK2, PFK2, GLK1, GPM1, PFK1, TPI1, TDH1, PGI1, ENO2, PDC1, ADH1, PDC5, CDC19, PGK1, TDH2
monosaccharide metabolic process (biological_process)	1.42e-21	19 of 35	123 of 6381	FBA1, TDH3, ENO1, HXK1, HXK2, PFK2, GLK1, GPM1, PFK1, TPI1, TDH1, PGI1, ENO2, PDC1, ADH1, PDC5, CDC19, PGK1, TDH2
carbohydrate catabolic process (biological_process)	1.97e-21	19 of 35	125 of 6381	FBA1, TDH3, ENO1, HXK1, HXK2, PFK2, GLK1, GPM1, PFK1, TPI1, TDH1, PGI1, ENO2, PDC1, ADH1, PDC5, CDC19, PGK1, TDH2
generation of precursor metabolites and energy (biological_process)	7.97e-18	19 of 35	190 of 6381	FBA1, TDH3, ENO1, HXK1, HXK2, PFK2, GLK1, GPM1, PFK1, TPI1, TDH1, PGI1, ENO2, PDC1, ADH1, PDC5, CDC19, PGK1, TDH2
single-organism carbohydrate metabolic process (biological_process)	1.60e-13	19 of 35	319 of 6381	FBA1, TDH3, ENO1, HXK1, HXK2, PFK2, GLK1, GPM1, PFK1, TPI1, TDH1, PGI1, ENO2, PDC1, ADH1, PDC5, CDC19, PGK1, TDH2
gluconeogenesis (biological_process)	3.72e-13	10 of 35	33 of 6381	FBA1, TDH3, ENO1, GPM1, TPI1, TDH1, PGI1, ENO2, PGK1, TDH2
hexose biosynthetic process (biological_process)	5.25e-13	10 of 35	34 of 6381	FBA1, TDH3, ENO1, GPM1, TPI1, TDH1, PGI1, ENO2, PGK1, TDH2
monosaccharide biosynthetic process (biological_process)	7.33e-13	10 of 35	35 of 6381	FBA1, TDH3, ENO1, GPM1, TPI1, TDH1, PGI1, ENO2, PGK1, TDH2

Exploring transcriptomics & proteomics datasets in FungiDB

Learning objectives:

- Query host-pathogen RNA-Seq data.
- Create a proteomics query and save this strategy to your account.

Transcriptomics.

There are different ways to search through transcriptomics datasets. The following search schemas can be used to explore the datasets in various ways:

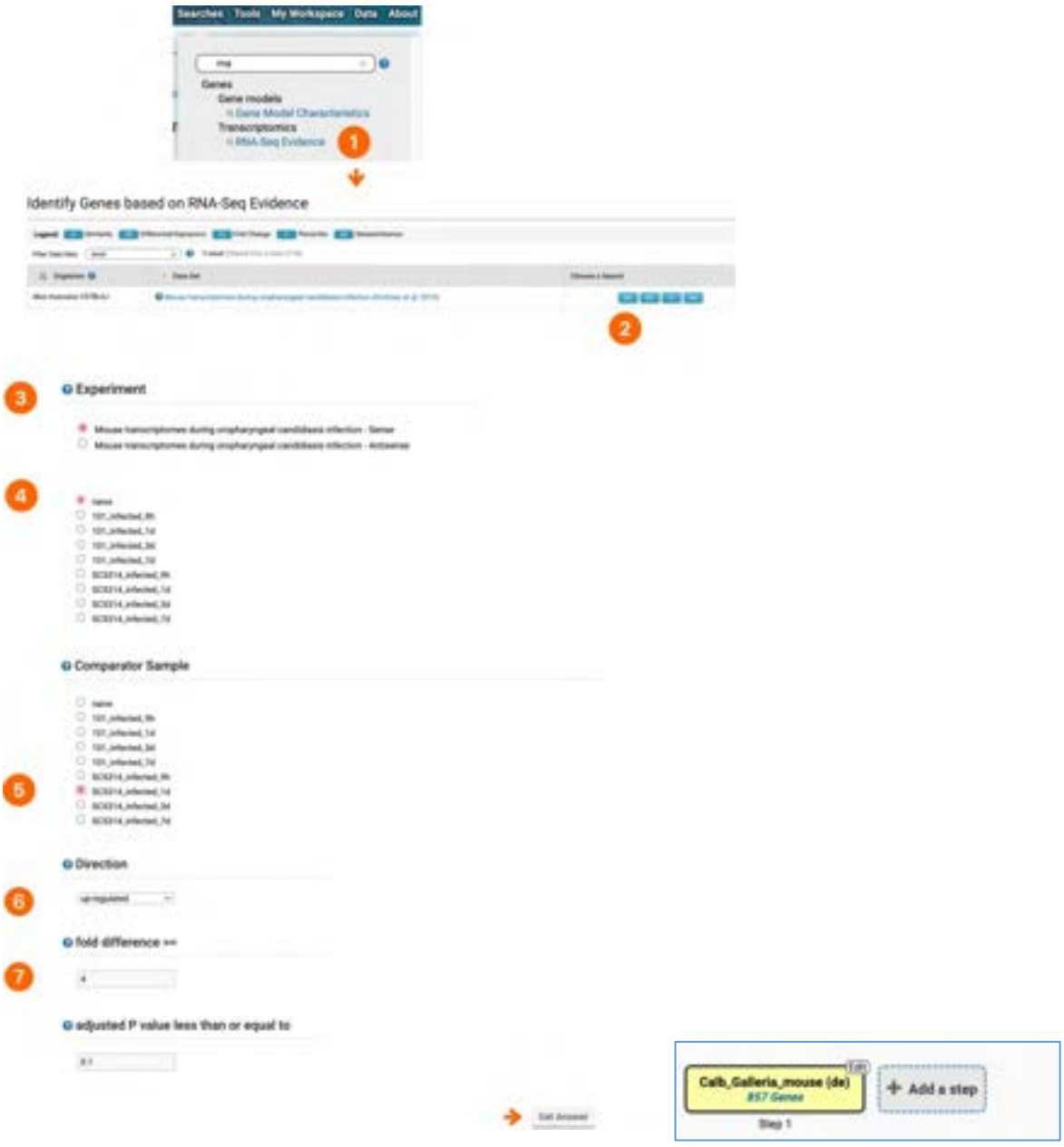
- **Coexpression.** Search for genes which have positive or negative correlations with a set of genes.
- **Similarity.** Search for genes which have a similar profile for an experiment.
- **Differential Expression (DE).** This search uses DESeq2 analysis results. You can choose the directionality and magnitude of the difference by setting both fold change and adjusted p values. For example, selecting up-regulated genes with a fold difference of 2 and an adjusted p-value cutoff of 0.1 will only show results where the comparator is twice that of the reference with an adjusted p-value of 0.1 or less.
- **Fold change (FC).** Find genes with changes in gene expression when statistical analysis in not available (e.g. no replicates). After selecting samples, you have the option to take the average, minimum, or maximum expression value within each group. If choosing only one sample from a group, the selected 'operation' will not affect your results. Time-series experiments will offer an extra parameter called "Global min/max" which allows you to filter your results further. Finally, you can choose the directionality and the magnitude of the difference (e.g., up/down regulated, fold difference of 2, etc.)
- **MetaCycle.** This search is applied to circadian datasets. For each study/experiment, you can choose either ARSER (Yang and Su 2010) or JTK_Cycle (Hughes et al. 2010), which are methods for detecting rhythmic signals. The search will return the corresponding period, amplitude, and p-value.
- **Percentile (P).** For each Experiment and Sample, genes are ranked by expression level (e.g., search for low/high gene expression levels).
- **Sense/antisense (SA).** This search is applied to stranded datasets. You can find genes that exhibit simultaneous changes in sense and antisense transcripts in the Comparison sample relative to the Reference Sample. For example, you could look for genes showing increasing antisense transcripts and decreasing sense transcripts, as might occur when antisense transcription suppresses sense transcription. The search will perform all pairwise comparisons between the Comparison and Reference samples.

For this exercise, we will query the host (mouse) and pathogen (*Candida albicans*) RNA-Seq data produced by Kirchner et al. in 2019. The study focuses on the oropharyngeal candidiasis experimental model in mice, which was used to examine *C. albicans'* interaction with the host at mucosal surfaces in vivo. The study involved two strains of *C. albicans*: SC5314, a virulent lab strain, and the persistent strain 101. A persistent strain can resist medical treatment, often leading to chronic or recurrent infections.

Objective: Identify differentially expressed genes in mice (HostDB.org) and Candida albicans SC5314 (FungiDB.org) during infection (1d).

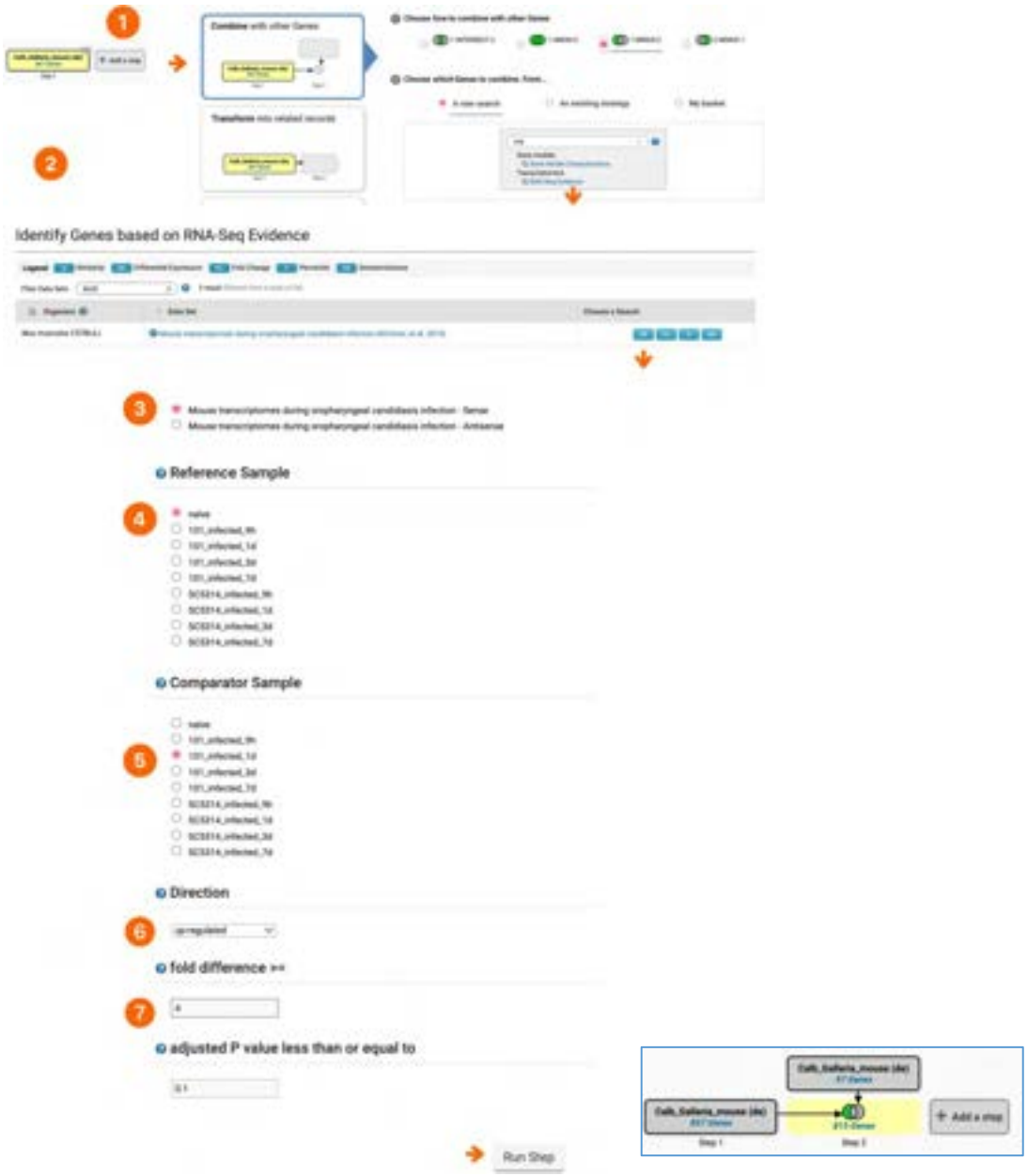
A. The next block of exercises will be carried out in HostDB.org

- **Identify genes up-regulated in mice infected with SC5314 at 1d**.
  1. Navigate to the RNA-Seq Evidence search and filter RNA-Seq datasets for “Kirch”.
  2. Click on the “DE” button.
  3. Choose to examine the sense strand.
  4. Select reference sample: naïve.
  5. Select comparator sample: SC5314_infected_1d.
  6. Look for up-regulated genes.
  7. Select magnitude of upregulation: 4 fold.



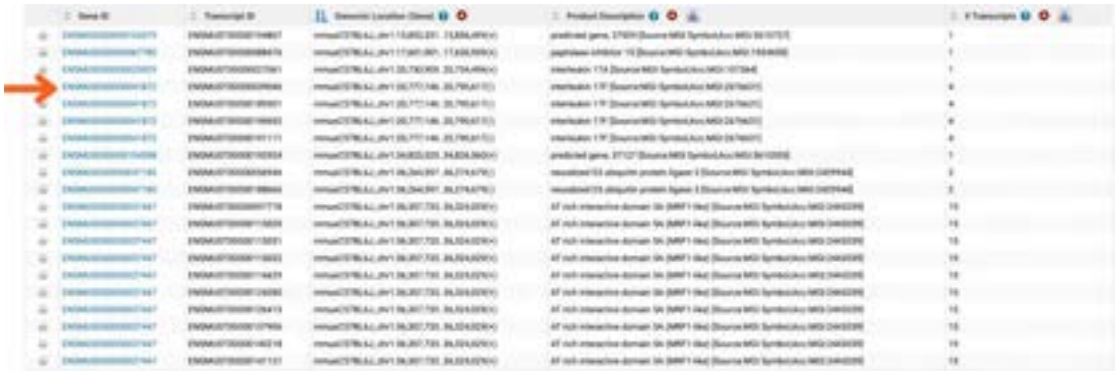
- **Identify host genes up-regulated by the SC5314 strain but not 101 at 1d of infection**.
    1. Click on the “Add Step” button.
    2. Navigate to the RNA-Seq Evidence search, select the “1 minus 2” Boolean operator, identify the RNA-Seq Evidence” search, filter for “Kirch” to quickly identify the dataset and click on the “DE” button.
    3. Choose to examine the sense strand.
    4. Select reference sample: naïve.
    5. Select comparator sample: 101_infected_1d.
    6. Look for up-regulated genes.
    7. Select magnitude of upregulation: 4 fold.

Note: The default Boolean operator is set to the “intersect” option. Make sure to select the correct Boolean operator for this search.

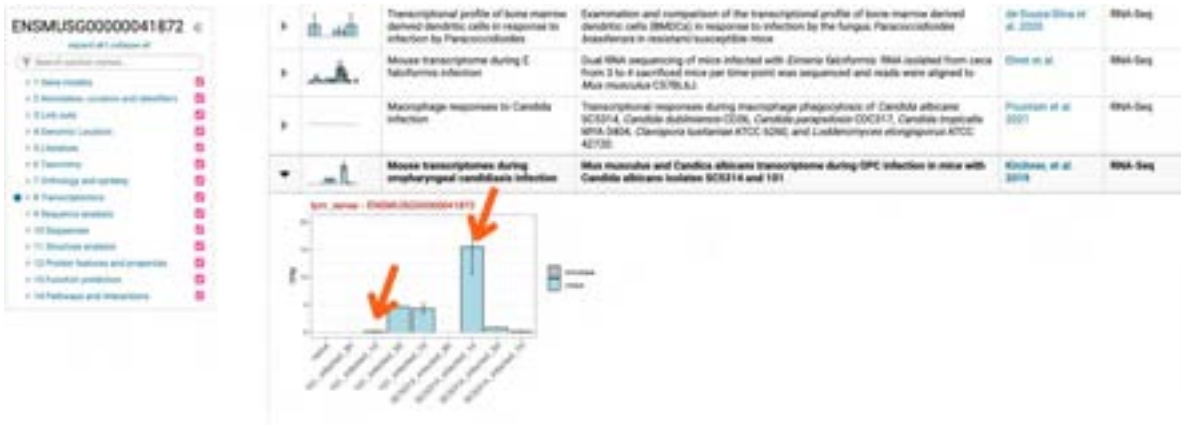


- **Examine the results in HostDB:**

    1. Click on the Gene ID link for “interleukin 17F” and navigate to the Transcript expression section within the gene record page.



Notice that the interleukin 17F response is much stronger at 1d in response to SC5314 infection. This is consistent with the delayed mouse response to C. albicans strain 101 compared to strain SC5314. Now, you may want to look back at gene enrichment signatures in fungi to learn more about SC5314 and 101-driven responses.

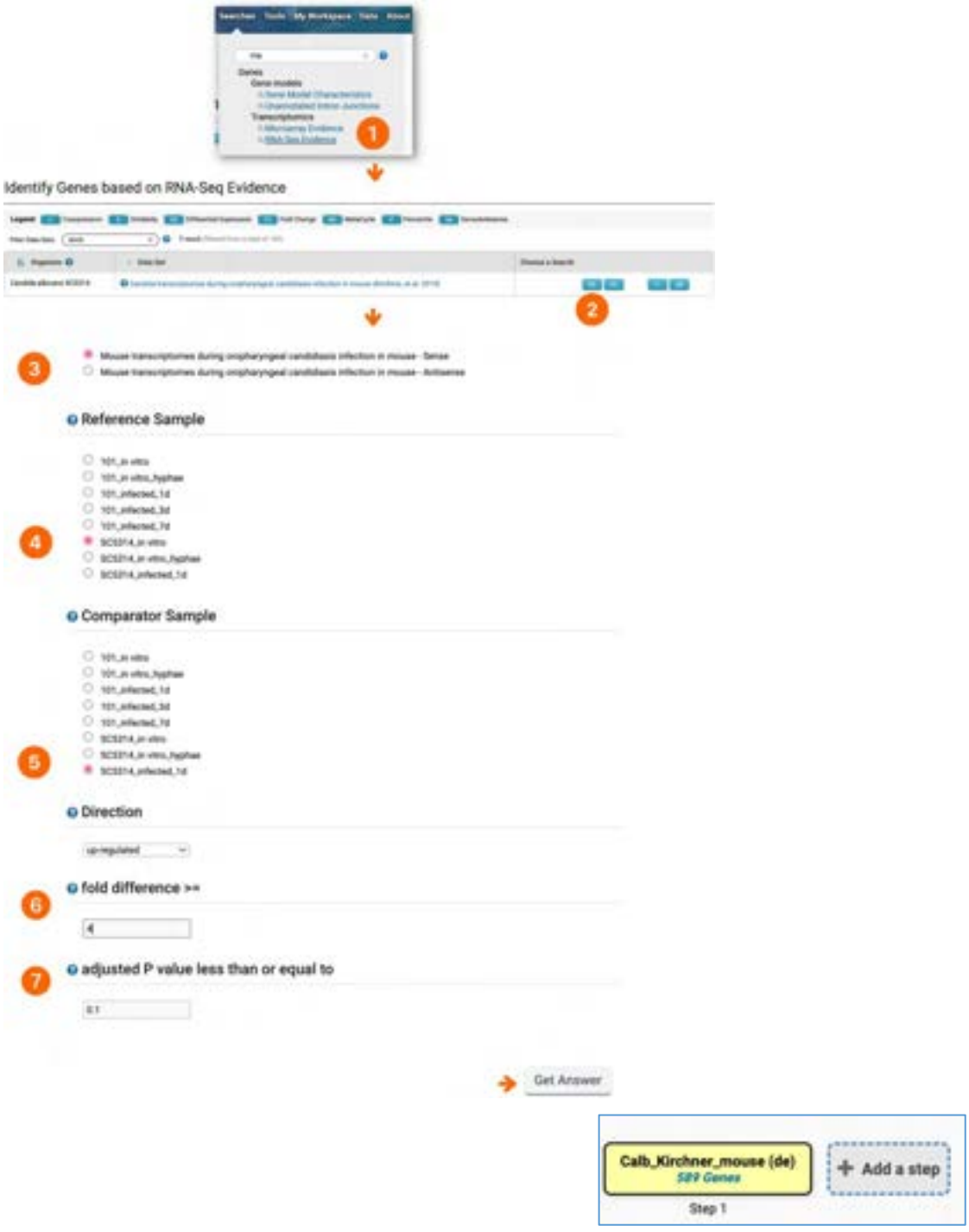


In summary, this strategy compared differentially expressed genes in mice in response to infection with SC5314 and 101 strains. It also identified genes up-regulated in response to SC5314 at 1d of infection while subtracting common genes upregulated in response to the exposure to the 101 strain.

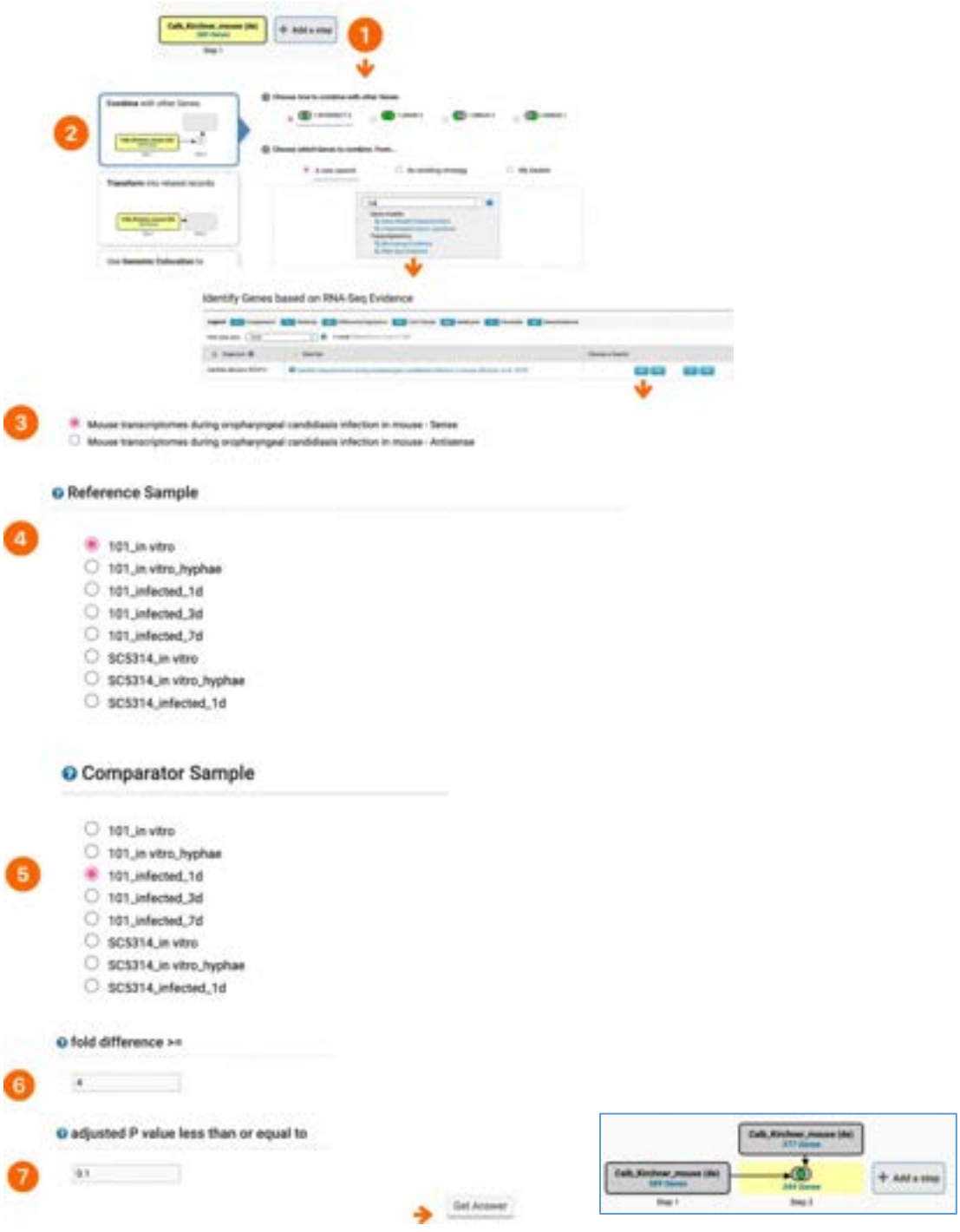
Strategy URL: https://hostdb.org/hostdb/app/workspace/strategies/import/de6763c0b7f9916c

B. The next block of exercises will be carried out in FungiDB.org.

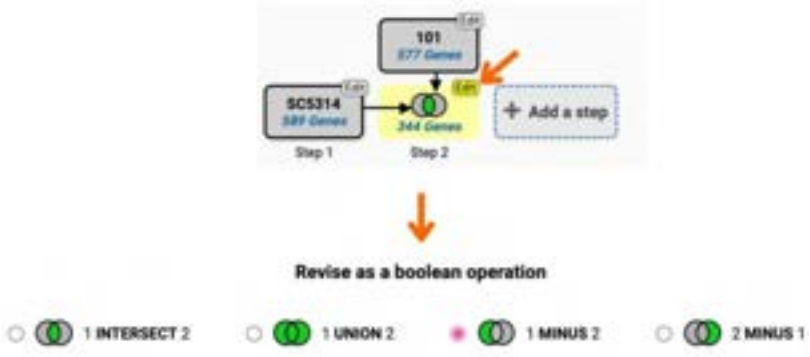
- **Identify genes up-regulated in SC5314 at 1d of infection**.
  1. Navigate to the RNA-Seq Evidence search and filter RNA-Seq datasets for “Kirch”.
  2. Click on the “DE” button.
  3. Choose to examine the sense strand.
  4. Select reference sample: SC5314_in vitro.
  5. Select comparator sample: SC5314_infected_1d.
  6. Look for up-regulated genes.
  7. Select magnitude of upregulation: 4 fold.



- **Identify genes up-regulated in SC5314 but not 101 strain at 1d of infection**.
    1. Click on the “Add Step” button.
    2. Navigate to the RNA-Seq Evidence search, filter for “Kirch” to quickly identify the dataset, and click the “DE” button.
    3. Choose to examine the sense strand.
    4. Select reference sample: 101_in vitro.
    5. Select comparator sample: 101_infected_1d.
    6. Look for up-regulated genes.
    7. Select magnitude of upregulation: 4 fold.



Note: You can always modify the Boolean operator by clicking on the Edit function as shown below:



In summary, this strategy compared differentially expressed genes in SC5314 and 101 strains. It also identified genes up-regulated in SC5314 at 1d of infection while subtracting common upregulated genes in the 101 strain background.

Note: The results of this analysis can be exported. FungiDB offers several download options, including viewing them within the browser or exporting them locally to your computer.

You can save the strategy by clicking on the floppy disk icon on the right. We will return to this strategy in the module on GO Enrichment analysis.

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

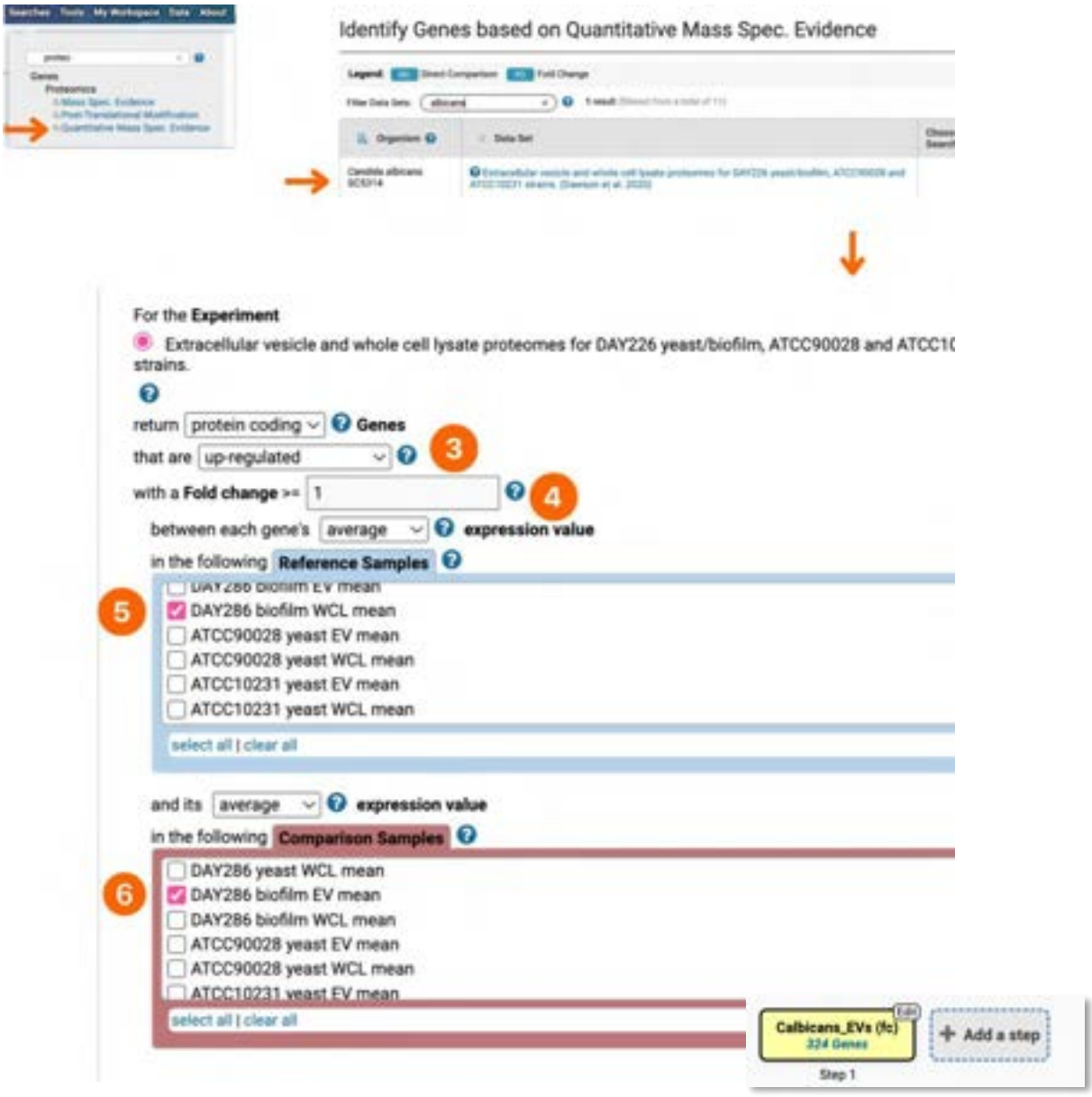
Proteomics

Objective: Query proteomics data for *Candida albicans*.

Fungal extracellular vesicles (EVs) have been implicated in host-pathogen and pathogen-pathogen communication. In this exercise, we will query quantitative mass spec data, compare protein abundance in EVs vs. whole cell lysate (WCL) in biofilm conditions, and cross-reference these results with the RNA-Seq evidence search created above.

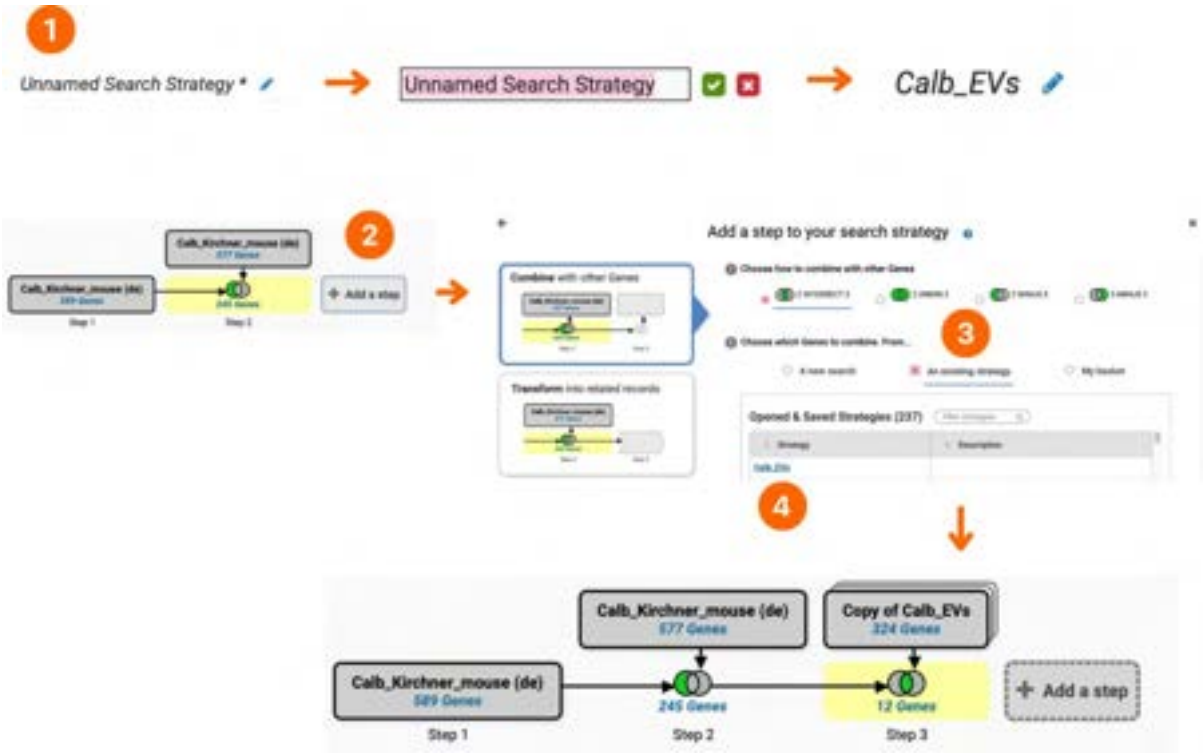
- **Identify proteins more abundant in EVs than whole cell lysate (WCL).**

1. Navigate to the “Quantitative Mass Spec. Evidence” search.
2. Filter for “albicans” and click the “FC” button for the Dawson et al. 2020 dataset.
3. Look for up-regulated genes.
4. With a Fold change >= 1.
5. Set Reference strain to DAY286 biofilm WCL mean.
6. Set Comparison Sample to DAY286 biofilm EV mean



- **Identify genes that are upregulated in SC5314 during infection and present in the EVs samples when Candida is grown in biofilm condition.**

1. Give your proteomics search a name (e.g. Calb_EVs)
2. Click on the previous RNA_Seq search to activate it and “Add a step”
3. Select to add an existing strategy
4. Click on the Calb_EVs proteomics strategy to import the results.



Strategy URL:
Calb_EVs: https://fungidb.org/fungidb/app/workspace/strategies/import/c971467cff5062fa
Transcriptomics & Proteomics query:
https://fungidb.org/fungidb/app/workspace/strategies/import/18b90faee40fe0db

References.

1. Hughes ME, Hogenesch JB, Kornacker K. 2010. JTK_CYCLE: an efficient non- parametric algorithm for detecting rhythmic components in genome-scale datasets. J Biol Rhythms. 25(5):372–380. doi:10.1177/0748730410379711.
2. Yang R, Su Z. 2010. Analyzing circadian expression data by harmonic regression based on autoregressive spectral estimation. Bioinformatics. 26(12):i168–i174. doi:10.1093/bioinformatics/btq189.

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Exploring Gene Models in JBrowse

Learning objectives:

- Leverage omics data (e.g., RNA-Seq) to evaluate gene models.
- Determine if a gene model is accurate or if alternate models are possible

In previous exercises, you spent some time learning about gene pages and examining genes in the context of the JBrowse genome browser. It is important to recognize that gene models (structural annotation) are often open to interpretation, especially with respect to:

● transcript initiation and termination sites (5' and 3' untranslated regions, or UTRs)

● alternative processing events … if you sequence deep enough, virtually all genes (in organisms that process transcripts) display alternative splicing, even for single exon genes.

● the potential significance of non-coding RNAs

Even heavily curated genomes do not fully reflect all available knowledge about stage-specific splicing, as new information is emerging all the time! In addition, many gene models were computationally derived using methods that may have not replied on experimental evidence supporting intron/exon boundaries (e.g. RNA-seq data).

In this exercise, we will explore genome browser track configuration options in greater detail, focusing on the interpretation of RNA-seq datasets and using this information to examine the genes in *Aspergillus fumigatus* Af293. The genes will be provided in class. Examine gene models and the underlying evidence as a group.

How to create your JBrowse view:

1. Navigate to JBrowse.
2. Select *Aspergillus fumigatus* Af293 from the dropdown menu.
3. Within JBrowse, click the 'Select tracks' tab and choose the 'Transcriptomics' category.
4. Select the dataset called 'Response to caspofungin'.
5. Choose to visualize 'unique' tracks from the 'RNA-Seq Alignment' category.
6. You may also want to activate the 'Syntenic sequences and genes' track to visualize gene conservation in other species.

During your discussion, evaluate gene models for missing or incorrectly annotated introns/exons, UTRs, merged genes or unannotated genes in the vicinity. Be prepared to discuss one gene per group in the classroom.

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Tools | My Workspace | Data | A | Genome | Track | View | Help

- Apollo
- BLAST (multi-query capable)
- Companion
- CRISPR guide design tool
- Galaxy
- Genome browser

- Aspergillus fischeri NRRL 181
- Aspergillus flavus NRRL3357
- Aspergillus flavus NRRL3357 2020
- Aspergillus fumigatus A1163
- **Aspergillus fumigatus Af293**

Select Tracks

- **My Tracks**
  - Currently Active
  - Recently Used
- **Category** X
  - 10 Transcriptomics
- **Subcategory**
  - 10 RNA-Seq
- **Dataset** X
  - 6 Adaptation to oxygen limitation
  - 7 Comparative transcriptomics of dormant and germinating conidia
  - 4 Determining Aspergillus fumigatus transcription factor expression and function during invasion of the mammalian lung
  - 22 Gene expression in *WT,* hrmA deletion, hrmA OE, hrmA_REV, EVOL under hypoxia and normoxia conditions
  - 6 Gene expression under oxidative and iron stresses
  - 6 Mycelial gene expression in response to treatment with 5,8-diHODE
  - 10 **Response to cas fungin**
  - 38 SensitiVity of transcription factor mutants of Aspergillus fumigatus to Congo Red
  - 6 Transcriptome analysis of conidium germination of Aspergillus fumigatus in different growth conditions
  - 10 Transcriptome of wild-type vs veA and mtf A deletion mutants
  - 6 Transcriptome under normoxia and hypoxia conditions
  - 8 Transcriptomes of *WT,* nctA, and nctB mutants in response to itraconazole.
  - **8** Transcriptomes of itraconazole-resistant strains
  - 10 Transcriptomics of Aspergillus fumigatus upon exposure to human airway epithelial cells
- **Track Type**
  - 8 Coverage
  - **1** Multi *'X:'f* plot
  - **1** Multi-Density
- RNA-Seq Alignment
  - 8 non-unique
  - 10 unique

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Name	C,
Response to caspofungin - 001.1 - WT_CSP (unique forward) Coverage	Tr
Response to caspofungin - 001.3 - WT_CSP (unique reverse) Coverage	Tr
Response to caspofungin - 002.1 - WLCT (unique forward) Coverage	Tr
Response to caspofungin - 002.3 - WT_CT (unique reverse) Coverage	Tr
Response to caspofungin - 003.1 - delta fhdA._CSP (unique forward) Coverage	Tr
Response to caspofungin - 003.3 - delta fhdA._CSP (unique reverse) Coverage	Tr
Response to caspofungin - 004.1 - delta fhdA._CT (unique forward) Coverage	Tr
Response to caspofungin - 004.3 - delta fhdA._CT (unique reverse) Coverage	Tr
Response to caspofungin Density - Uniciue Only	Tr
Response to caspofungin XYPlot - Unique Only	Tr

Select Tracks

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  - Currently Active
  - Recently Used
- **cat..,iry**

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Name

category

2 unique and non-uniq

40 (no data)
1 Com aratlve Genomlcs
21 Epigenomlcs
3 Gene Models

Syntenk Sequences and Genes
(Shaded C
by Orthology)
ompara

Exercise: Ensembl Fungi whole-genome alignments

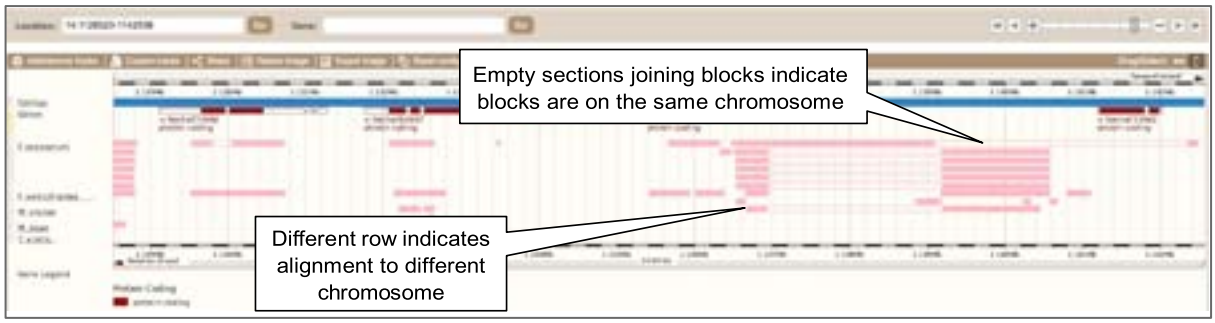
Links to be clicked shown in blue, text to be entered shown in red.

Ensembl Fungi contains whole genome alignments for pairs of key species, generated using LastZ. Let's look at some of these comparative genomics views in the Location tab.

(a) Find the region 14:1128520-1142558 in *Fusarium solani* and go to the Region in detail page. This region includes four genes we identified from our first BioMart query: *PEP5*, *PDA1*, *ESP3* and *PEP5*.

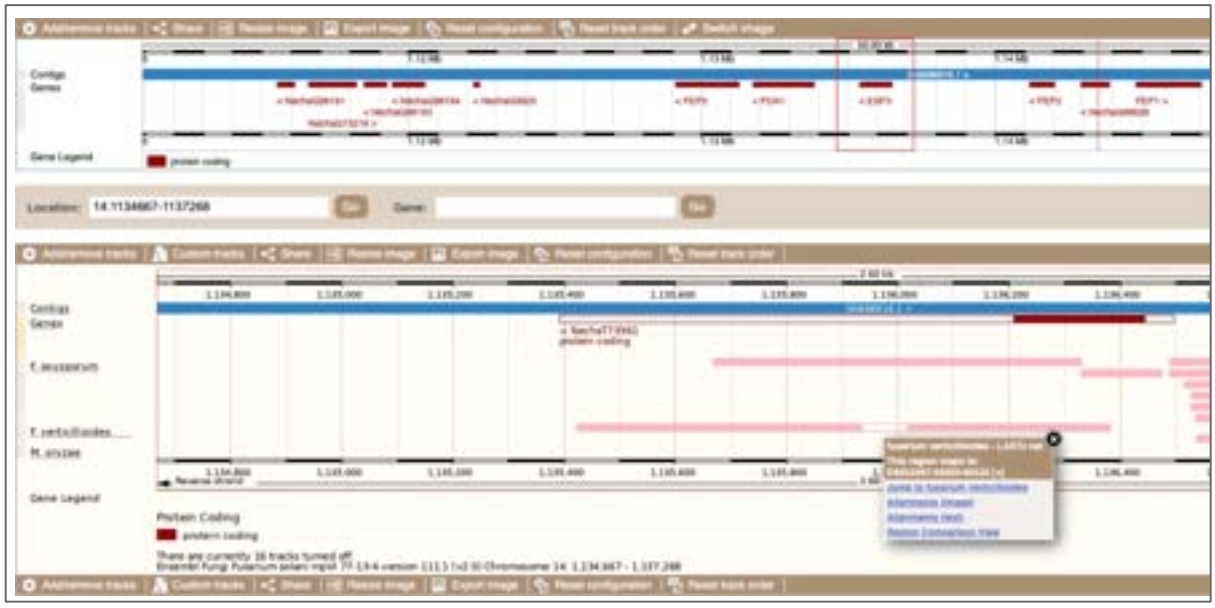
We can look at individual species' comparative genomics tracks in this view by clicking on Configure this page. In the 'Comparative genomics' section, turn on all of the available species' alignments in the normal style.

We can now see some pink alignments shown on the display. Alignments to the same chromosome are presented in a single row, and gaps in the alignment are shown by linking blocks. If there are alignments to multiple chromosomes in the aligned species these are represented on different rows.



(b) Looking at the pink alignment blocks, does this region in F. solani s align to multiple different chromosomes in the other species?

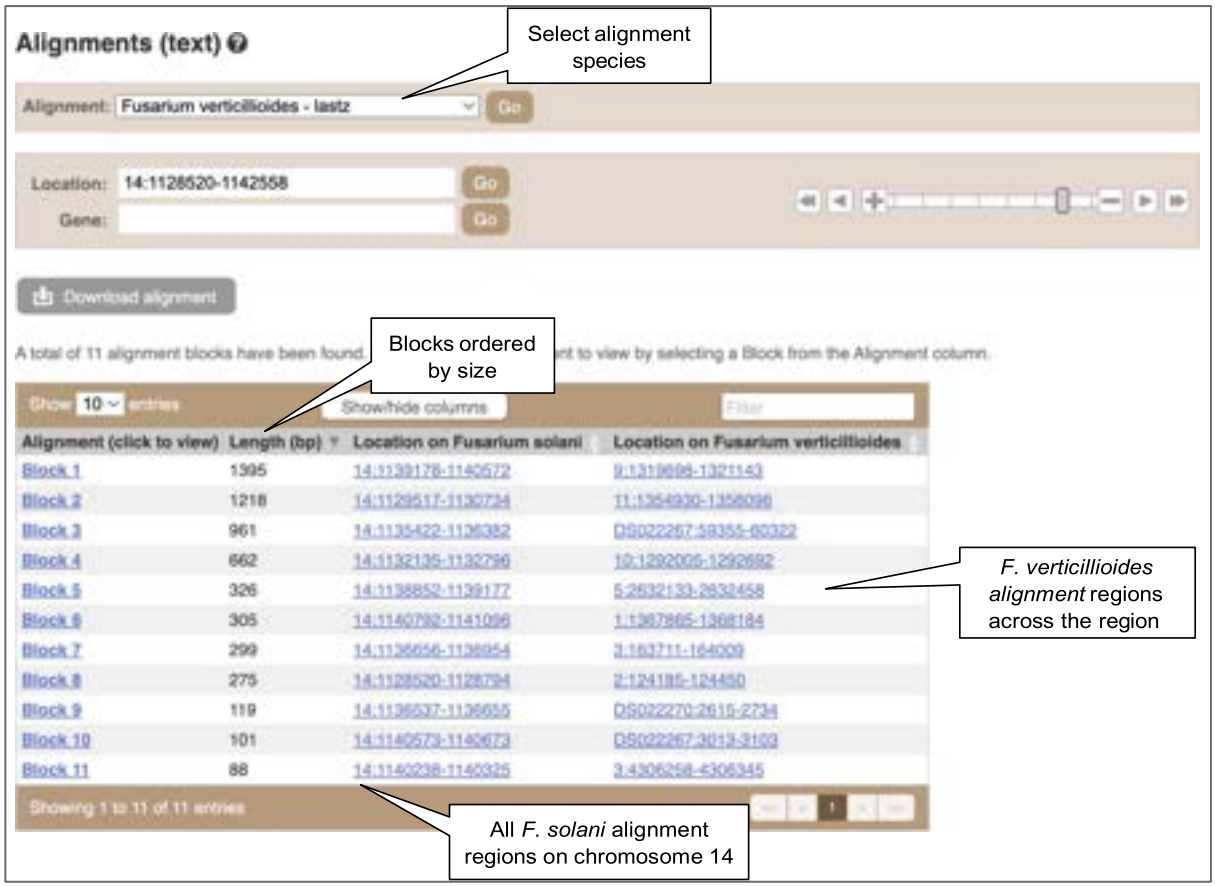
(c) Which chromosome(s) does the *F. solani ESP3* gene align to in *F. verticillioides*?



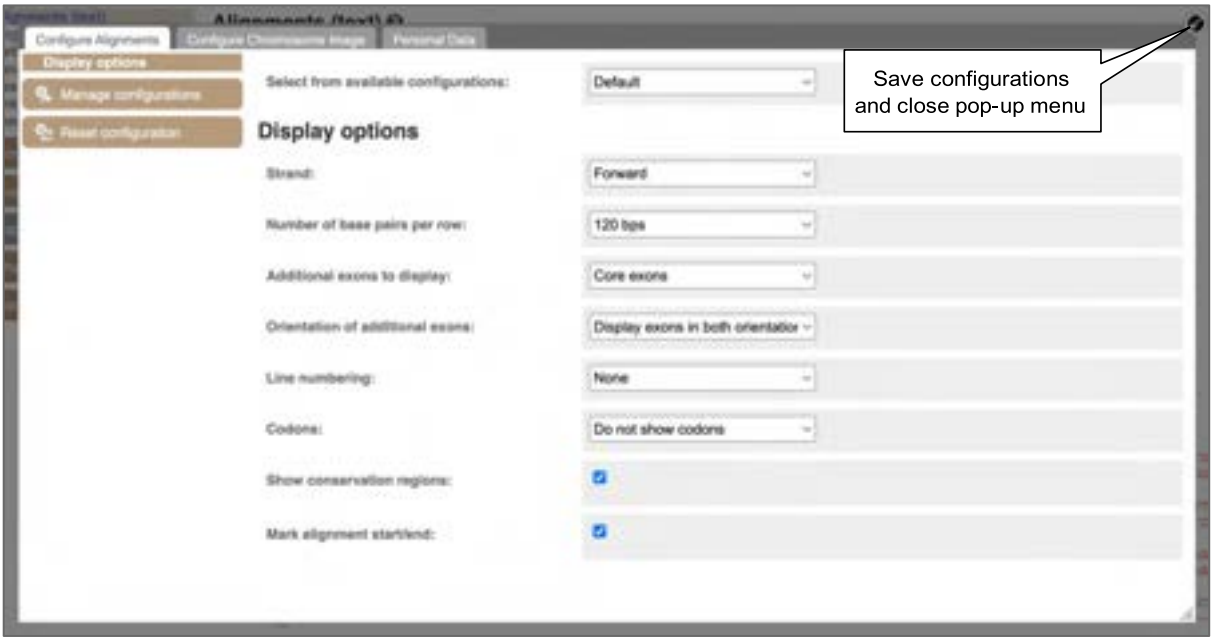
We can see that alignments in this region are quite poor for these species, with alignments spanning different chromosomes. This supports the lack of orthologues between these species.

We can view more detailed alignments in the alignment's text/image and region comparison views. Let's first view a text alignment in this region. Click on Alignments (text) on the left and choose *Fusarium verticillioides* from the drop-down menu.

Because this single chromosome region in *F. solani* aligns to regions that are far spread in other genomes, you need to select a specific block for the alignment, as we cannot display a single sequence alignment from more than one region.

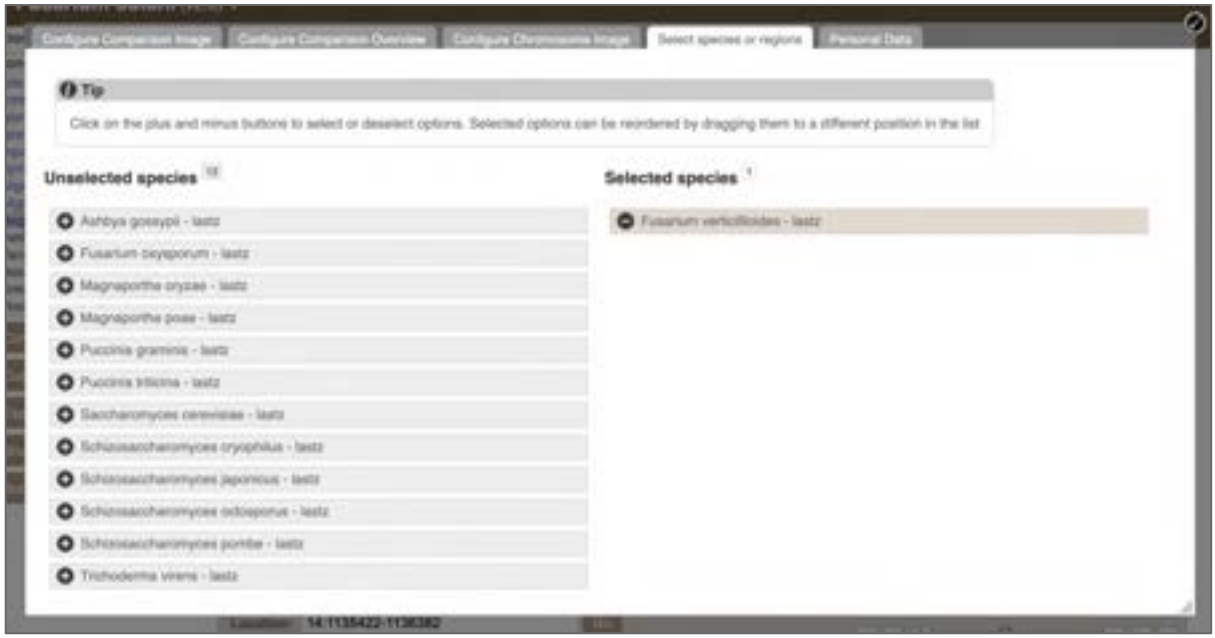


Let's click on Block 3. This takes you to a new page with a sample of the aligned sequence. Then click the button to Display full alignment. You will see a list of the regions aligned, followed by the sequence alignment. Exons are shown in red. Click on Configure this page, you can turn on the options to view Show conservation regions and Mark alignment start/end. Remember to click the tick at the top right when closing this window to save your choices. This will add highlights where the sequence matches.

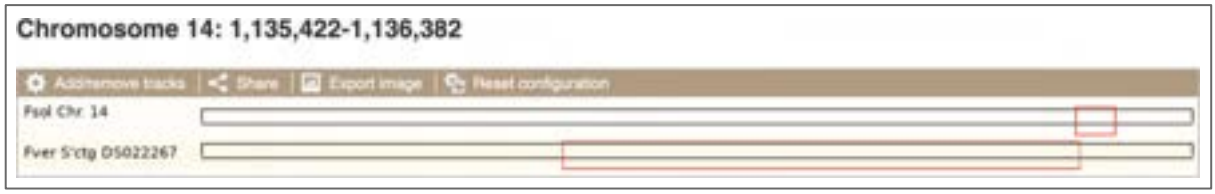


To view an image of the alignments, click on Region comparison in the left-hand navigation panel. This view is like the 'Region in detail' page as it shows three images of the genome at different scales. You can add multiple species to this view.

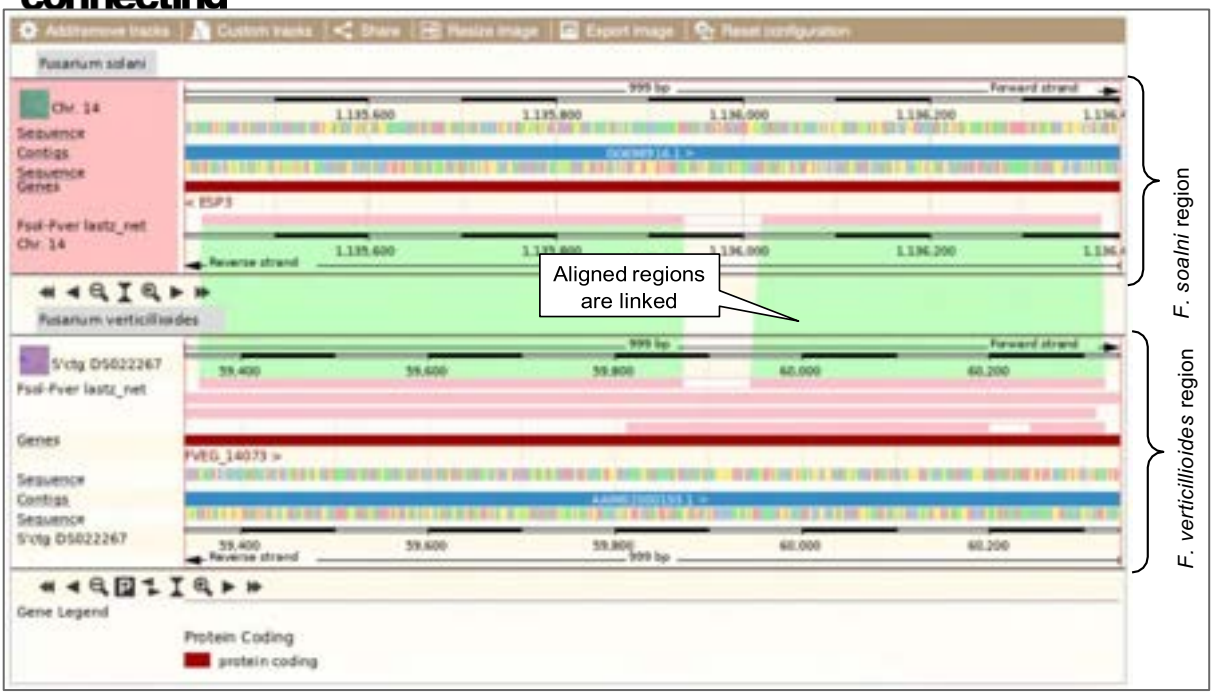
Click on the brown Select species or regions button. In the pop-up menu, select *Fusarium verticillioides* from the list. Close the window.



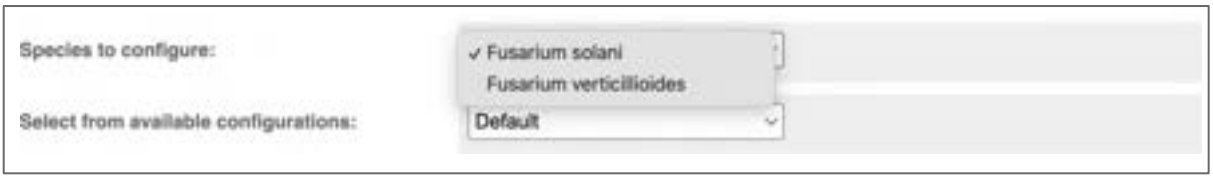
This page, similar to the region in detail page, shows the chromosome positions first. We can see the location of this alignment on the scaffold in *F. verticillioides*.



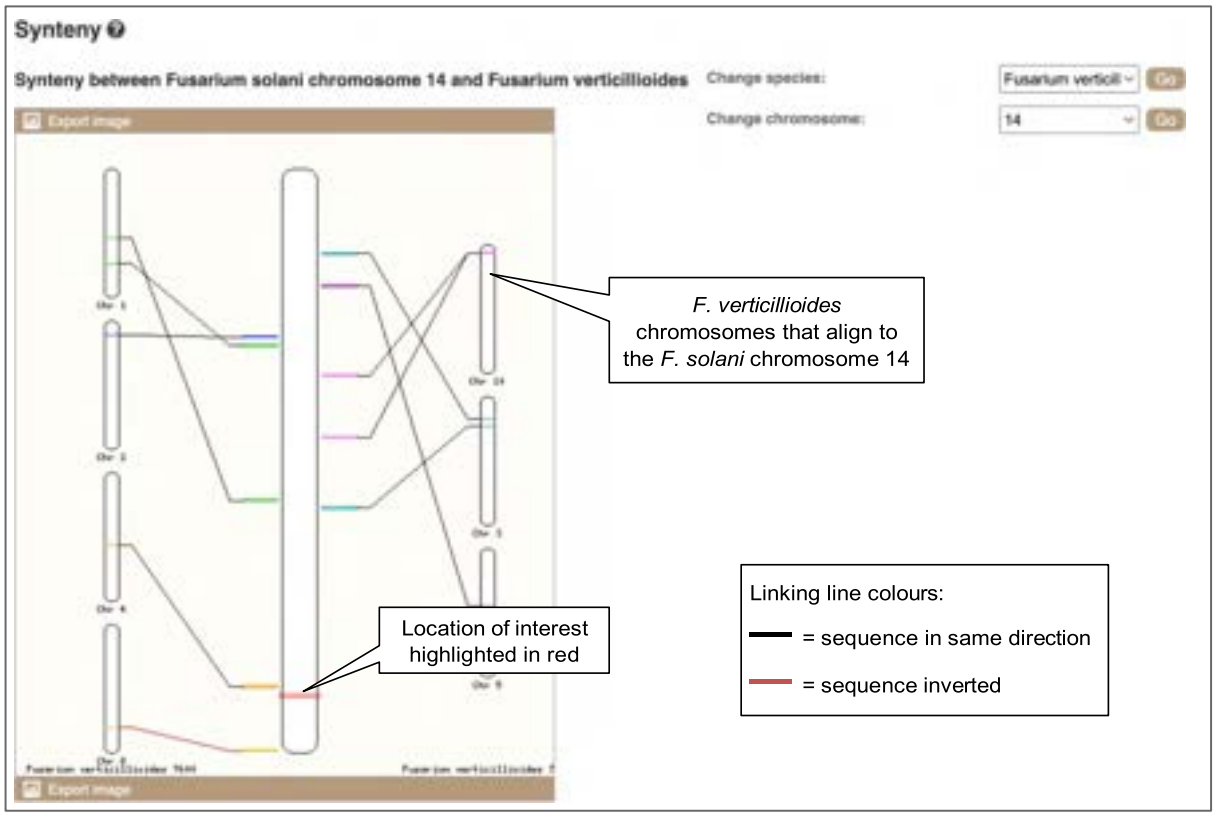
Scroll down to the most detailed image. An example image (of another alignment block) is below, and you should see something similar on your browser.



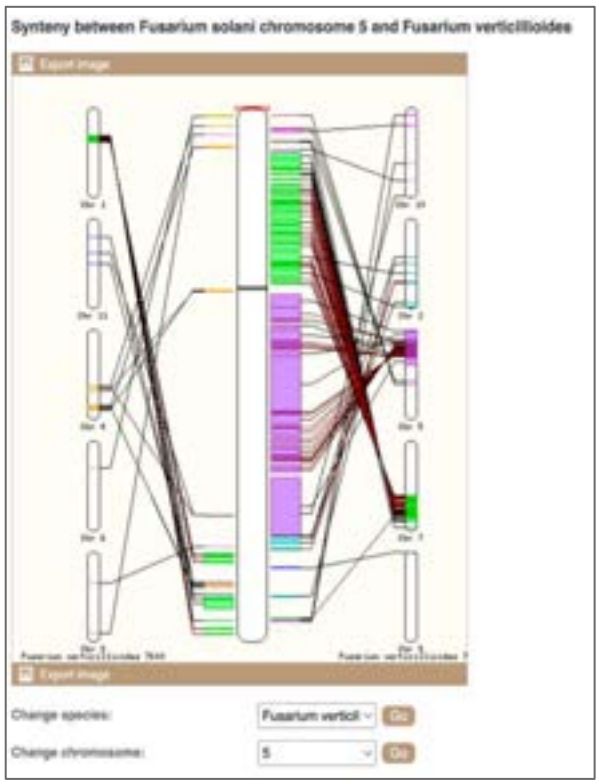
You can add data to both of these views with the same options you had in the 'Region in detail' page. Click on Configure this page and look at the top of the menu.

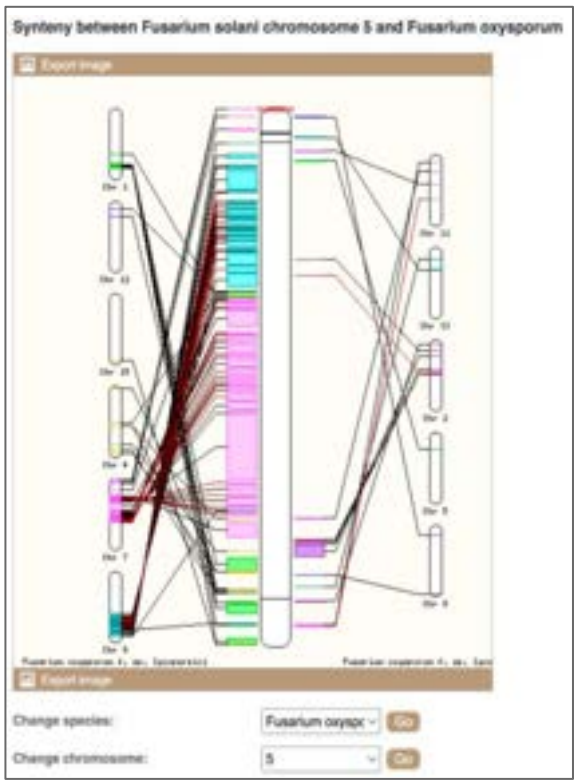


We can view chromosomal rearrangements in the 'Synteny' view. Click on Synteny in the left-hand navigation panel.



(d) Which chromosome in *F. verticillioides* is most similar to *F. solani* chromosome 5? Change the display to show *F. oxysporum*. Does this give you the same answer as for *F. verticillioides*?





Additional Exercise - Rearrangements in *Magnaporthe* species

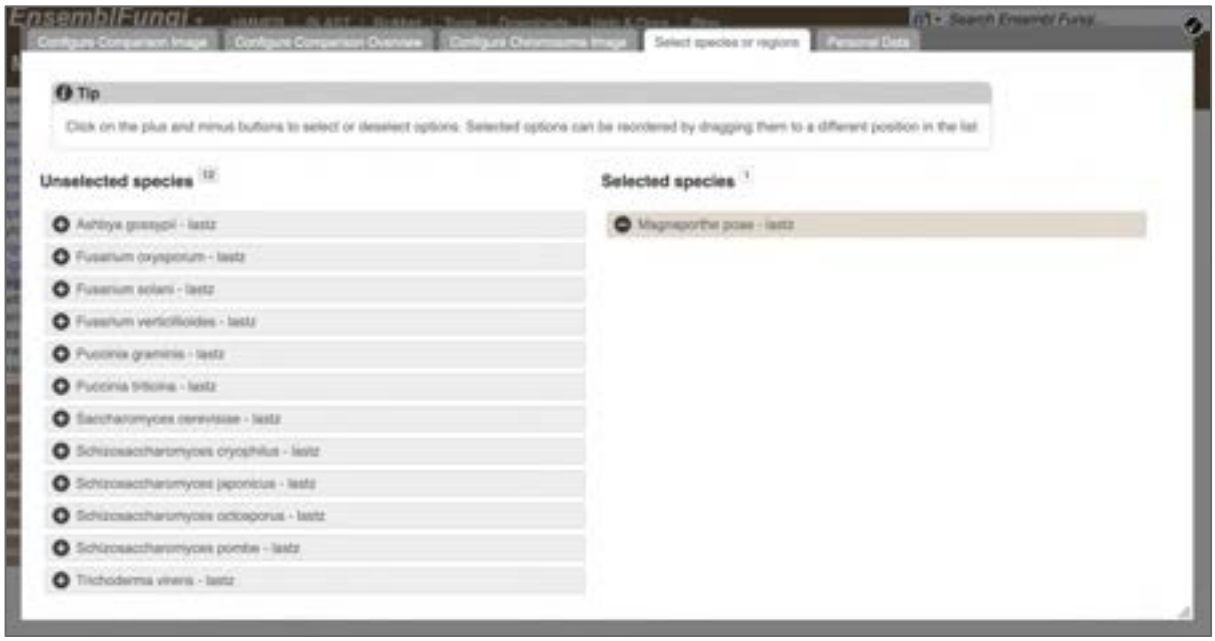
In the publication '[PacBio sequencing reveals transposable elements as a key contributor to genomic plasticity and virulence variation in *Magnaporthe oryzae*]', Bao et al (2017) identified a region on chromosome 1 that is shown to be a region of inter-chromosomal rearrangement and inversion. We're going to take a look at this region and see how it looks in *Magnaporthe oryzae* and *Magnaporthe poae*.

(a) Search for the region 1:5603535-5611402 in *Magnaporthe oryzae*.
(b) Click on Region comparison and choose *Magnaporthe poae* from the Select species or regions pop-up to display an alignment.
(c) Scroll down to the most detailed image. To what region (chromosome/scaffold/contig) does this region align to on the *M. poae* assembly?
(d) Which genes are present in the aligned region for *M. oryzae* and *M. poae*? What are their biotypes?

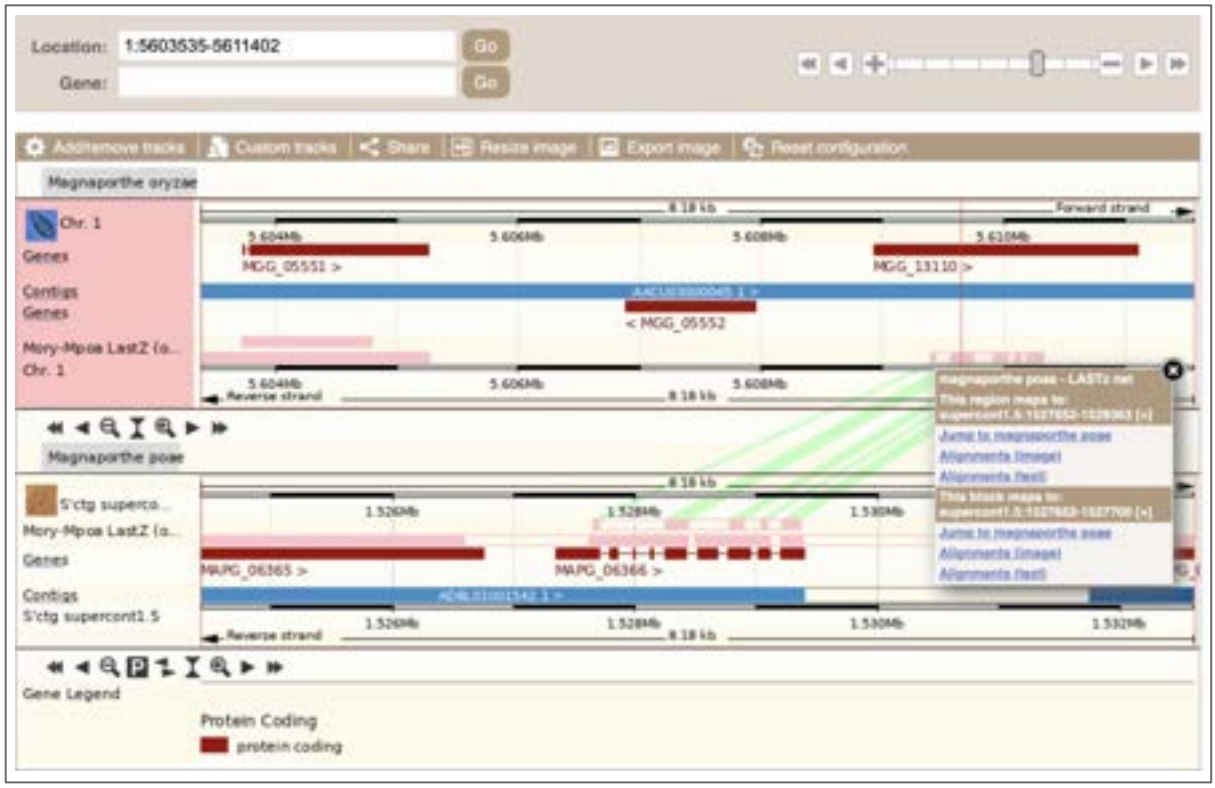
Answer - Rearrangements in *Magnaporthe* species

(a) Go to fungi.ensembl.org in your browser and search for the region 1:5603535-5611402 in *Magnaporthe oryzae*.

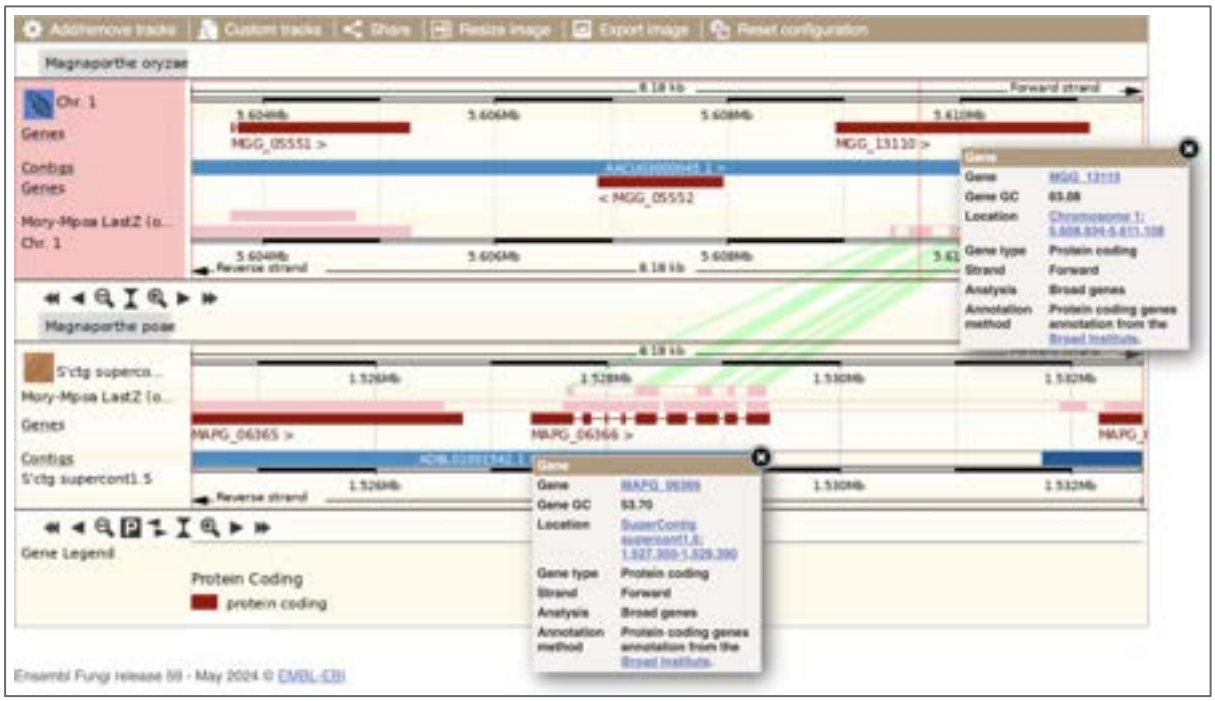
(b) Click on Region Comparison in the left-hand panel. Click on Select species or regions and select *Magnaporthe poae* - lastz in the pop-up menu to display the alignment.



(c) Scroll down to the ‘Region in detail’ view. The region aligns to SuperContig (S’ctg) supercont1.5 in the *M. poae* assembly.



(d) In the ‘Genes’ track, find out which features overlap the alignment regions. Click on the feature to find out more information. In *M. oryzae*, the gene MGG_13110 is present. In
M. poae, the gene MAPG_06366 is present. Both genes are protein coding.


Add/remove tracks
Custom tracks
Share
Resize image
Export image
Reset configuration
Magnaporthe oryzae
Chr. 1
Genes
Contigs
Genes
Mory-Mpoa LastZ (o...
Chr. 1
MGG_05551 >
MGG_13110 >
< MGG_05552
Forward strand
Reverse strand
Gene
Gene MGG_13110
Gene GC 65.08
Location Chromosome 1: 5,608,934-5,611,108
Gene type Protein coding
Strand Forward
Analysis Broad genes
Annotation method Protein coding genes annotation from the Broad Institute.
Magnaporthe poae
S'ctg superco...
Mory-Mpoa LastZ (o...
Genes
Contigs
S'ctg supercont1.5
MAPG_06365 >
MAPG_06366 >
Gene
Gene MAPG_06369
Gene GC 53.70
Location SuperContig supercont1.5: 1,927,300-1,929,300
Gene type Protein coding
Strand Forward
Analysis Broad genes
Annotation method Protein coding genes annotation from the Broad Institute.
Gene Legend
Protein Coding
protein coding
Ensembl Fungi release 59 - May 2024 © EMBL-EBI

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MycoCosm: Comparative Analysis of Gene Families

Objective: Compare genomes of wood decay fungi to identify gene families which can be used to distinguish white rot and brown rot fungi

Many fungi of the phylum Basidiomycota are capable of degrading wood, including the recalcitrant polymer lignin, which gives wood its structural strength and resistance to microbial attack (Floudas et al. 2012; Riley et al. 2014). These wood decaying fungi are often classified as either **white rot**, in which lignin is completely degraded and cellulose is left somewhat intact; or **brown rot**, in which cellulose is degraded and lignin is left somewhat intact. While the precise enzymatic mechanisms vary from one fungus to another, in general white rot genomes encode class II peroxidase enzymes to break down lignin, carbohydrate-binding motif enzymes to bind cellulose, and glycoside hydrolases to break down cellulose. By contrast, brown rot genomes tend to have relatively reduced numbers of these enzymes, or even lack them entirely.

Suppose we are comparing the genomes of four wood decaying fungi: *Auricularia subglabra*, *Calocera cornea*, *Gloeophyllum trabeum*, *Phanerochaete chrysosporium* RP-78. Suppose, also, that we don't know which of them are white-rot or brown-rot fungi. How can we use MycoCosm to make predictions about their mode of decay?

Start by going to the genome group page created for this example (in real life we would use a similar genome group page, but with a larger, ecologically- or phylogenetically-relevant selection of organisms):

https://mycocosm.jgi.doe.gov/WR_BR_example_2017/

##	Name	Assembly Length	# Genes	Published
1	Auricularia subglabra v2.0	76,853,599	25,459	Floudas D et al., 2012
2	Calocera cornea v1.0	33,244,933	13,177	Nagy LG et al., 2016
3	Gloeophyllum trabeum v1.0	37,181,821	11,846	Floudas D et al., 2012
4	Phanerochaete chrysosporium RP-78 v2.2	35,149,519	13,602	Ohm RA et al., 2014

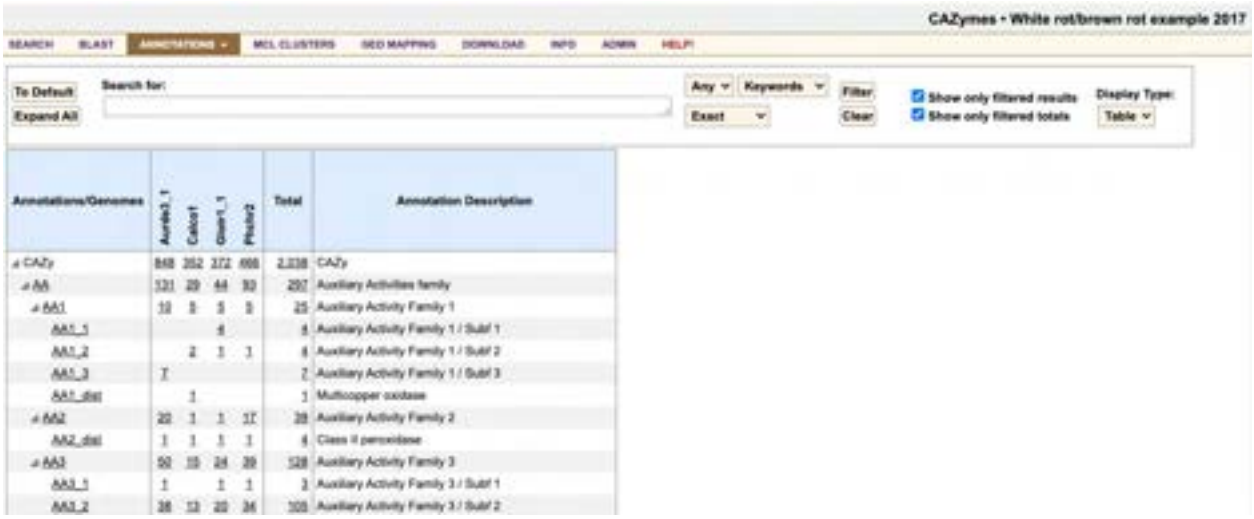
CAZy browser

CAZymes (Carbohydrate-Active Enzymes) are enzymes that degrade, modify, and/or create glycosidic bonds (Levasseur et al. 2013). They can be classified into families of structurally-

related catalytic and carbohydrate-binding modules (or functional domains). The classifications used by the CAZy database are incorporated into MycoCosm for comparative analyses.

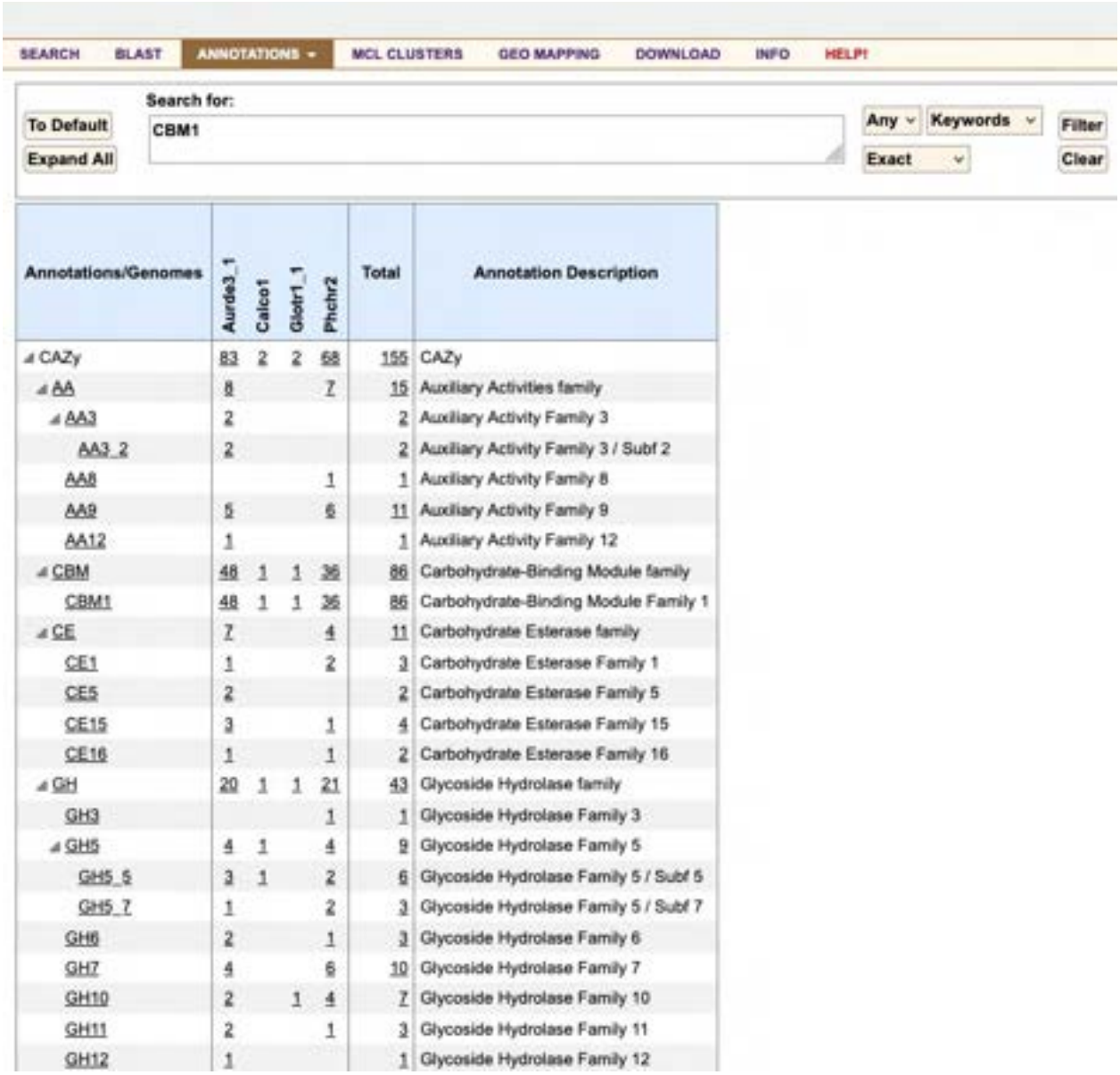
Click on the CAZYMES item under ANNOTATIONS in the Main menu.

Here you will see a table representation of the predicted CAZymes in each species. The organisms are labeled along the top by genome portal identifier (“portal ID”). The CAZymes are organized hierarchically by family and labeled along the sides: CAZy family identifier on the left, and family description on the right. The numbers in the table represent how many proteins from each organism’s gene catalog were annotated with a given CAZyme, with a total provided for each row. Notice that the CAZymes are hierarchically organized: you can see the total number of genes assigned to the top level enzyme category (e.g. “AA”). To view family (e.g. “AA1”, “AA2”) and subfamily (e.g. “AA1_1”, “AA1_2”) designations, click on the small arrow to the left of each category, or use the “Expand All” button at the top of the page.



If we read Levasseur et al. 2013, we know that the AA2 family consists of peroxidases that may degrade lignin. Browsing the table, we see that *P. chrysosporium* and *A. subglabra* possess 20 and 17 copies of AA2, whereas *G. trabeum* and *C. cornea* each possess only one copy of AA2. This might suggest that the former two are white rot fungi and the latter two brown rot fungi!

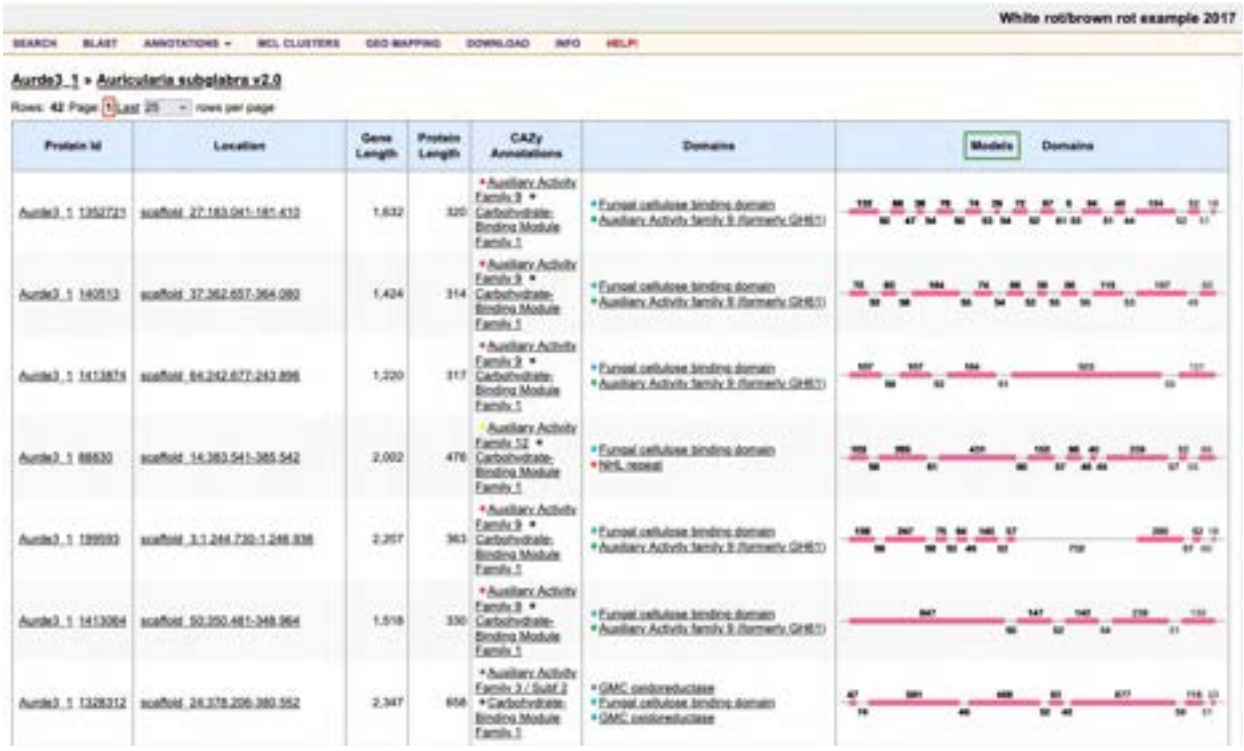
What about the carbohydrate binding motifs, CBM1? Let's say we don't want to scroll through the entire list of CAZymes. Type "CBM1" into the "CAZY terms" search box and click "Filter". This will limit the view to only those CAZymes that have a CBM1. Why do so many CAZymes besides CBM1 show up? Because CBM1 co-occurs on the same protein chain with many other CAZymes of diverse function. The numbers in the table will now show, for each CAZyme's row, the number of proteins that also have a CBM1.



Annotations/Genomes	Aurde3_1	Calco1	Glotr1_1	Phchr2	Total	Annotation Description
CAZy	83	2	2	68	155	CAZy
AA	8			7	15	Auxiliary Activities family
AA3	2				2	Auxiliary Activity Family 3
AA3_2	2				2	Auxiliary Activity Family 3 / Subf 2
AA8				1	1	Auxiliary Activity Family 8
AA9	5			6	11	Auxiliary Activity Family 9
AA12	1				1	Auxiliary Activity Family 12
CBM	48	1	1	36	86	Carbohydrate-Binding Module family
CBM1	48	1	1	36	86	Carbohydrate-Binding Module Family 1
CE	7			4	11	Carbohydrate Esterase family
CE1	1			2	3	Carbohydrate Esterase Family 1
CE5	2				2	Carbohydrate Esterase Family 5
CE15	3			1	4	Carbohydrate Esterase Family 15
CE16	1			1	2	Carbohydrate Esterase Family 16
GH	20	1	1	21	43	Glycoside Hydrolase family
GH3				1	1	Glycoside Hydrolase Family 3
GH5	4	1		4	9	Glycoside Hydrolase Family 5
GH5_5	3	1		2	6	Glycoside Hydrolase Family 5 / Subf 5
GH5_7	1			2	3	Glycoside Hydrolase Family 5 / Subf 7
GH6	2			1	3	Glycoside Hydrolase Family 6
GH7	4			6	10	Glycoside Hydrolase Family 7
GH10	2		1	4	7	Glycoside Hydrolase Family 10
GH11	2			1	3	Glycoside Hydrolase Family 11
GH12	1				1	Glycoside Hydrolase Family 12

Notice the abundance of CBM1-encoding genes in *P. chrysosporium* and *A. subglabra*, while *G. trabeum* and *C. cornea* have only a single CBM1-encoding gene each (co-occuring with GH5_5 and GH10 proteins). All of this indicates that we might be looking at two white-rot and two brown-rot fungi.

Click on the number (e.g., 48 for Aurde3_1) to see the CBM1-containing proteins of *A. subglabra* in more detail. Notice a variety of CAZymes co-occur with CBM1, including GH5 (various subfamilies), GH6, and many others.



As an exercise, repeat the same search with GH6, GH7, and also the AA9 family of lytic polysaccharide monoxygenases, which may oxidatively act on lignin (Levasseur et al. 2013). Do the presence/absence patterns of these genes indicate the same conclusions about these fungi's mode of decay as we found with AA2 and CBM1? Is it a strict dichotomy, or are there some grey areas in the distribution of these genes?

(Answer: *P. chrysosporium* and *A. subglabra* induce white rot wood decay; *G. trabeum* and *C. cornea* brown rot. Notice that brown rot *G. trabeum* has a few AA9 genes, however, indicating that these genes may play a role in brown rot, not just white rot, where AA9s are expanded.)

Cluster page

Now that we have an idea which fungus uses which decay mode, let's ask the reverse question: what are the genes present in one lifestyle, and absent in the other? To do this, click the 'MCL CLUSTERS' item of the Main menu. Here you will see the results of protein sequence clustering by the TRIBE-MCL method (Enright et al. 2002). As with the CAZy browser, the columns indicate organisms. Each row indicates a single protein cluster (analogous to a protein family),

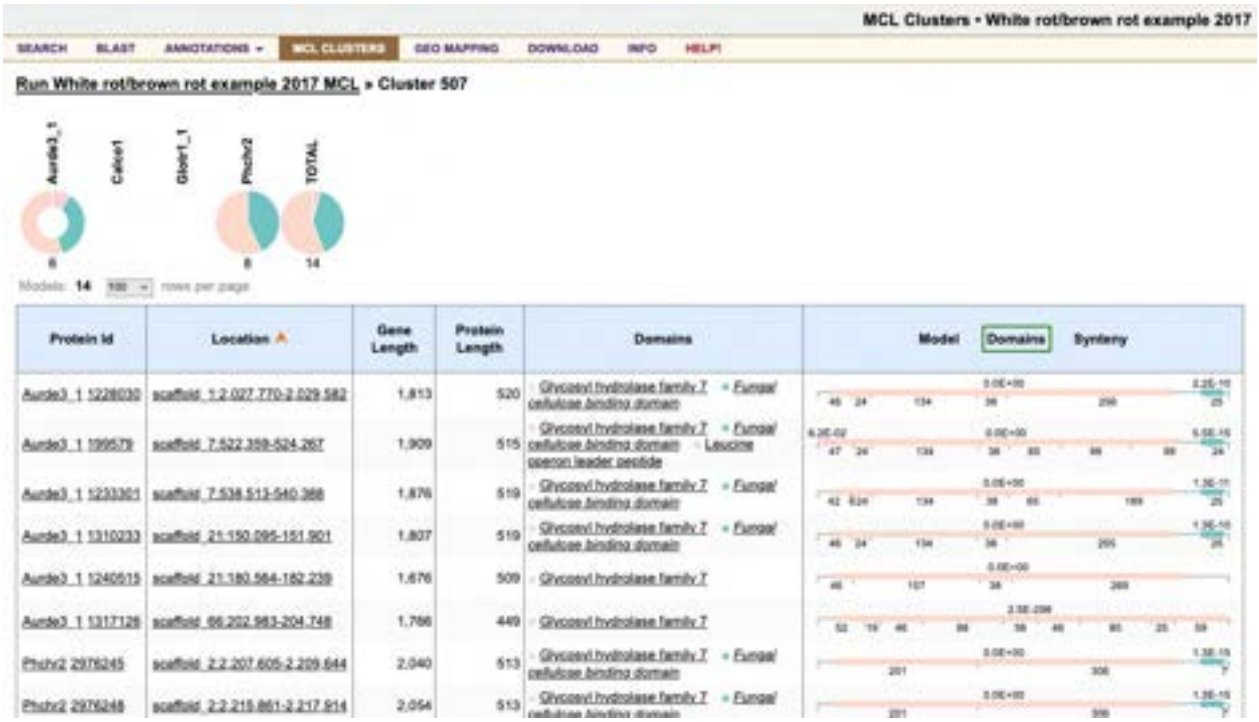
where the number corresponds to the proteins from each organism in the cluster. The donut charts provide visualizations for the relative number of proteins and functional content provided by each organism in the cluster. See the HELP Menu for a full explanation of the cluster page.

Notice that under each organism label is a button “any” that can be used to filter clusters by the number of proteins that organism contributes to a cluster, and thus limit which clusters are shown. As an experiment, set the white rot fungi (Aurde3_1 and Phchr2) to “1+” and the brown rot fungi (Calco1 and Glotr1_1) to “=0”. Doing so will return only those clusters which are present in Aurde3_1/Phchr2 and absent in Calco1/Glotr1_1.

150 clusters fit these criteria. These clusters might include genes important to the white rot decay mode, because they are present in white rot fungi and absent in brown rot fungi. However, some of these clusters might have no functional connection to wood decay mode - they are present/absent from the respective kinds of wood decay fungi merely by chance. These clusters nevertheless represent candidates for further analysis of possible connections to decay mode.

How does one begin interpreting the results? To help with this, each cluster row shows the Pfam domains (https://www.ebi.ac.uk/interpro/entry/pfam) that are found in that cluster. Notice that the third row has a “Peroxidase” (PF00141) domain. Notice that the numbers are very close to what we found for the AA2 class II peroxidases in the CAZy browser. It turns out that PF00141 is a superfamily that includes the AA2 enzymes, but it is important to note that not all members of PF00141 can degrade lignin - some have other functions.

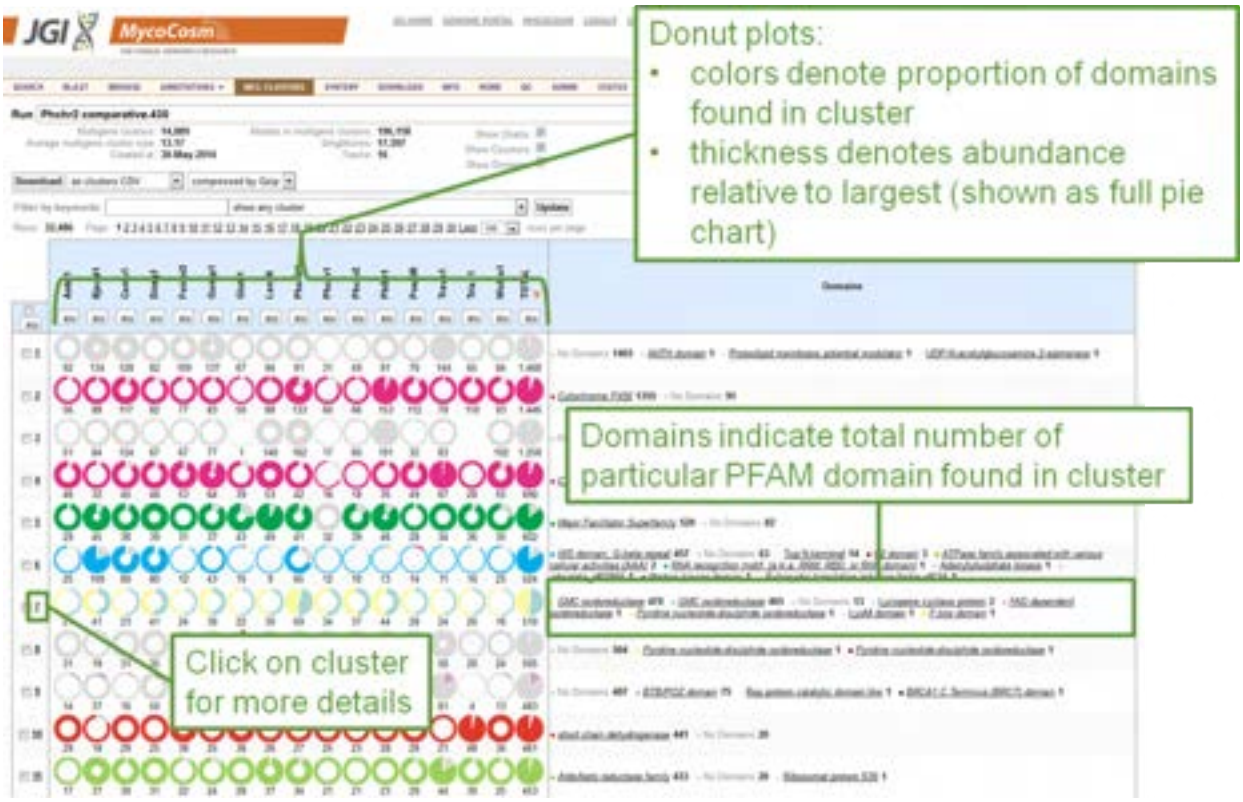
Scroll through the rest of the 150 clusters and you will see domains such as “Glycosyl hydrolase family 7” and “Fungal cellulose binding domain” in cluster 507, which roughly overlap with the GH7 and CBM1 families from the CAZy exercise. Click the “507” to explore that cluster in more detail. On the cluster detail page, a table is presented with one protein per row. Click the “Domains” view on the rightmost column to see the domain structure of each protein. Notice that all of the proteins have the GH7 domain, and that most (but not all) have a single CBM1 motif at the C-terminus.



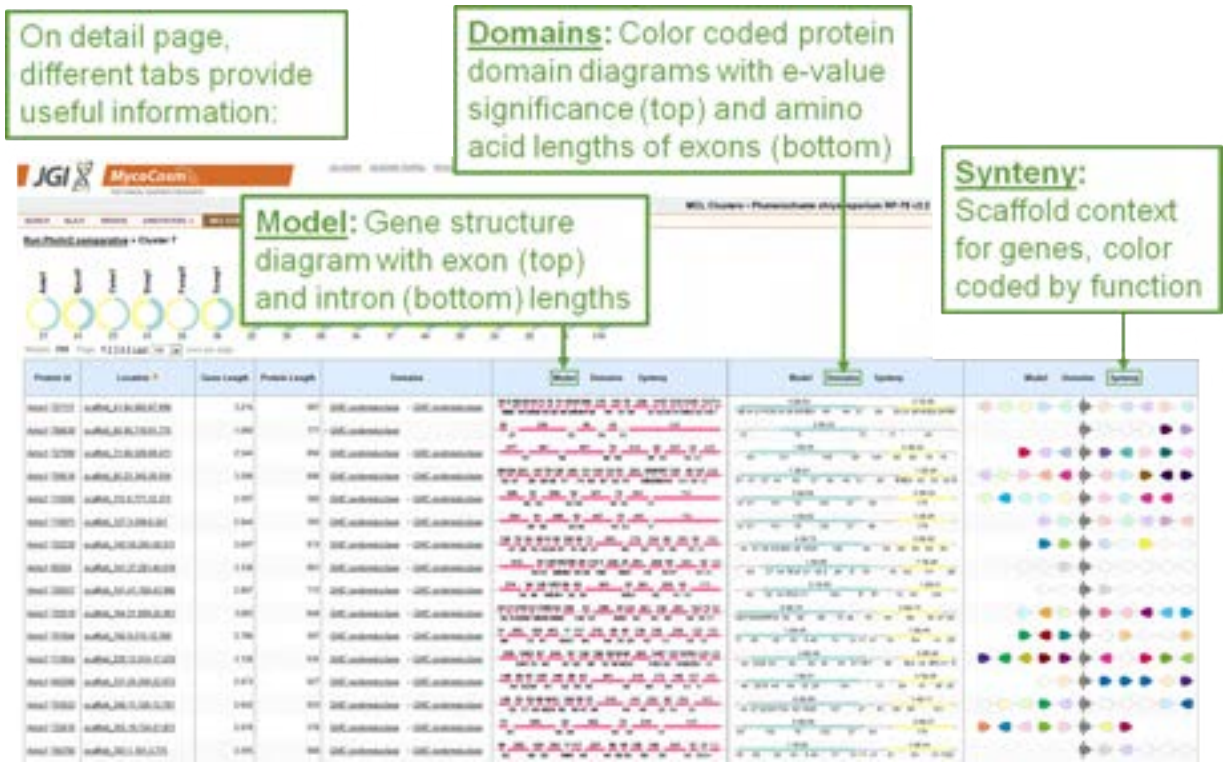
Let’s look at what other proteins have the CBM1 carbohydrate-binding motifs in them. Returning to the cluster run page (click the “MCL CLUSTERS” tab). Enter the phrase “fungal cellulose binding domain” (be sure to include the quotes) into the “filter by keywords” field and select “Update”. This returns some 26 clusters, all of which have the Pfam domain CBM_1 (PF00734). We see that CBM1 motifs occur in a wide array of domain combinations: often with GMC oxidoreductases, AA9 lytic polysaccharide monoxygenases (formerly Glycosyl hydrolase family 61), and many hydrolytic enzymes such as GH5, GH6, and GH7. Notice that while these proteins typically are found in expanded copy number in the white rot fungi (Aurde3_1 and Phchr2) they are sometimes found, albeit in lower copy number, in the brown rot fungi (Calco1 and Glotr1_1).

As additional exercises you can (a) search for gene families absent in both white rot fungi; (b) find gene families absent in white rot but present in both brown rot fungi and look at functional domains associated with these families; (c) check if any of these domains are present only in brown rot fungi by resetting filters back to “any” and searching for names of these domains.

A summary of tools available in MCL clustering are shown below:



Clicking in Cluster number provides additional tools as shown below:



References:

- Floudas, D., Binder, M., Riley, R., Barry, K., Blanchette, R. A., Henrissat, B., Martinez, A. T., Otillar, R., Spatafora, J. W., Yadav, J. S., Aerts, A., Benoit, I., Boyd, A., Carlson, A., Copeland, A., Coutinho, P. M., de Vries, R. P., Ferreira, P., Findley, K., Foster, B., Gaskell, J., Glotzer, D., Gorecki, P., Heitman, J., Hesse, C., Hori, C., Igarashi, K., Jurgens, J. A., Kallen, N., Kersten, P., Kohler, A., Kues, U., Kumar, T. K., Kuo, A., LaButti, K., Larrondo, L. F., Lindquist, E., Ling, A., Lombard, V., Lucas, S., Lundell, T., Martin, R., McLaughlin, D. J., Morgenstern, I., Morin, E., Murat, C., Nagy, L. G., Nolan, M., Ohm, R. A., Patyshakuliyeva, A., Rokas, A., Ruiz- Duenas, F. J., Sabat, G., Salamov, A., Samejima, M., Schmutz, J., Slot, J. C., St John, F., Stenlid, J., Sun, H., Sun,
  S., Syed, K., Tsang, A., Wiebenga, A., Young, D., Pisabarro, A., Eastwood, D. C., Martin, F., Cullen, D., Grigoriev, I. V., & Hibbett, D. S. 2012. The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science, 336(6089): 1715-1719.
- Riley, R., Salamov, A. A., Brown, D. W., Nagy, L. G., Floudas, D., Held, B. W., Levasseur, A., Lombard, V.,
  Morin, E., Otillar, R., Lindquist, E. A., Sun, H., LaButti, K. M., Schmutz, J., Jabbour, D., Luo, H., Baker, S. E., Pisabarro, A. G., Walton, J. D., Blanchette, R. A., Henrissat, B., Martin, F., Cullen, D., Hibbett, D. S., & Grigoriev, I. V. 2014. Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white- rot/brown-rot paradigm for wood decay fungi. Proc Natl Acad Sci U S A, 111(27): 9923-9928.
- Levasseur, A., Drula, E., Lombard, V., Coutinho, P. M., & Henrissat, B. 2013. Expansion of the enzymatic repertoire of the CAZy database to integrate auxiliary redox enzymes. Biotechnol Biofuels, 6(1): 41.
- Enright, A. J., Van Dongen, S., & Ouzounis, C. A. 2002. An efficient algorithm for large-scale detection of protein families. Nucleic Acids Res, 30(7): 1575-1584.

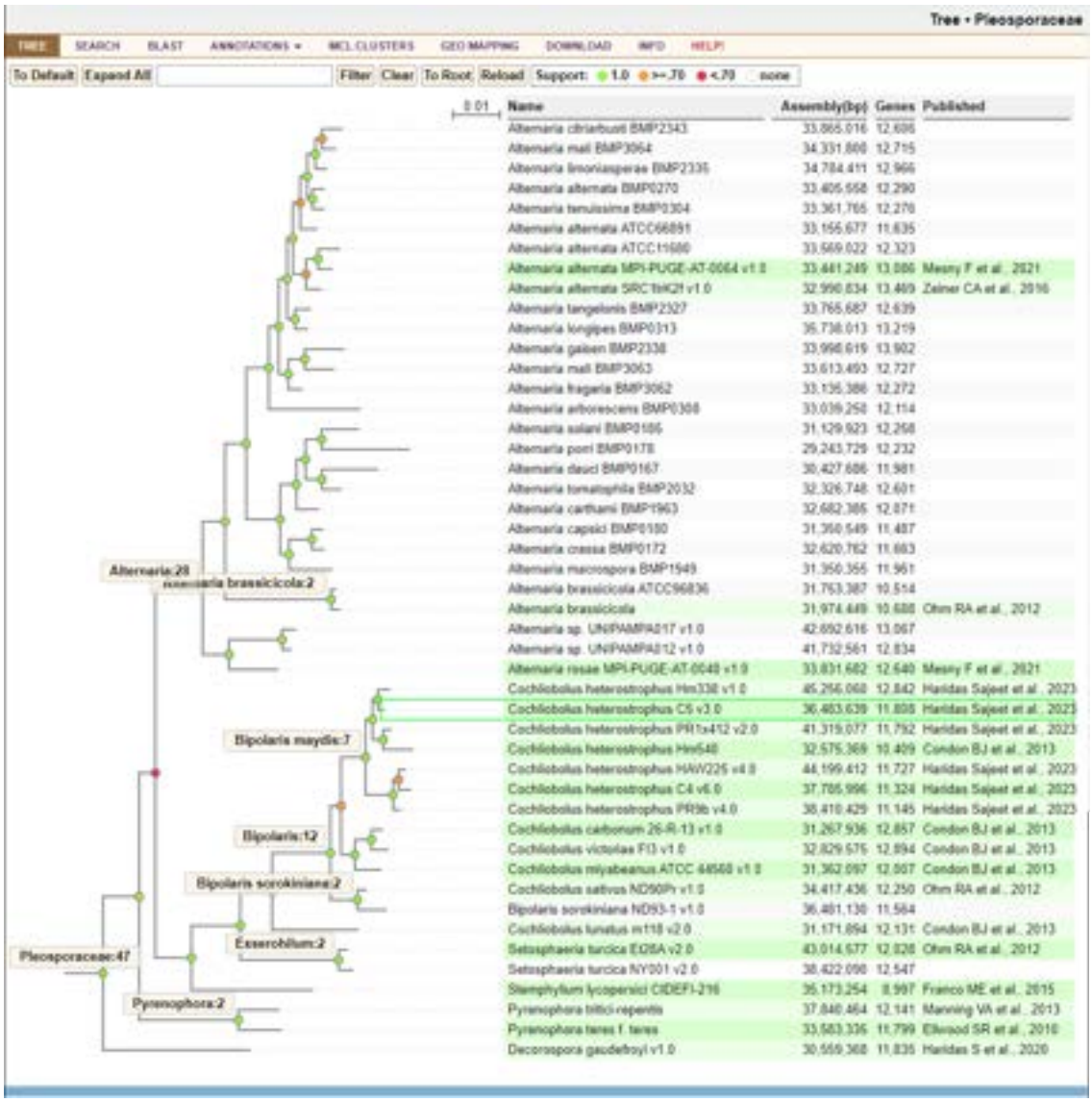
MycoCosm: Synteny Tutorial

<u>Objective</u>: Explore genome synteny of *Cochliobolus heterostrophus* C5 with related genomes using the Pleosporaceae group page and the *Cochliobolus heterostrophus* C5 genome portal.

The SYNTENY tab is used for pairwise whole genome comparisons, enabling visual comparative analysis of complete genome assemblies at different levels of resolution. Since this uses one genome as the comparator, the SYNTENY tab is only available on single genome portals (i.e., absent from groups).

First, go to the Pleosporaceae group page at https://mycocosm.jgi.doe.gov/Pleosporaceae

Click on the TREE tab and locate *Cochliobolus heterostrophus* C5 in the tree.



Note the green selection box while mousing over the tree. Left-clicking will collapse and expand the selection box. Shift+clicking will isolate the selection in a new view. To restore the default view, click the TREE tab (the browser back button does not work on the tree page). Click on "*Cochliobolus heterostrophus* C5" to go to the organism genome portal. Ideally, you should do this in another tab or window so that you can follow the exercises below keeping the phylogenetic placement of this organism in mind.

Click on the SYNTENY tab in the organism portal (*Cochliobolus heterostrophus* C5).

Genomic synteny is displayed in three collapsible panels in the Synteny Browser:

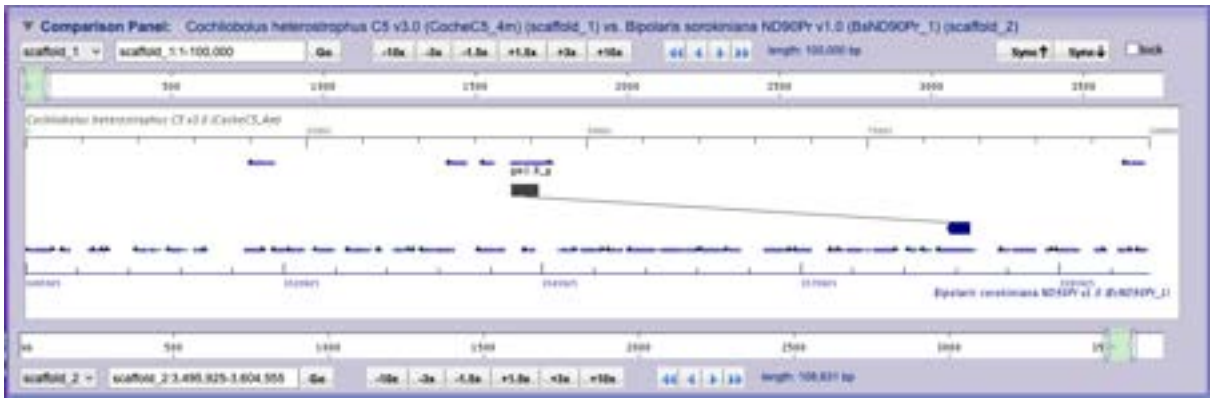
A. the Genome Panel
B. the Chromosome Panel
C. the Comparison Panel.

The compared genome can be changed from the dropdown menu and clicking "Submit".

A. The Genome Panel depicts alignment density for all scaffolds in the reference genome against all chromosomes in the compared genome. Here, alignment density is defined for a region in the reference genome as the number of syntenic regions in the compared genome. Darker regions in the image have higher density of coverage. Clicking on a particular scaffold selects that for the Chromosome and Comparison panels below.

B. The Chromosome Panel shows all of the alignments in the compared genome to a particular interval on a single chromosome in the reference genome. Synteny is depicted as "blocks" along the reference-genome interval. Each block represents an alignment of two sequences, where the position of the block indicates the alignment's location on the reference genome and the color of the block indicates the chromosome where the match is found on the compared genome. Click on Legend (green arrow) to reveal the color-coding schema. The blocks appear stacked on top of each other when a fragment of the reference genome has synteny with multiple locations in the compared genome. The navigation buttons along with the chromosome slider (red arrow) allow for zooming and panning along the interval of the reference chromosome. A protein model (blue arrow) leads to the protein page, which shows annotations and a link to the genome browser.

C. The Comparison Panel zooms further to depict synteny between a specific interval on the reference genome and a specific interval on the compared genome. In this view, each aligned region is depicted as a pair of blocks, one along the reference chromosome (grey) and one along the compared chromosomes (colored), connected by a line. Also displayed in the Comparison Panel are gene model tracks (if available) for the reference and compared chromosomes.



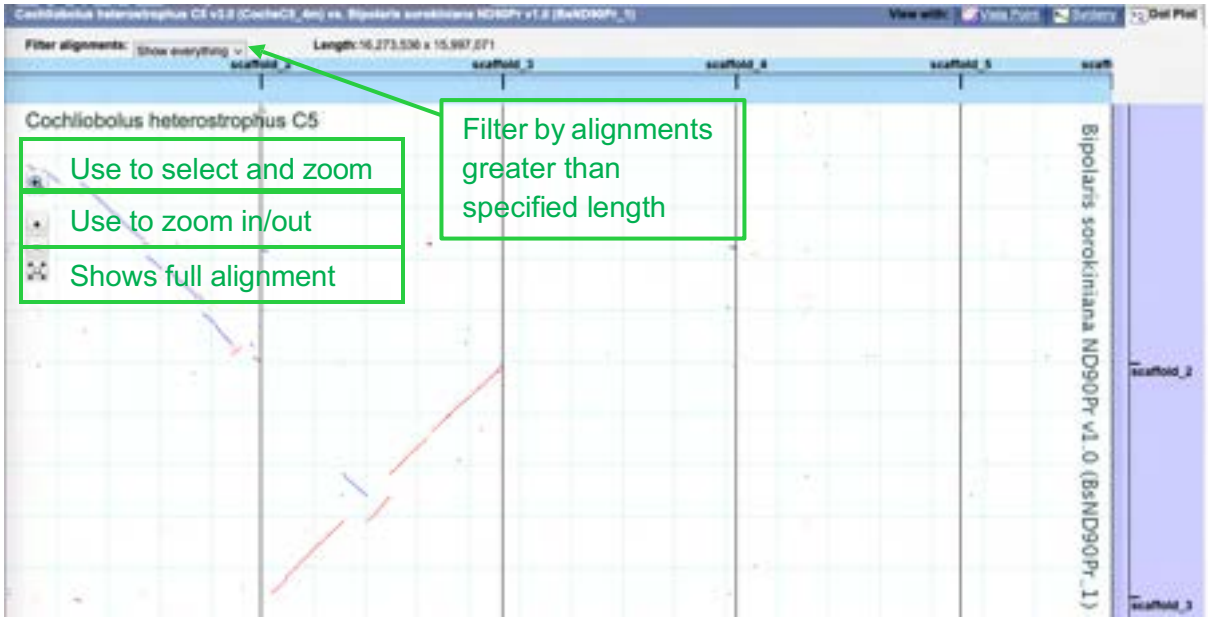
Syntenic blocks and gene models are both interactive, as described above for the Chromosome Panel. Navigation controls allow the user to switch chromosomes, zoom and pan independently over the reference and compared genomes.

The SYNTENY page also allows whole genome pairwise comparison and comparison of one-to-many using the ‘Dot Plot’ and ‘Vista Point’ views respectively.

‘Dot Plot’ is an interactive tool that enables users to look at the DNA conservation between two genome assemblies at different levels of resolution and across multiple chromosomes/scaffolds.

In the main view window, DNA coordinates of the reference genome are presented on the X axis, and DNA coordinates of the compared genome are presented on the Y axis. All chromosomes or scaffolds are concatenated together, usually in a descending order by size. The diagonal lines in the image display the homologous regions between the two genomes. If the line is blue, the regions are on the same strand. If the line is red, the regions are on opposite strands. The grid in black lines indicates scaffold/chromosome boundaries. Use the

toolbar on the left to zoom or select specific regions on the plot. The map can also be navigated using click+drag similar to google maps. A cutoff control above the main window allows you to filter alignments to show only syntenic regions greater than a specified length.



'Dot Plot' hides the genome portal navigation bar. You can click the "Synteny" view to restore it.

'Vista Point' shows multiple genome alignment using "peaks and valleys" graph as seen on the genome browser. Regions of high conservation are colored according to the annotation as exons (dark blue), UTRs (light blue) or non-coding (pink). The thresholds that determine what gets colored, as well as minimum and maximum percentage bounds can be adjusted by the user. The order of the curves and the zoom can be adjusted using drag-and-drop and click-and-drag respectively.

Exercises:

1. Study the phylogenetic tree of the Pleosporaceae.
2. Use the SYNTENY tab in the *Cochliobolus heterostrophus* C5 genome portal and compare it to the genome of *Cochliobolus heterostrophus* C4. Compare the *Cochliobolus heterostrophus* C5 genome with other genomes of increasing phylogenetic distance like *Cochliobolus sativus*, *Setosphaeria turcica* and *Alternaria brassicicola*. Increase the viewed area by dragging the slider to cover a greater percentage of the scaffold. Note how increasing the cutoff from the default (50bp) can remove spurious alignments often caused by repeats.
3. Use the 'Dot Plot' view to study the high congruence between the two *Cochliobolus heterostrophus* assemblies. Compare the *Cochliobolus heterostrophus* C5 genome with other genomes of increasing phylogenetic distance as above. Note the breakdown of large scale synteny with increasing phylogenetic distance into mesosynteny as described by Ohm et al. (2012). In mesosynteny, genes are conserved within homologous chromosomes (scaffolds), but with randomized orders and orientations. Mesosynteny becomes more pronounced moving further phylogenetically to *Stagonospora nodorum* (Phaeosphaeriaceae). Ohm et al. showed that this type of genome evolution can be explained by repeated intra-chromosomal inversions.

Reference:

- Ohm RA, Feau N, Henrissat B, Schoch CL, Horwitz BA, et al. (2012) Diverse Lifestyles and Strategies of Plant Pathogenesis Encoded in the Genomes of Eighteen Dothideomycetes Fungi. PLOS Pathogens 8(12): e1003037.

wellcome connecting science

Exploring protein domains and clusters across species in Ensembl and MycoCosm

Links to be clicked shown in blue, text to be entered shown in red.

We're going to use the HMMER tool, embedded in Ensembl Fungi, with the Ensembl Fungi species as reference proteomes and standard search parameters. You can use the full tool here: https://www.ebi.ac.uk/Tools/hmmer/search/phmmer if you want to work with other species and set your own thresholds. This tool uses protein sequences as input and finds domains within them and maps against all species, like a more powerful, domain oriented BLAST. We'll then explore this at a finer scale looking at a handful of closely related species in the MycoCosm Cluster tool.

In the BioMart exercise, we found genes that were associated with reduced virulence in *Fusarium solani*, and which did not have an orthologue in *F. oxysporum*. We're going to explore one of these genes in more detail: *PEP2* or NechaG73962.

a) Search *Fusarium solani* for NechaG73962 at fungi.ensembl.org. Navigate to the Transcript tab and either export the protein sequence in FASTA format or highlight and copy it.

b) Using the link in the Ensembl Fungi header navigate to the HMMER tool. Paste in your sequence and click Submit.
   I. What is the PFAM domain identified in this sequence?
   II. Hover over the domain image, what is the length of the aligned region with our submitted protein sequence?

c) In the 'Significant Query Matches' table at the bottom of the page, click on the black Customise button and add Phylum to the table.
   I. To which phylum do the top hits belong to?
   II. Which species is reported as the top hit? Is this the same as *Fusarium solani* (think about the reproductive cycle...)?

d) We can explore the taxonomy more broadly elsewhere. Click on the Taxonomy tab just above the domain image.
   I. How many hits were there in the *Basidiomycota*?
   II. Click to expand the *Agaricomycetes* node by clicking on the arrow, and then the *Agaricales*. Which families are represented?

   NOTE: You may need to click on the node name (e.g., *Agaricales*), to reposition the image.

e) Let's explore this protein domain further using the JGI MycoCosm cluster tool. This will enable us to compare the presence genes containing this domain across a closely related group. Navigate to mycocosm.jgi.doe.gov on your browser and search for *Fusarium solani.* Select *Fusarium solani* FSSC 5 v1.0, then click on the MCL clusters option at the top of the page. Search for the protein domain we identified, SnoaL_4.
   I. For the first cluster, 4,213, which species is missing any hits?

II. Click on the link for the domain (4213) to explore in more detail. We can see from the donut plots that only 4 species contain this SnoaL-like domain.
III. Which species have the most similar protein lengths, and contain the SnoaL-like domain?

f) Click on Synteny in the final column.
I. Are the clusters around this conserved between these species? Click on them to highlight the occurrence in the image.
II. Which other species has the most similar synteny to *Fusarium solani* (Fusso1) protein 495533? Why do you think this is?

Answers

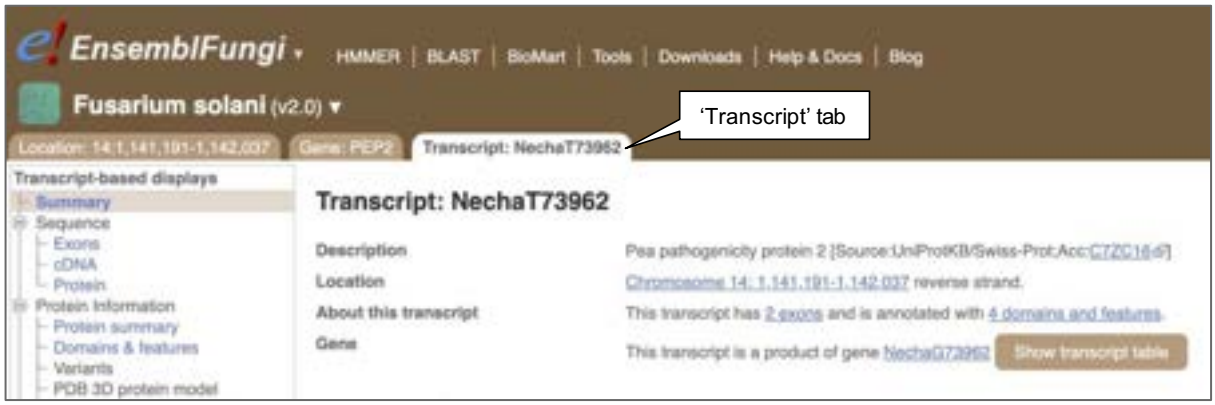
We're going to use the HMMER tool, embedded in Ensembl Fungi, with the Ensembl Fungi species as reference proteomes and standard search parameters. You can use the full tool here: https://www.ebi.ac.uk/Tools/hmmer/search/phmmer if you want to work with other species and set your own thresholds. This tool uses protein sequences as input and finds domains within them and maps against all species, like a more powerful, domain oriented BLAST. We'll then explore this at a finer scale looking at a handful of closely related species in the MycoCosm Cluster tool.

In the BioMart demo, we found genes that were associated with reduced virulence in *Fusarium solani*, and which did not have an orthologue in *F. oxysporum*. We're going to explore one of these genes in more detail: *PEP2* or NechaG73962.

a) Search *Fusarium solani* for NechaG73962 at fungi.ensembl.org. Navigate to the Transcript tab and either export the protein sequence in FASTA format, or highlight and copy it.

Answer: Go to fungi.ensembl.org. From the homepage select *Fusarium solani* from the drop-down list and type in NechaG73962. Hit Go.

Click on the gene name hyperlink on the results page, this will take you to the gene tab. Click on the transcript tab Transcript: NechaT73962 to go to the transcript tab.

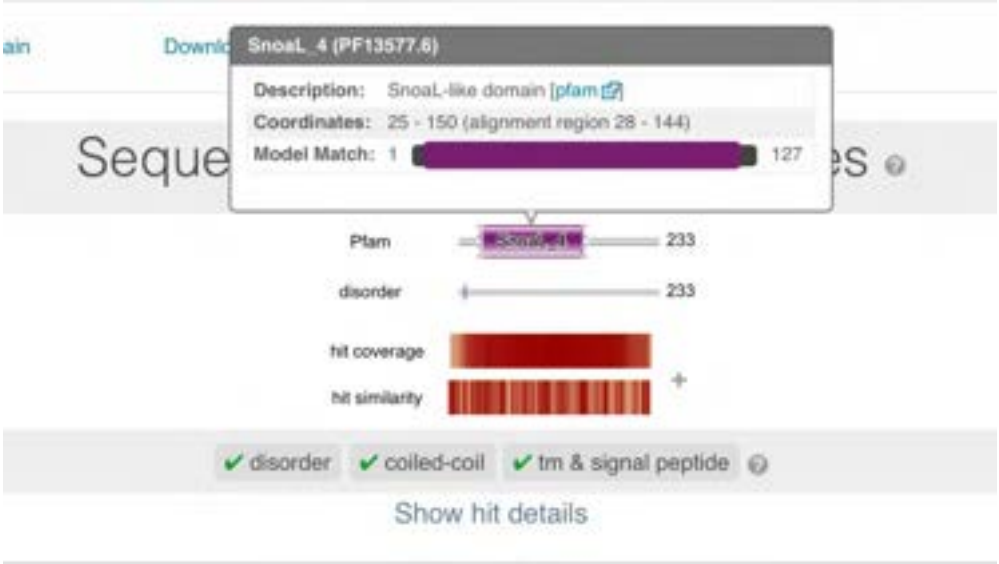


On the left-hand navigation panel there is a link for Protein under the Sequence header. Highlight the protein sequence and copy it.

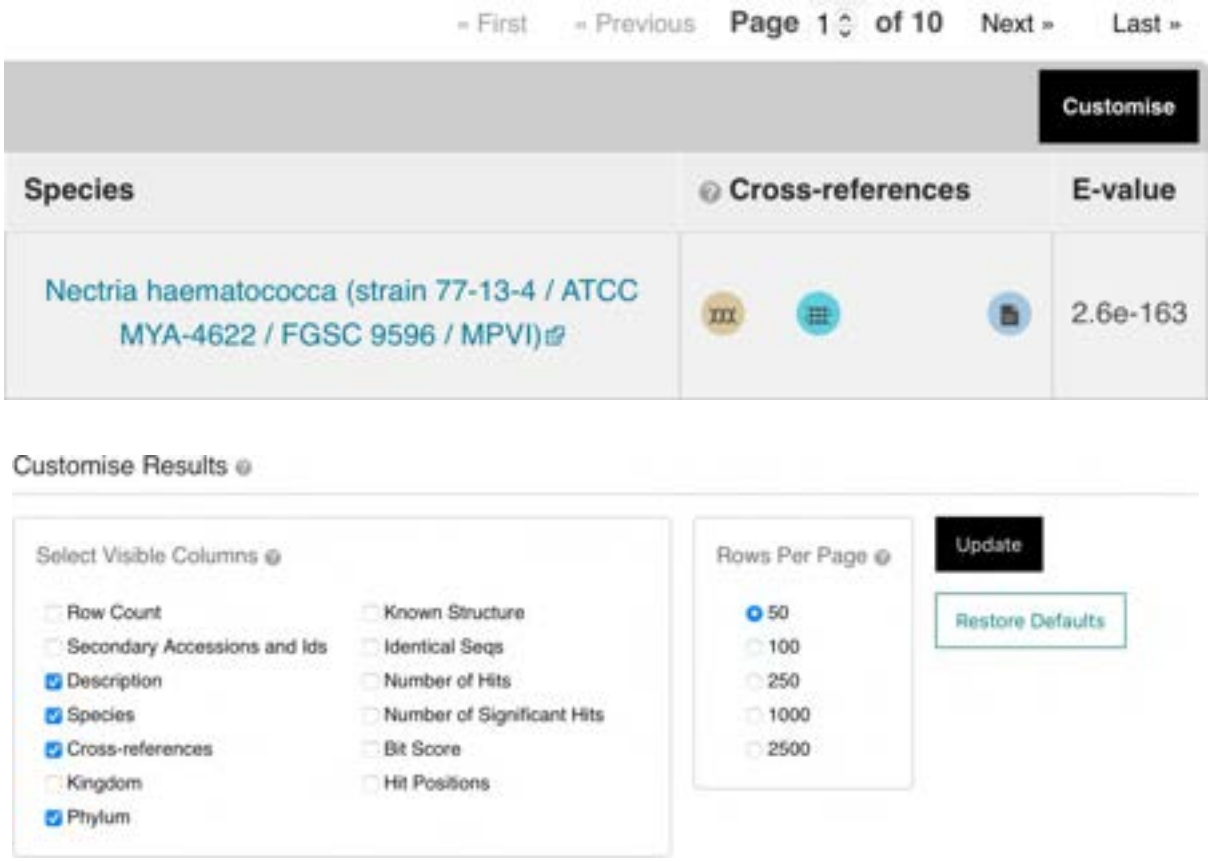
Using the link in the Ensembl Fungi header navigate to the HMMER tool. Paste in your sequence and click Submit.

III. What is the PFAM domain identified in this sequence?
IV. Hover over the domain image, what is the length of the aligned region with our submitted protein sequence?

Answers: The image shown in the centre middle of the page shows the domain (or domains) matched in your sequence. Hovering over the domain will give you some summary information, including the length of the overlapping sequence.



b) In the Significant Query Matches table at the bottom of the page, click on the black Customise button and add ‘Phylum’ to the table.

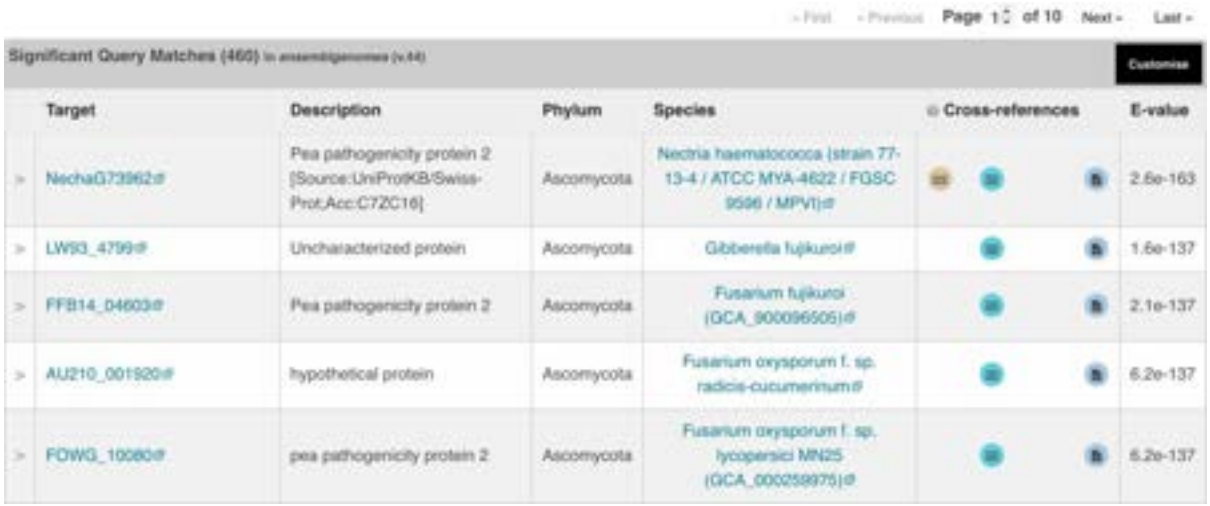


I. To which Phylum do the top hits belong to?

Answer: We can see that the column of the first hits are all listed as 'Ascomycota'

II. Which species is reported as the top hit? Is this the same as *Fusarium solani* (think about the reproductive cycle...)?

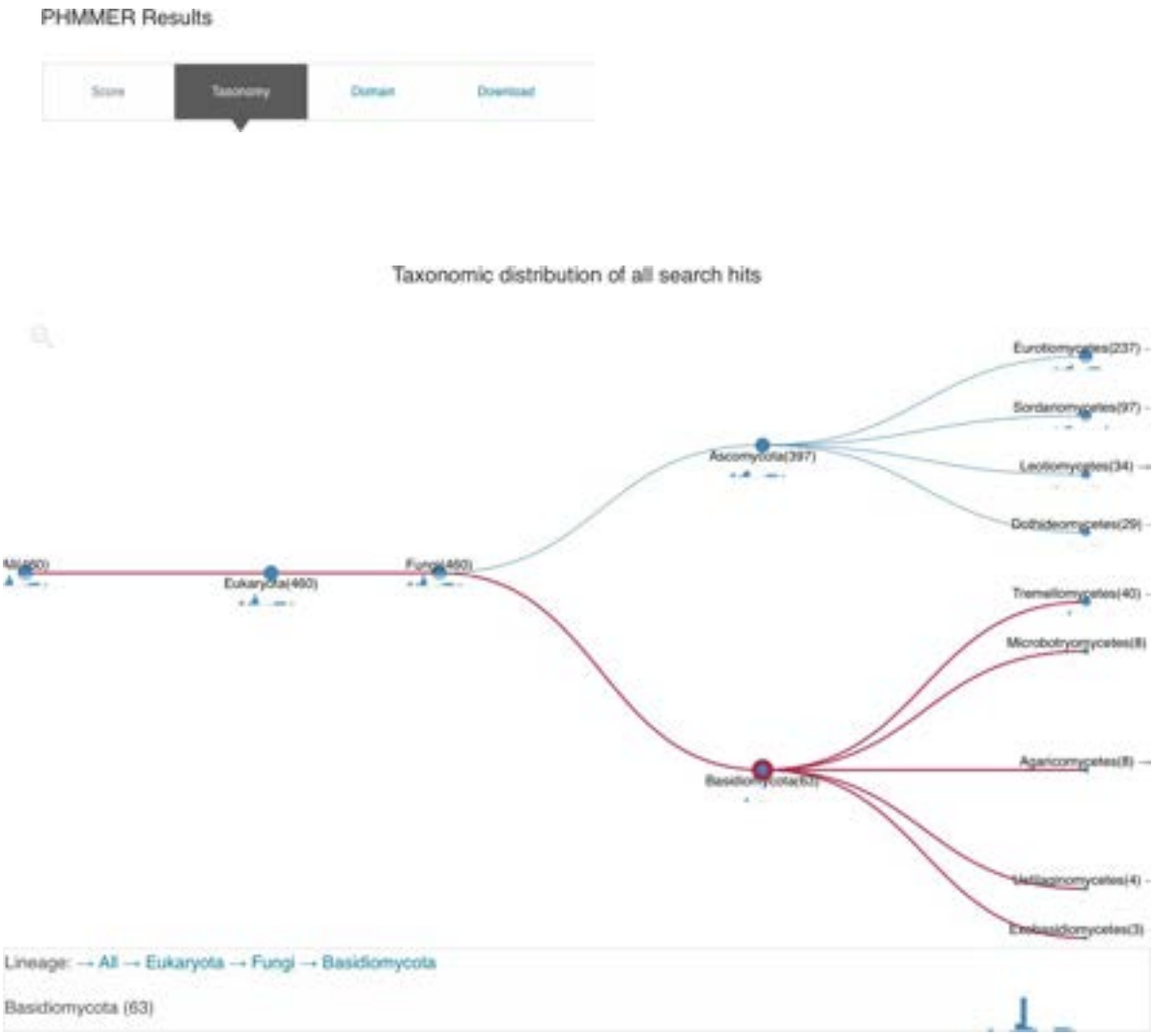
Answer: The sexual form (teleomorph) of *Fusarium solani* (the anamorph) is *Nectria haematococca*. Note that *Fusarium vanettenii* is another name for *Nectria haematococca.*



c) We can explore the taxonomy more broadly elsewhere. Click on the Taxonomy tab just above the domain image.

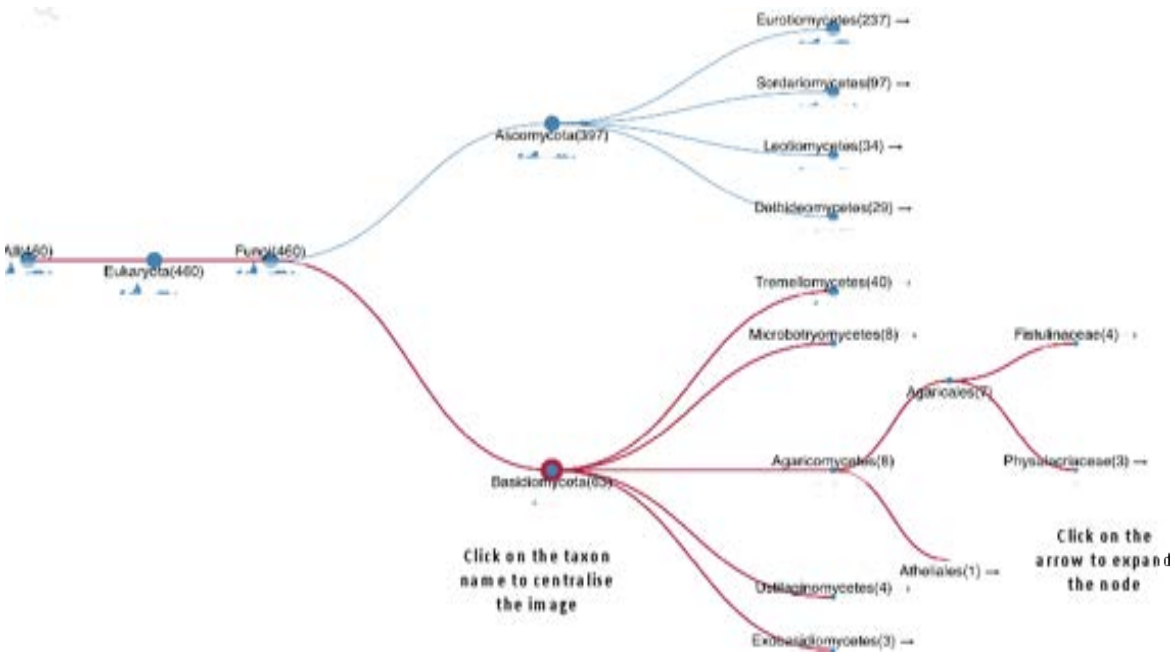
I. How many hits were there in the Basdiomycota?

Answer: We can see from the number in the parentheses that there are 63 hits.

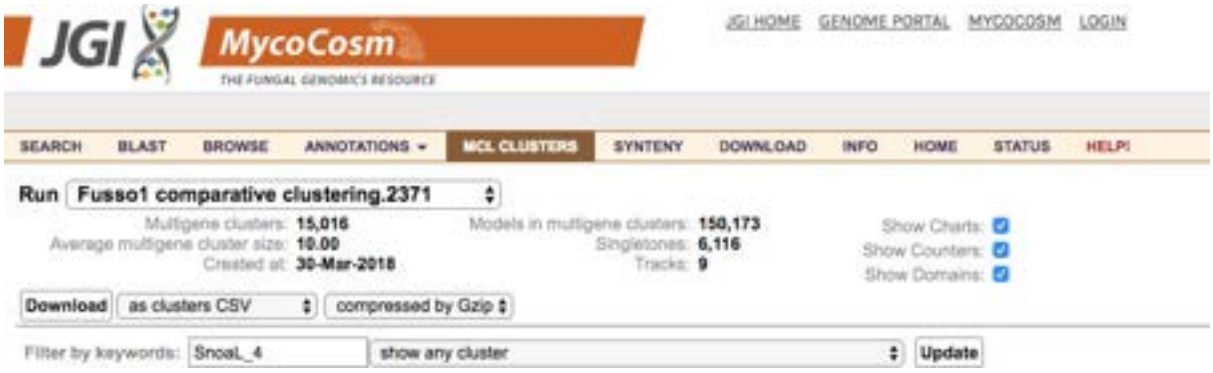


II. Click to expand the Agaricomycetes node by clicking on the arrow, and then the Agaricales. Which families are represented?

Answer: Fistulinaceae and Physalacriaceae families are shown here with 4 and 3 members respectively.
NOTE: You may need to click on the node name (e.g. Agaricales), to reposition the image.



d) Let's explore this protein domain further using the JGI MycoCosm cluster tool. This will enable us to compare the presence genes containing this domain across a closely related group. Navigate to mycocosm.jgi.doe.gov and search for *Fusarium solani.* Select Fusarium solani FSSC 5 v1.0, then click on the MCL clusters option at the top of the page. Search for the protein domain we identified, SnoaL_4.



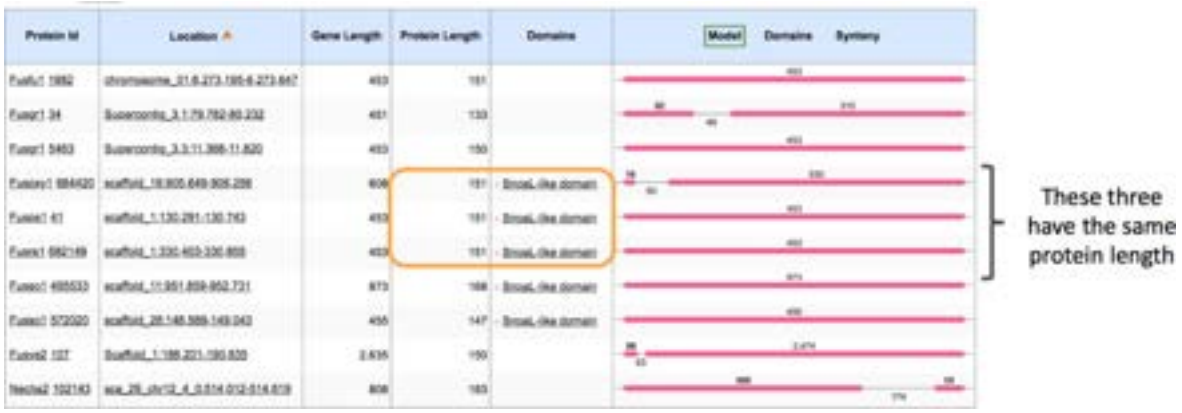
I. For the first cluster, 4,213, which species is missing any hits?

Answer: There is no 'donut' in the first row for the species Fusox2. Hover over the name or look at the list below the table to see what this species/assembly full name is, it is *Fusarium oxysporum* f. sp. lycopersici 4287 v2 ExternalModels.

II. Click on the link for the domain (4213) to explore in more detail. We can see from the donut plots that only 4 species contain this SnoaL-like domain.

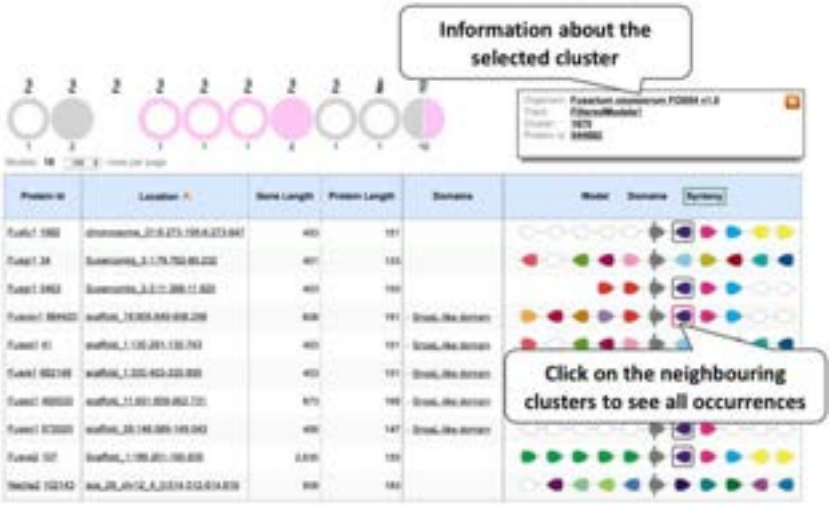
Answer: The pink colour corresponds to the SnoaL-like domain.

III. Which species have the most similar protein lengths, and contain the SnoaL-like domain?



e) Click on Synteny in the final column.

I. Are the clusters around this conserved between these species? Click on them to highlight the occurrence in the image.



II. Which other species has the most similar synteny to *Fusarium solani* (Fusso1) protein 495533? Why do you think this is?

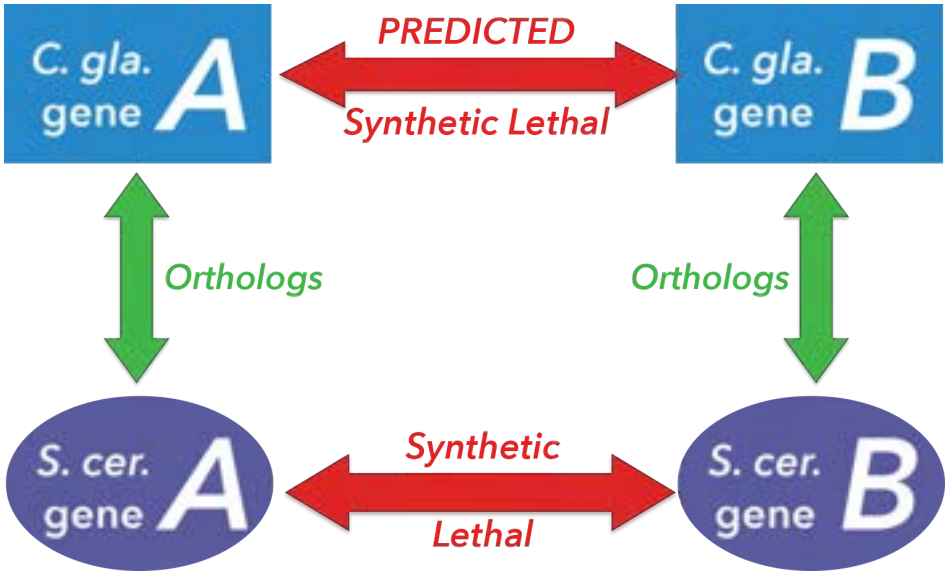
Answer: *Nectria haematococca* v2.0 FilteredModels1. We know this to be the sexual form of *F. solani* so this is expected.

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.

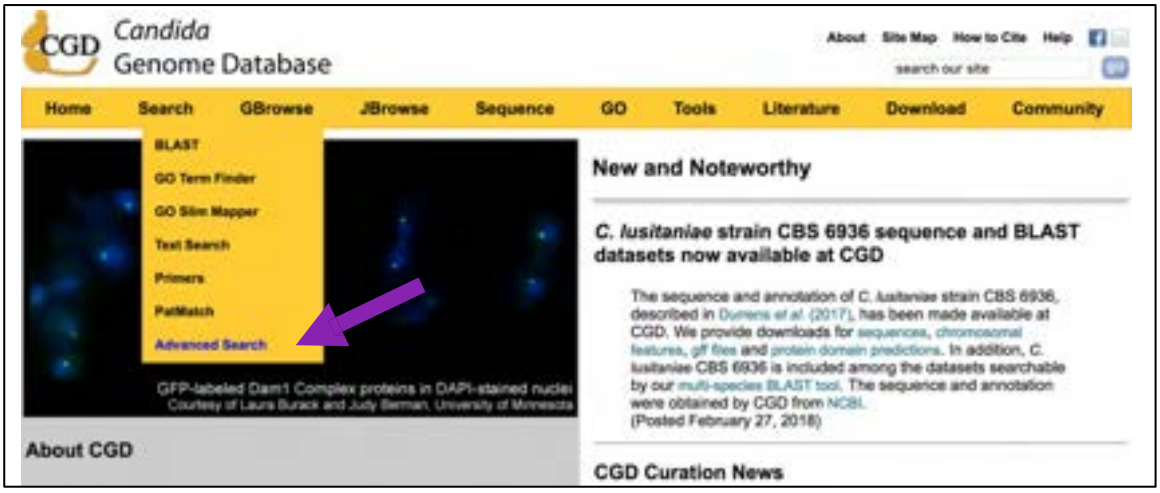
Genetic interactions such as synthetic lethality are richly documented for the budding yeast *S. cerevisiae*, but not as much for many other fungal species. By examining known genetic interactions in *S. cerevisiae*, we can predict synthetic lethal relationships in *C. glabrata* and other fungal pathogens.



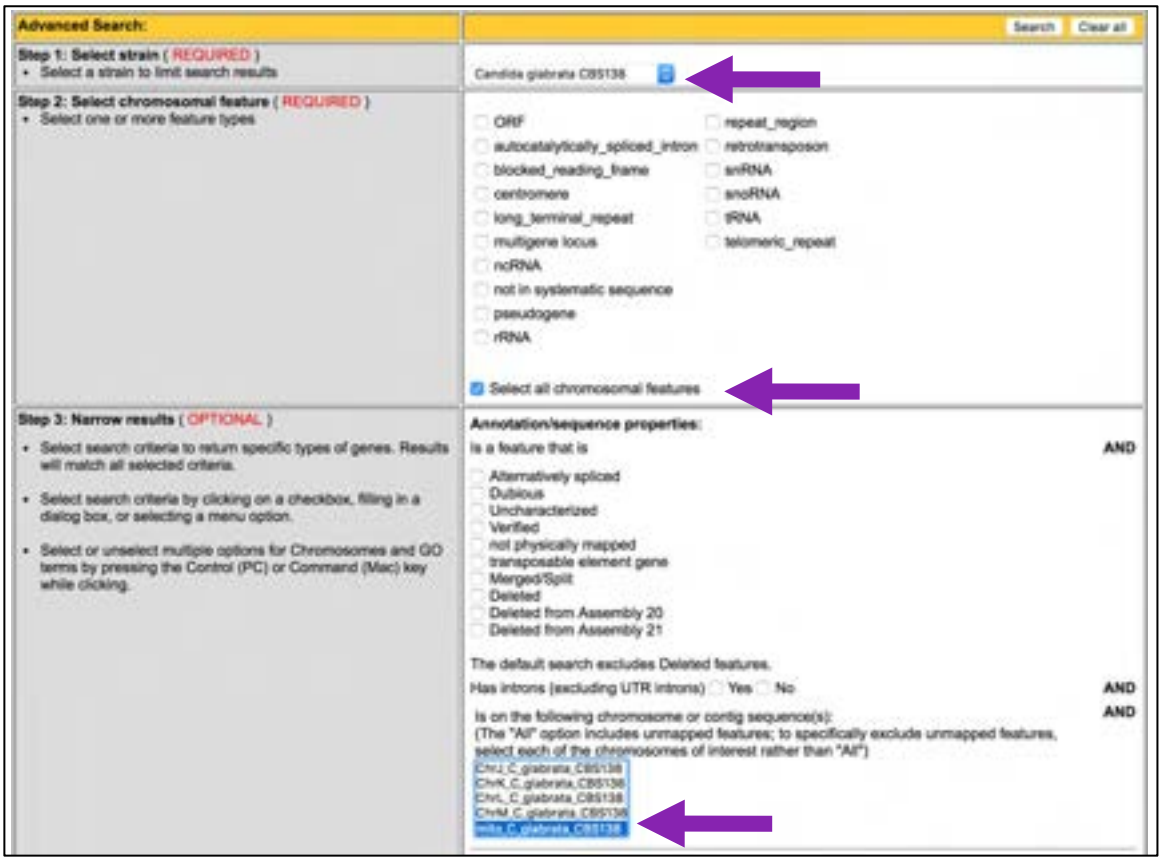
If conserved, these synthetic lethal interactions may reveal future antifungal targets for use against azole-resistant strains in the clinic. Using known synthetic lethal interactions in the *S. cerevisiae* genome, predict potentially conserved synthetic lethal interactions for mitochondrial genes in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

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

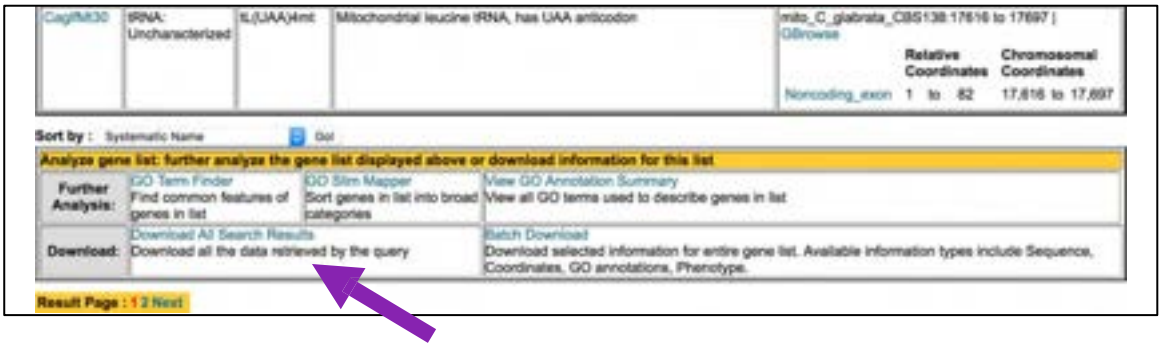
- On the CGD homepage (**http://www.candidagenome.org**), open the Search tab in the yellow toolbar and select **Advanced Search**.



- 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.

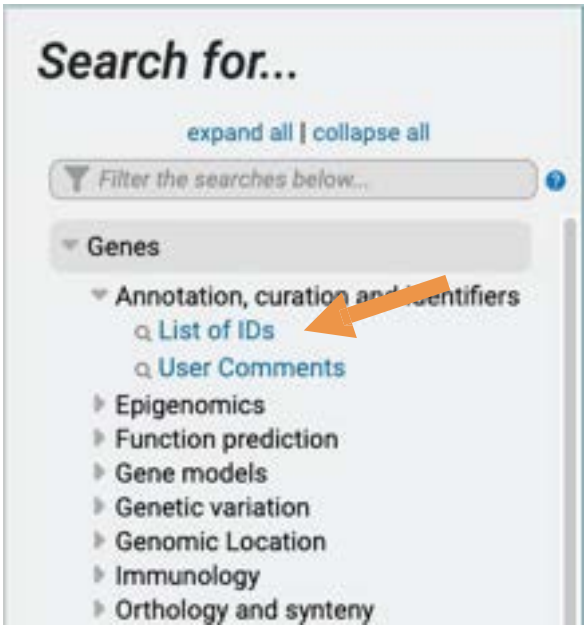


- Click on "Search" (bottom left). A results page will follow, listing out 37 features in the *C. glabrata* mitochondrial genome.
- Scroll to the bottom of the page and click on the "**Download All Search Results**" link. The results will download in an Excel sheet.

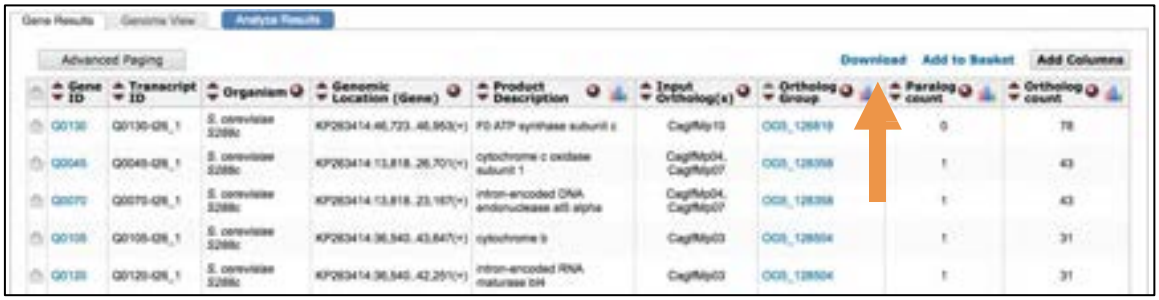


2. Use FungiDB to find *S. cerevisiae* orthologs of *C. glabrata* mitochondrial genes:

- Open the FungiDB homepage (**http://fungidb.org/**). In the "**Search for…**" section on the left, select "**Genes**," then "**Annotation, curation and identifiers**" section and click on "**List of IDs**".



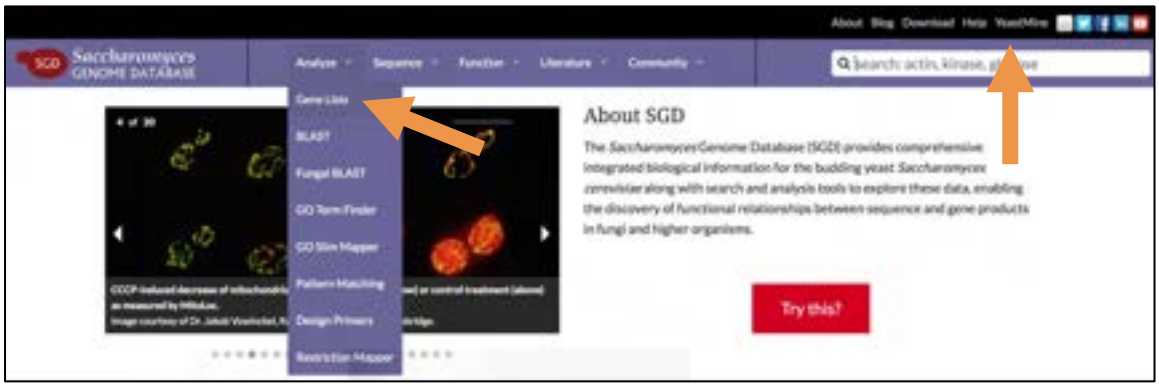
- Using your exported file from CGD, copy and paste the ORF names of the *C. glabrata* mitochondrial genes into the box. Click on "**Get Answer**".
- In the Search Strategy panel, click on the "**Add Step**" button. In the resulting pop-up window, click on "**Transform into related records**" and then "**Orthologs**."
- In the "**Organism**" list, search for "cerevisiae". Select "Saccharomyces cerevisiae S288C", click "select only these," 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.



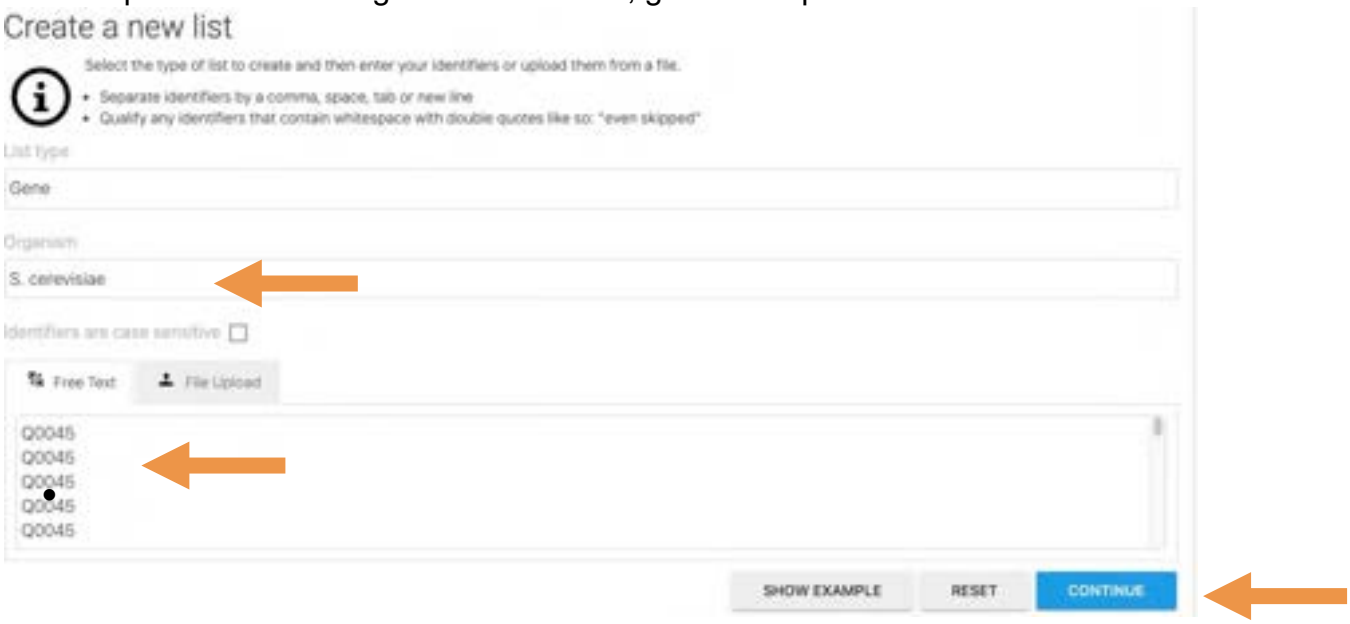
- In the download options menu, select “**Tab delimited (Excel) – choose a pre- configured table**”. Set the Download Type as **Excel File**, then hit **Get**.

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

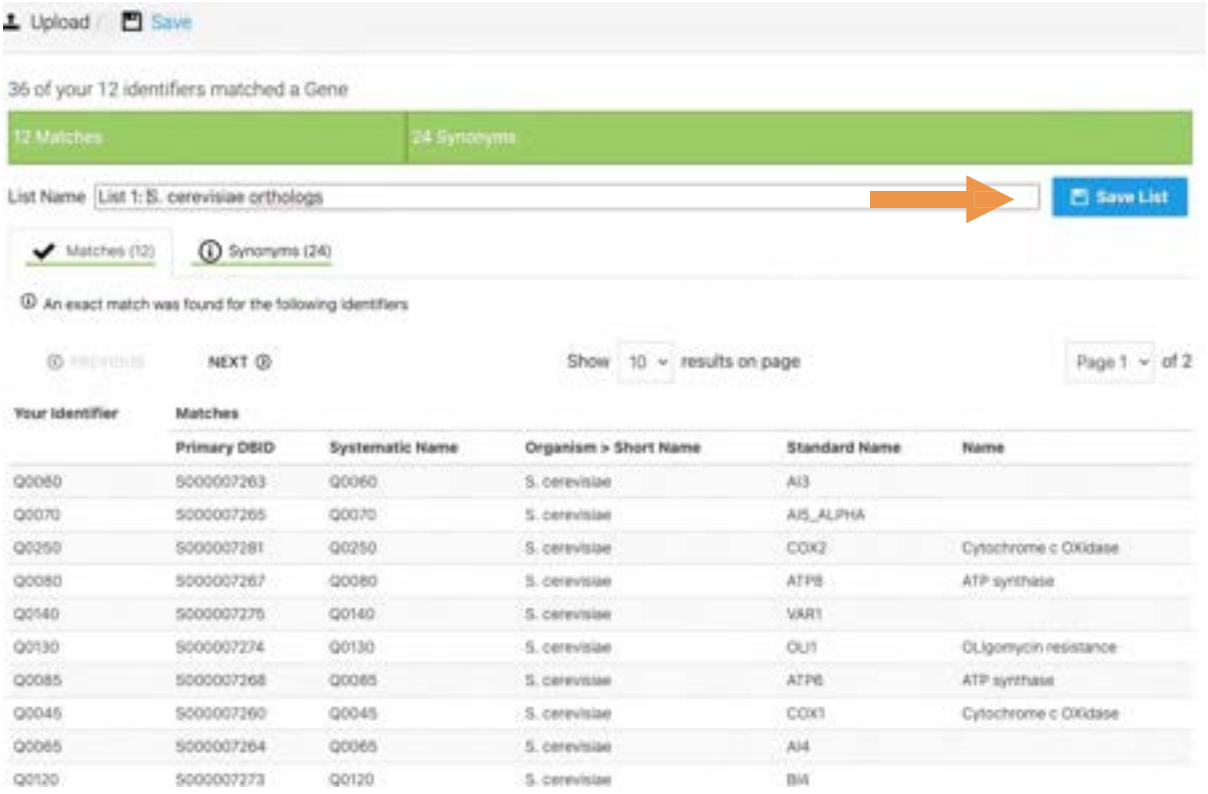
- Open the YeastMine homepage. You can access YeastMine from SGD by opening the Analyze tab and selecting **Gene Lists**, clicking the YeastMine link in the upper right corner of the homepage, or by entering in the URL:https://yeastmine.yeastgenome.org



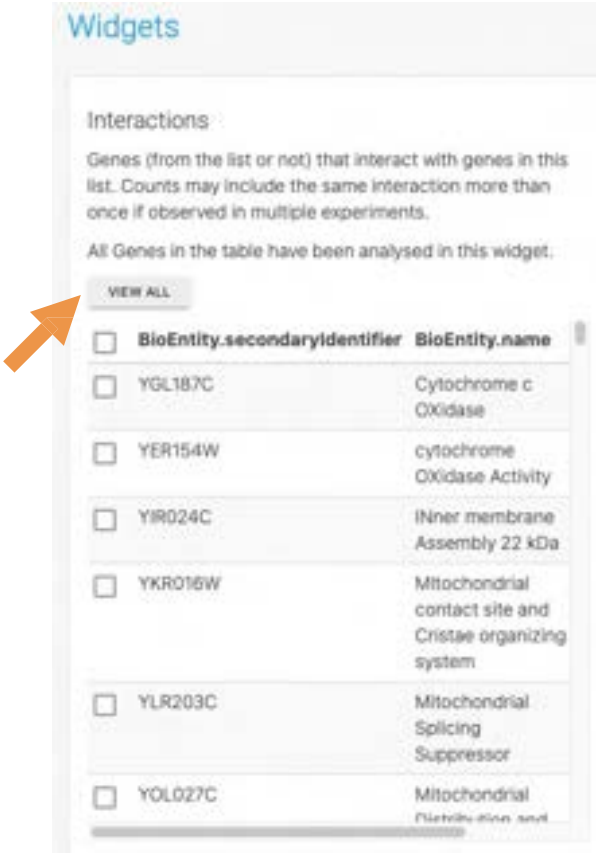
- Open the Excel file of *S. cerevisiae* orthologs that you downloaded earlier. To import these orthologs into YeastMine, go to the Upload tab and then create



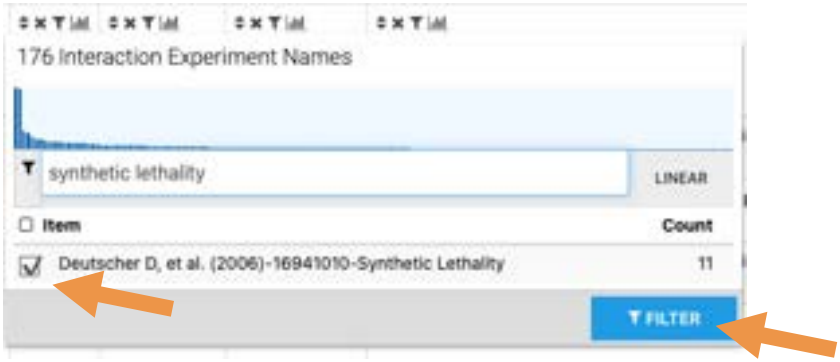
- You can save this list of genes as “**List 1: S. cerevisiae orthologs**”. Click on the blue “**Save List**” button.



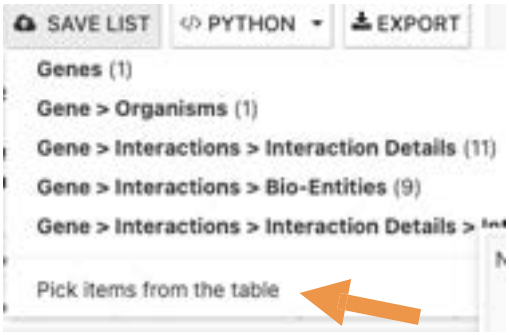
4. After you save the list, you'll get query results with options for running searches. In the **Widgets** section below the table, click "view all." You'll get roughly 750 results.

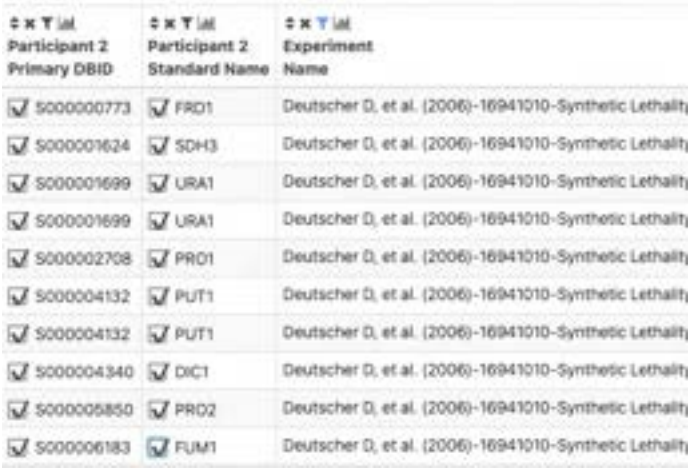


- In the column for "**Interaction Experiment Names**," search for "synthetic lethality," click the box next to any results, and then click **Filter**.

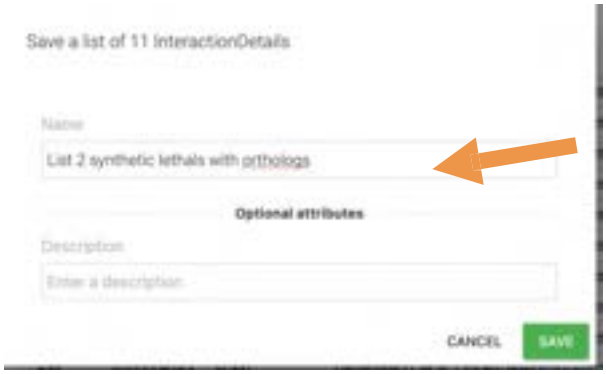


- You'll get a list of eleven genes that are synthetically lethal with a member of your original ortholog list.
- Click "**Save List**" at the top right and then choose "**Pick items from the table**." Radio buttons will appear in the table and you want to check all the items in the "**Participant 2 Standard Name"** column (this will automatically select the DBID as well). These are the genes known to be synthetically lethal with the list you used as input.

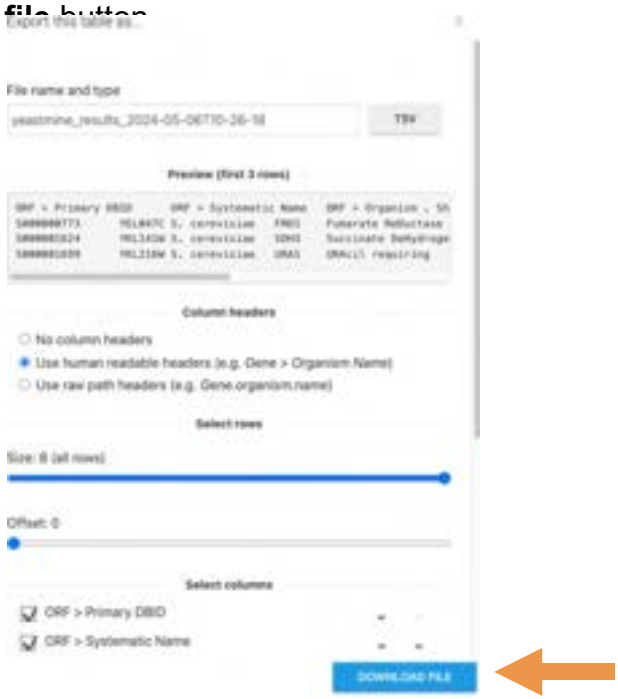




- Save this list as "List 2 synthetic lethals with orthologs"



- Access your new gene list by clicking on the **Lists** link in the top purple toolbar and selecting your new list name.
- 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:

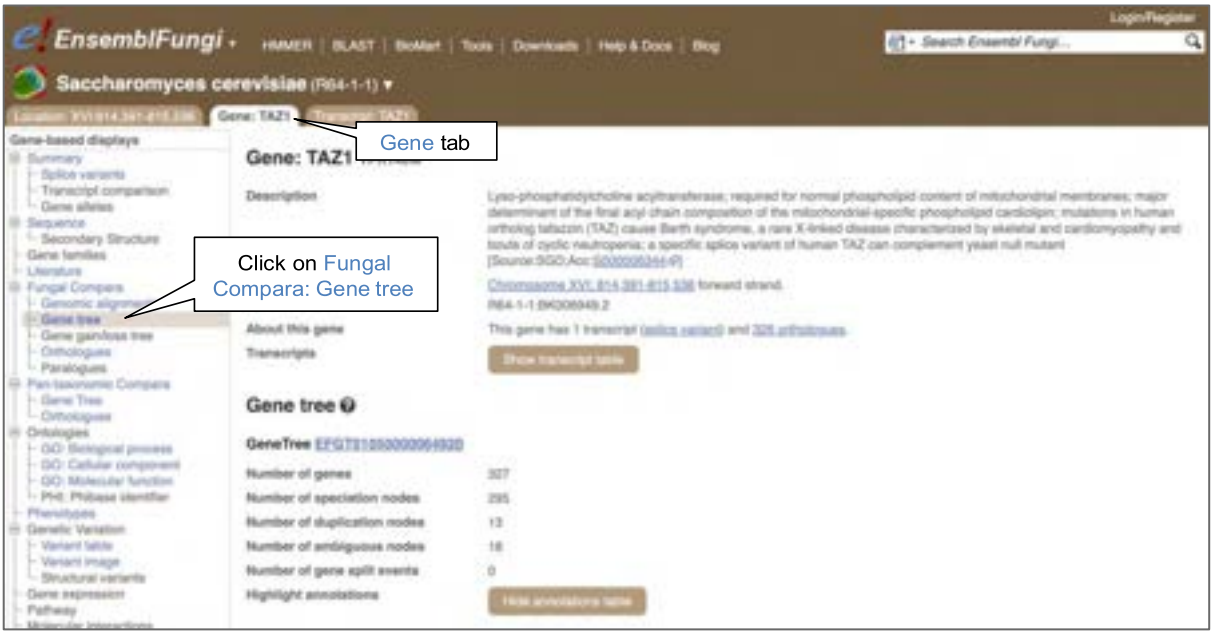
- Open the FungiDB homepage (**http://fungidb.org/**). In the "**Search for…**" section on the left, select **"Genes**," then "**Annotation, curation and identifiers**" section and click on "**List of IDs**".
- Using your exported file from Yeastmine, copy and paste the ORF names of the *S. cerevisiae* interactors (e.g. YEL047C, YKL141W, etc.) into the ID box. Click on "**Get Answer**".
- In the Search Strategy panel, click on the "**Add Step**" button. In the resulting pop-up window, click on "**Transform into related records**" and then "**Orthologs**."
- In the "**Organism**" list, search for "glabratus". Select "Nakaseomyces glabratus CBS 138 [Reference]," then hit "**Run Step**".
- 9 orthologs of the *S. cerevisiae* interactors will be returned. These are *C. glabrata* genes are predicted to have synthetic lethal interactions with *C. glabrata* mitochondrial genes. You can download this list.

- Then, 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?

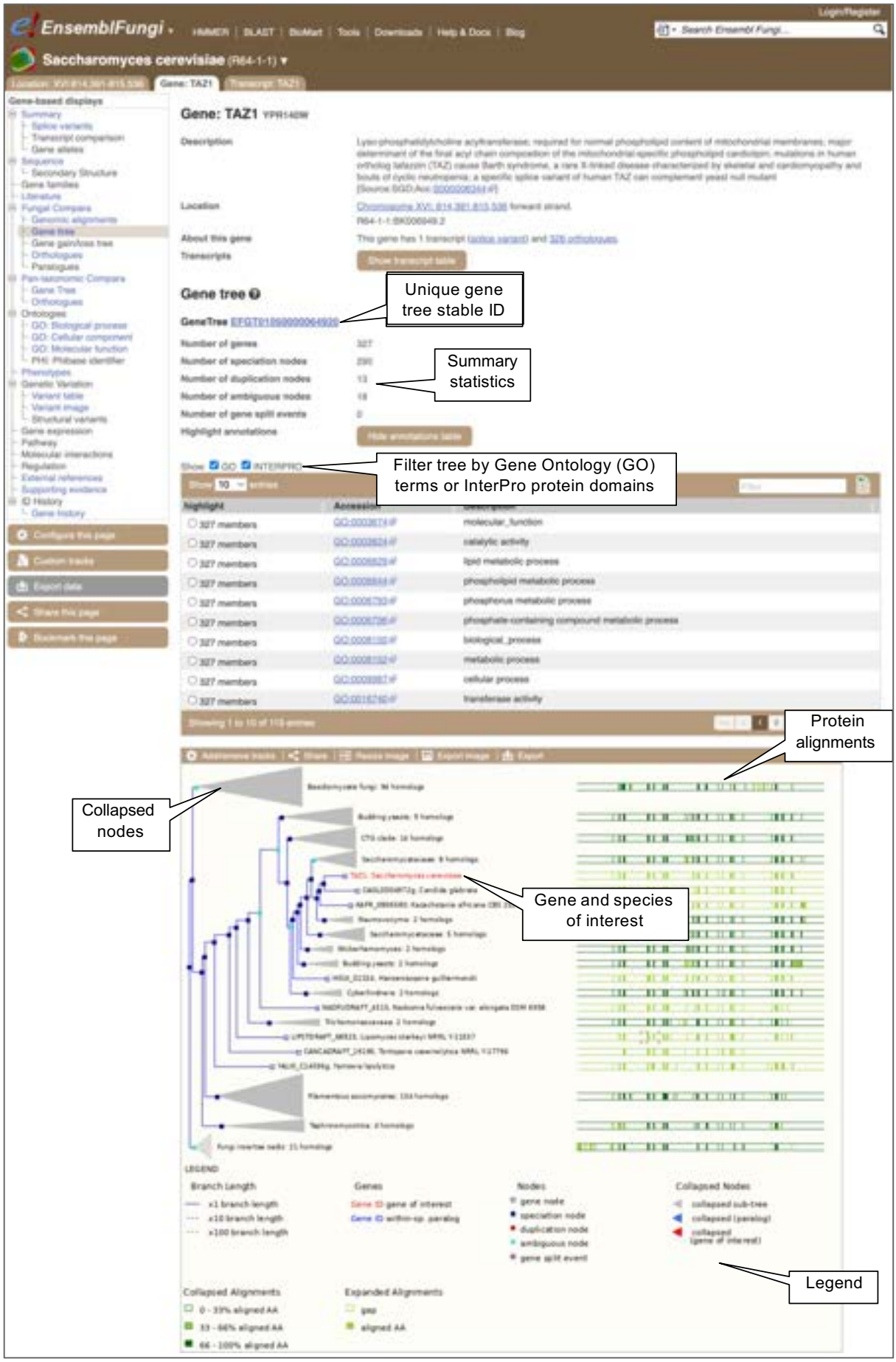
Exercise: Ensembl Fungi Gene Trees and Homologues

Links to be clicked shown in blue, text to be entered shown in red.

Let's look at the homologues of *Saccharomyces cerevisiae* (R64-1-1) *TAZ1* (gene stable ID: YPR140W). This gene is involved in stress response and conserved across different taxonomic domains. Click on the gene ID YPR140W to open the 'Gene' tab.

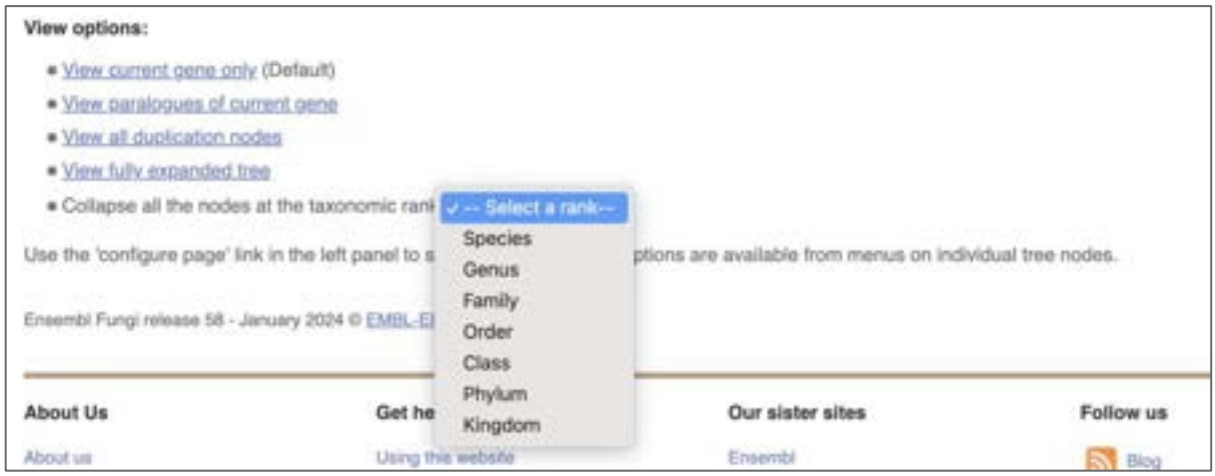


Click on Fungal Compara: Gene tree on the left-hand menu, which will display the current gene (in the context of a phylogenetic tree) used to determine orthologues and paralogues


Unique gene tree stable ID
Summary statistics
Filter tree by Gene Ontology (GO) terms or InterPro protein domains
Protein alignments
Collapsed nodes
Gene and species of interest
Legend

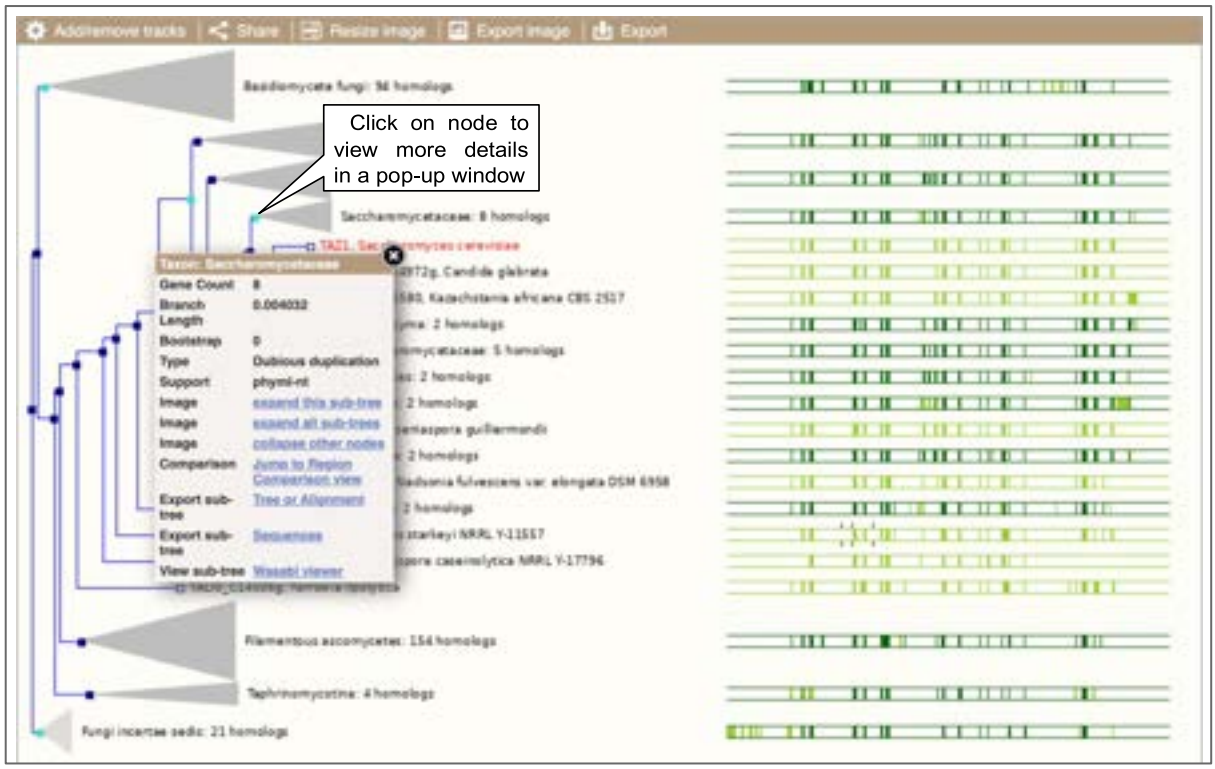
(a) How many duplication events are there in this tree?

Scroll to ‘View options’ at the bottom of the page. Here, you can find some quick filtering options. You can view paralogues and quickly expand or collapse nodes based on class, phylum etc.



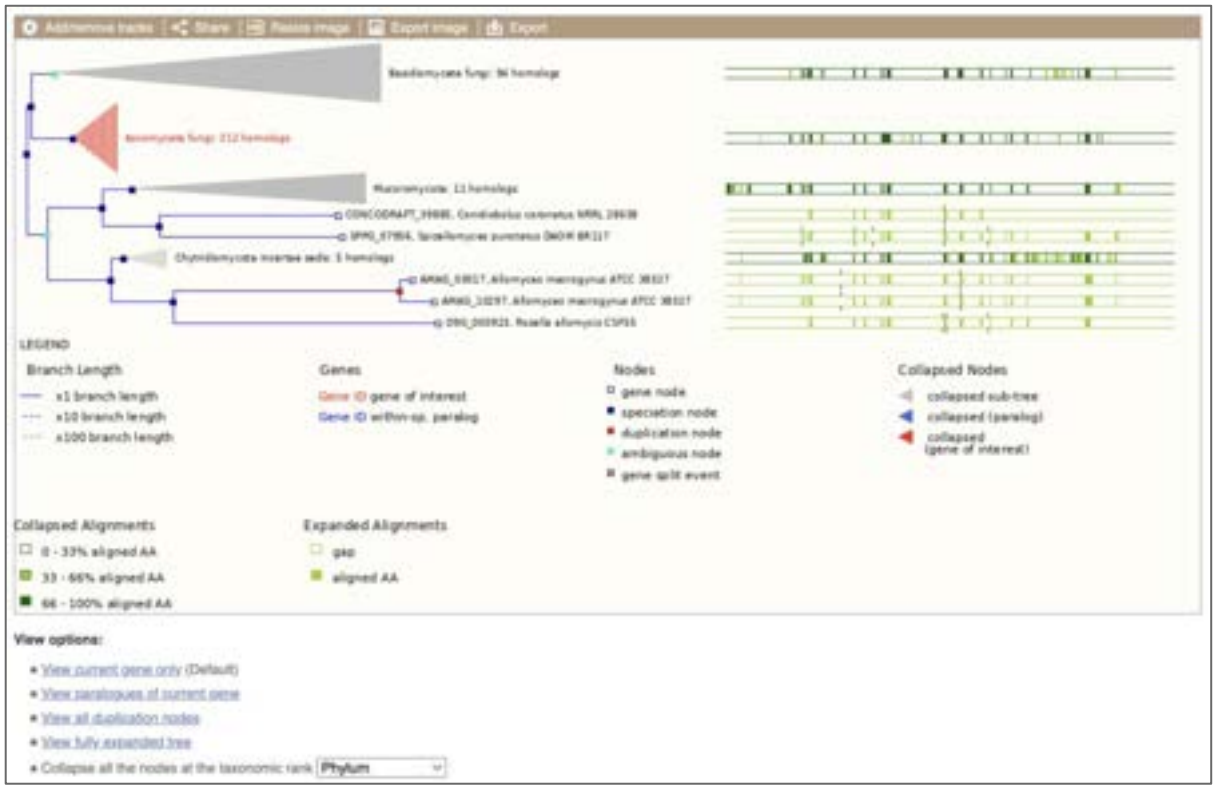
Click on View all duplication nodes. This will expand the tree so that all duplication nodes are visible. Count the number of red nodes. There are 13 duplication events in the tree.

Funnels indicate collapsed nodes. Click on a node (coloured square) to open a pop-up window, which tells you what type of node it is, some statistics and options to expand or export the sub-tree:



(b) What is the Phylum with the highest number of *TAZ1* homologues?

Under 'View options', collapse all nodes at the taxonomic rank Phylum.

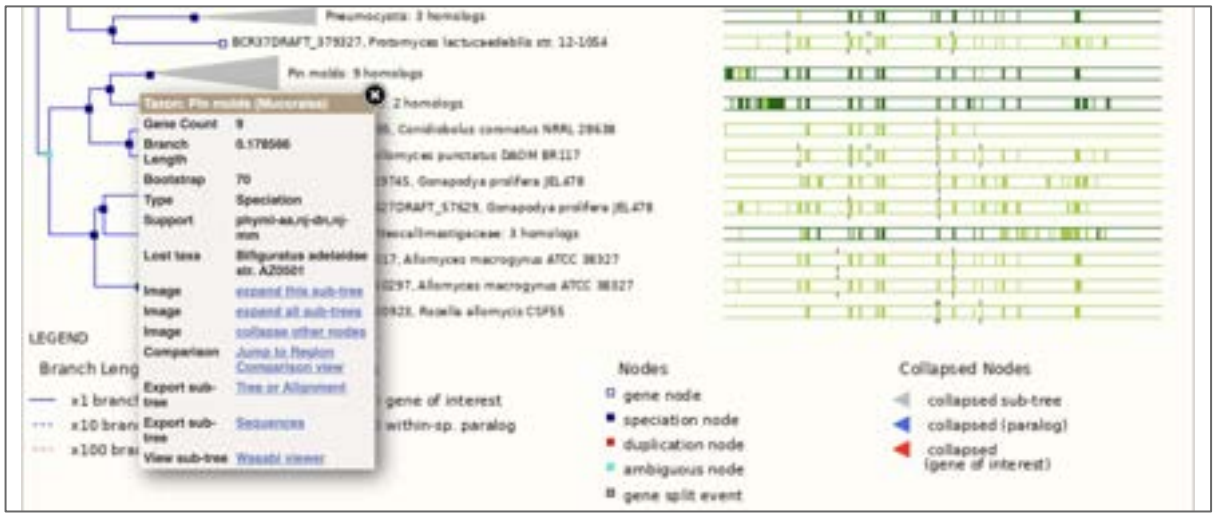


The phylum with the highest number of *TAZ1* homologues is Ascomycete fungi.

(c) What is the bootstrap support of the pin moulds (*Mucorales*) class in this tree?

Bootstrap values in a phylogenetic tree indicate that out of 100, how many times the same branch is observed when repeating the generation of a phylogenetic tree on a resampled set of data. Bootstrap values in Ensembl gene trees are calculated using a tool called TreeBeST, and the final consensus trees consist of clades chosen to minimise the number of duplications, losses inferred and have the highest bootstrap support. More on this process is available at https://www.ensembl.org/info/genome/compara/homology_method.html.

Click on the Pin molds node to view more details. In the pop-up window, you will find the bootstrap value to be 70.

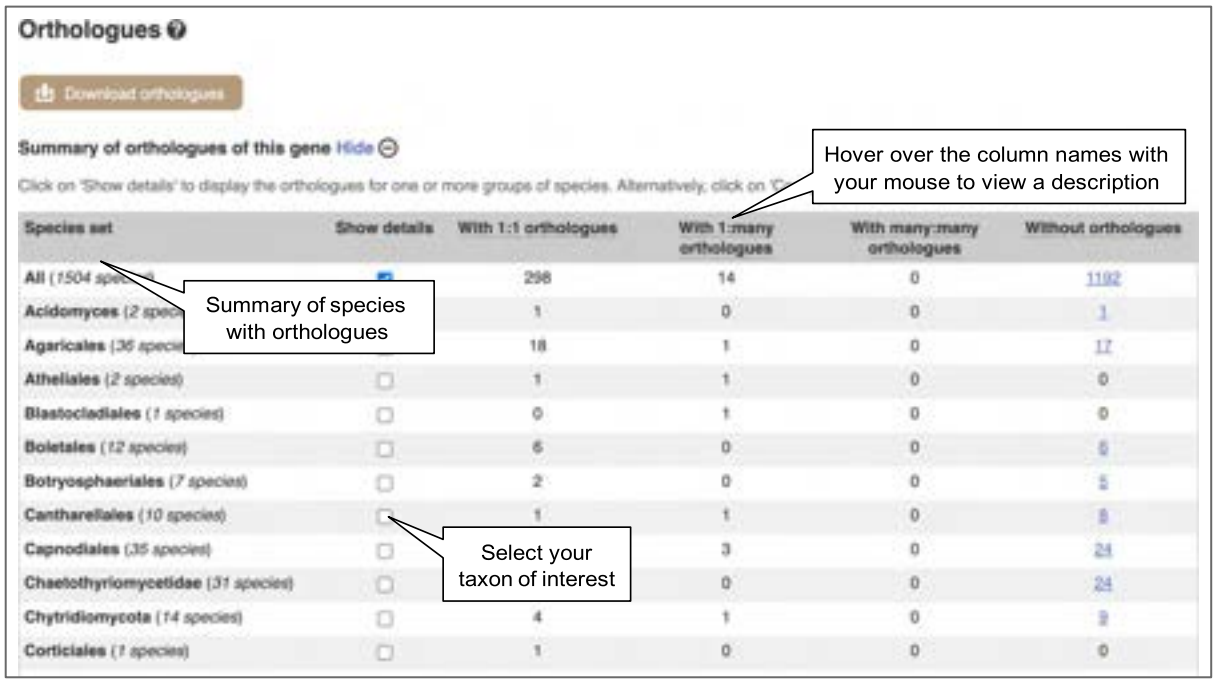


(d) Can you display the sequence alignment of all the homologues in this Class (*Hint: Use the Wasabi viewer*)?

Wasabi is an open-source, web-based environment for visualising sequence data alongside phylogenetic trees. You can read more about the platform in this publication: https://europepmc.org/article/MED/26635364.

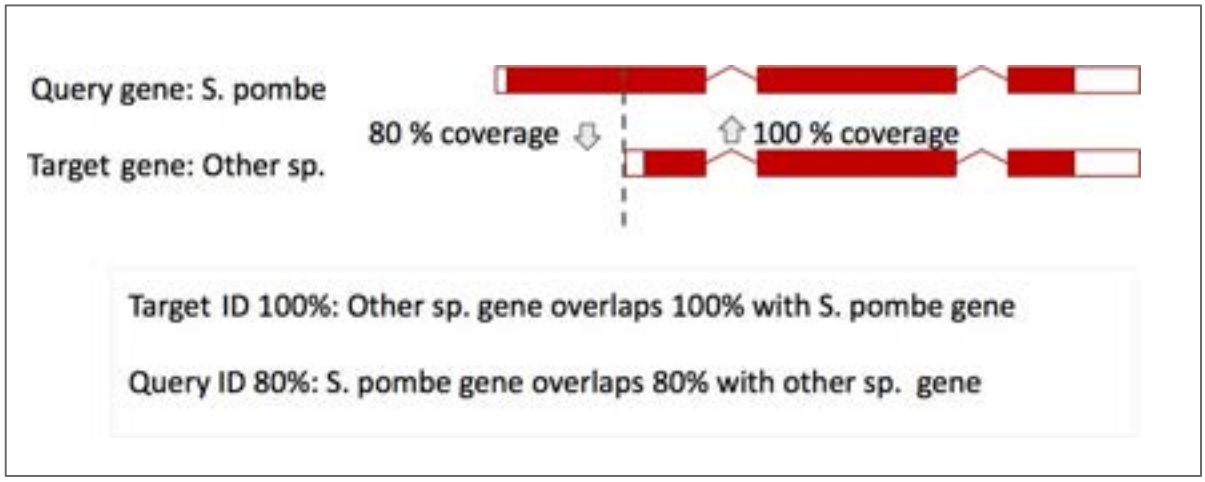
You can download the tree in a variety of formats. Click on the Export icon  in the bar at the top of the image. This opens a pop-up window where you can choose your format. You can preview this file before you download it.

We can look at homologues in the Orthologues and Paralogues pages, which can be accessed from the left-hand menu. If there are no orthologues or paralogues, then the link(s) will be greyed out. Click on Orthologues to see the orthologues available.

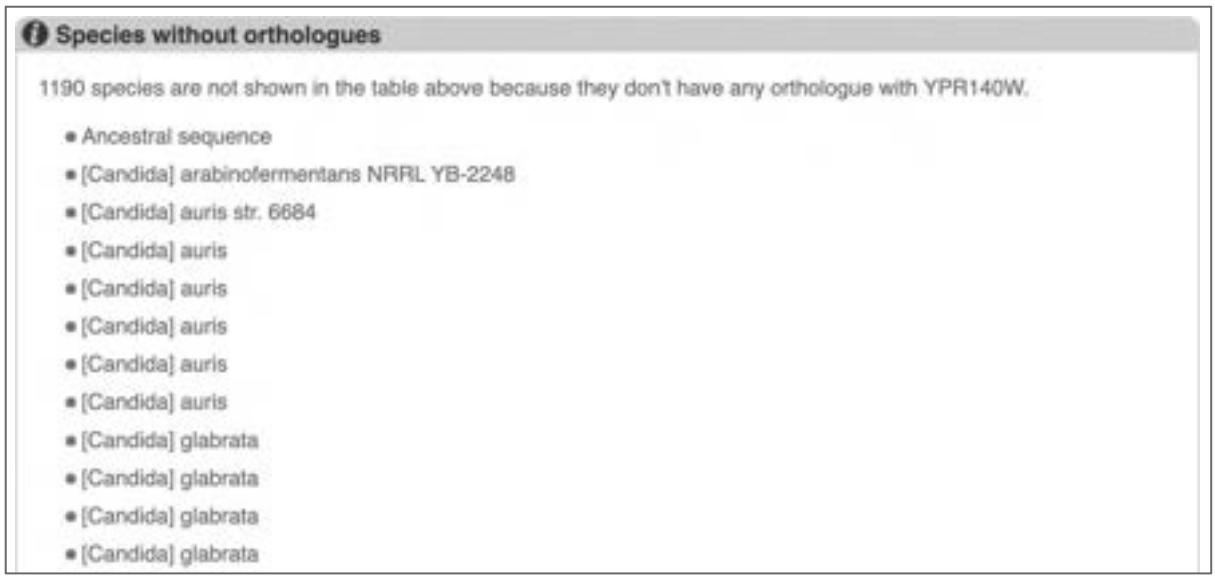


(e) What is the difference between Target %id and Query %id? (*Hint: Mouse over*)

The sequence identity is reported in two ways. Target %id is how much of the orthologue (target gene) overlaps with the query gene (our *S. cerevisiae* gene). The Query %id is the inverse of this. For example:

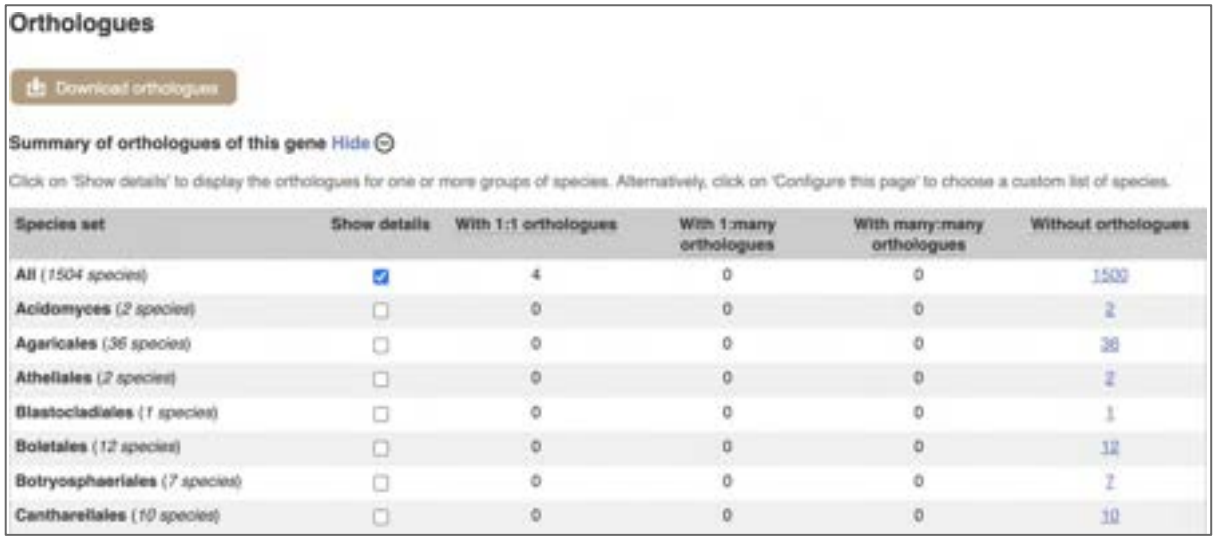


Click on Hide ⊖ above the table or scroll to the bottom of the page to see a list of the species that do not have any orthologues with *TAZ1* in *S. cerevisiae*… there are a lot!



S. cerevisiae is part of Ensembl's pan-taxonomic-compara (often shortened to pan-compara), which compares a subset of fungal species with representative species from other taxa, such as plants, protists, bacteria and vertebrates. This offers a broad view of homologous relationships from across the taxonomy. Go to Pan-taxonomic Compara: Gene Tree. Let's look at the pan-taxonomic tree with nodes collapsed at the Kingdom rank.

Click on Pan-taxonomic Compara: Orthologues.



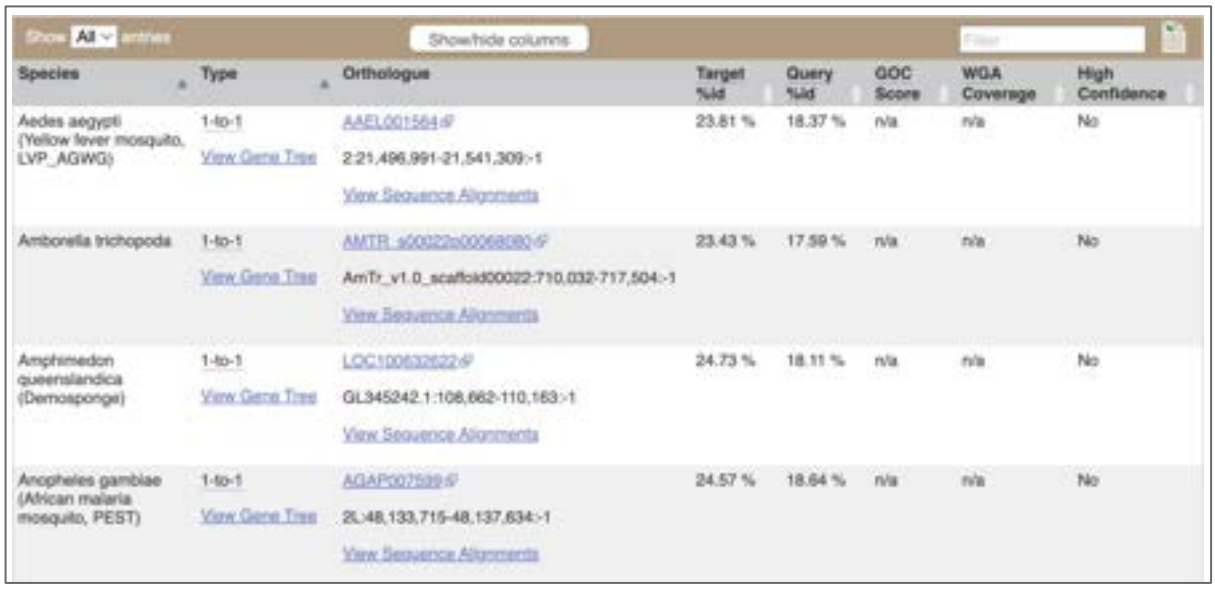
Orthologues

Download orthologues

Summary of orthologues of this gene Hide

Click on 'Show details' to display the orthologues for one or more groups of species. Alternatively, click on 'Configure this page' to choose a custom list of species.

Species set	Show details	With 1:1 orthologues	With 1:many orthologues	With many:many orthologues	Without orthologues
All (*1504 species*)	☑	4	0	0	1500
Acidomyces (*2 species*)	☐	0	0	0	2
Agaricales (*36 species*)	☐	0	0	0	36
Athelilales (*2 species*)	☐	0	0	0	2
Blastocladiales (*1 species*)	☐	0	0	0	1
Boletales (*12 species*)	☐	0	0	0	12
Botryosphaeriales (*7 species*)	☐	0	0	0	7
Cantharellales (*10 species*)	☐	0	0	0	10



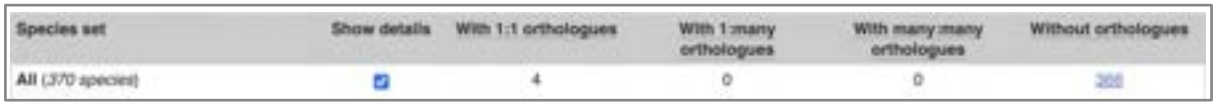
Species	Type	Orthologue	Target %id	Query %id	GOC Score	WGA Coverage	High Confidence
Aedes aegypti (Yellow fever mosquito, LVP_AGWG)	1-to-1	AAEL001564	23.81 %	18.37 %	n/a	n/a	No
	View Gene Tree	2:21,496,991-21,541,309:-1					
		View Sequence Alignments					
Amborella trichopoda	1-to-1	AMTR_s00022p00068080	23.43 %	17.59 %	n/a	n/a	No
	View Gene Tree	AmTr_v1.0_scaffold00022:710,032-717,504:-1					
		View Sequence Alignments					
Amphimedon queenslandica (Demosponge)	1-to-1	LOC100632622	24.73 %	18.11 %	n/a	n/a	No
	View Gene Tree	GL345242.1:108,662-110,163:-1					
		View Sequence Alignments					
Anopheles gambiae (African malaria mosquito, PEST)	1-to-1	AGAP007599	24.57 %	18.64 %	n/a	n/a	No
	View Gene Tree	2L:48,133,715-48,137,634:-1					
		View Sequence Alignments					

(f) How many species with predicted orthologues for this gene are there in Fungal Compara? What about in Pan-compara?

Fungal Compara:

Species set	Show details	With 1:1 orthologues	With 1:many orthologues	With many:many orthologues	Without orthologues
All (370 species)	☑	298	14	0	58

Pan-taxonomic Compara:

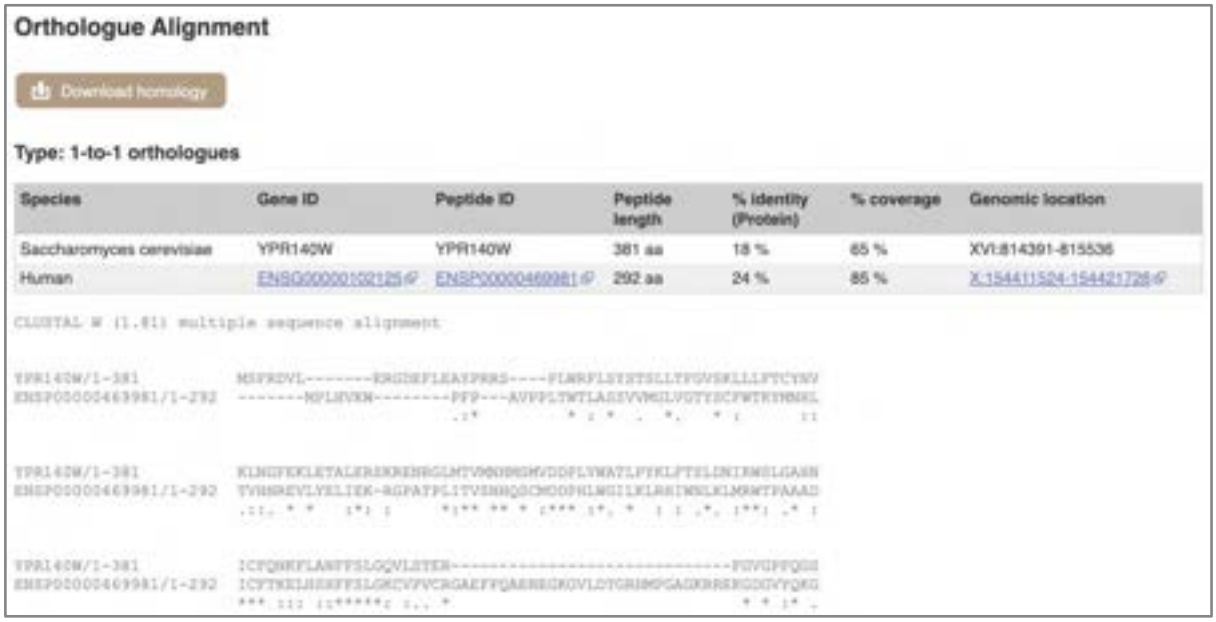


Species set	Show details	With 1:1 orthologues	With 1:many orthologues	With many:many orthologues	Without orthologues
All (370 species)	☑	4	0	0	368

(g) How many animal orthologues are there? Does this number agree with the Pan- taxonomic tree above? Hint: Click the Show details box for Vertebrates and Metazoa, and count the number of orthologues in the table below).

(h) Filter the second table to view the human orthologue. How much sequence identity does the human protein have to the *S. cerevisiae* one? Is it a high-confidence homologue? Click on the View Sequence Alignment link in the 'Orthologue' column to View Protein Alignment in ClustalW format. Does it support your conclusions?


Selected orthologues Hide
Show All entries
Show/hide columns
human
Species
Type
Orthologue
Target %id
Query %id
GOC Score
WGA Coverage
High Confidence
Human
1-to-1
TAFAZZIN (ENSG00000102125)
24.66 %
18.90 %
n/a
n/a
No
View Gene Tree
X:154,411,524-154,421,726:1
View Sequence Alignments
View Protein Alignment
View cDNA Alignment
Pediculus humanus
1-to-1
PHUM309640
18.90 %
n/a
n/a
No
View Gene Tree
DS235308:45,836-47,144:1
View Sequence Alignments


Orthologue Alignment
Download homology
Type: 1-to-1 orthologues
Species
Gene ID
Peptide ID
Peptide length
% identity (Protein)
% coverage
Genomic location
Saccharomyces cerevisiae
YPR140W
YPR140W
381 aa
18 %
65 %
XVI:814391-815536
Human
ENSG00000102125
ENSP00000469981
292 aa
24 %
85 %
X:154411524-154421726
CLUSTAL W (1.81) multiple sequence alignment
YPR140W/1-381
ENSP00000469981/1-292

Additional Exercise 1: *Zymoseptoria* Orthologues

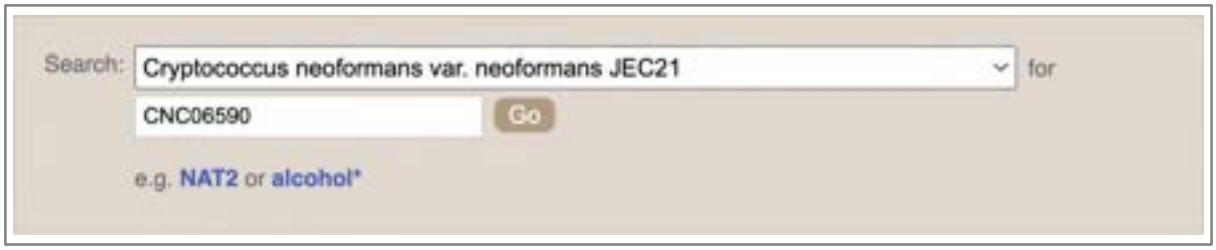
We will now explore an orthologue that we identified using BioMart (additional exercise 1 in the BioMart module). We identified 18 genes associated with the GO term detoxification in *Zymoseptoria tritici*. We then found a single high-confidence orthologue in *Cryptococcus neoformans* (CNM01690) which we will now explore further.

Search for CNM01690 in *Cryptococcus neoformans* var. *neoformans* JEC21 and go to the gene page.

(a) Does this gene in *C. neoformans* have a UniProtKB-Gene Ontology annotation?
(b) Find the *Z. tritici* orthologue in the Orthologues page and view a protein alignment.
(c) At which end of the protein (N- or C-terminus) does the alignment between these two genes become worse?

Additional Exercise 1 Answer: *Zymoseptoria* Orthologues

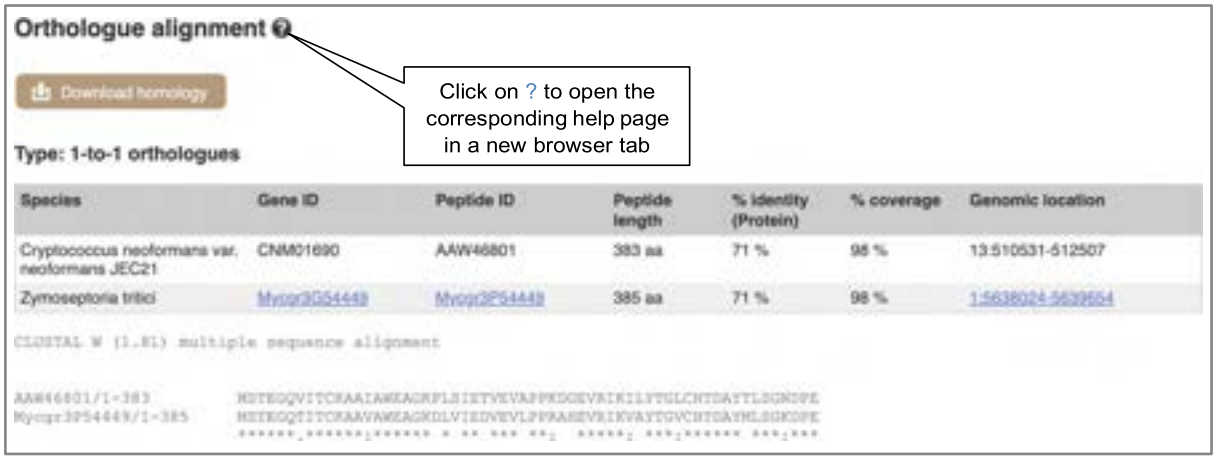
Go to fungi.ensembl.org in your browser. In the species-specific search box, select *Cryptococcus neoformans* var. *neoformans* JEC21 from the drop-down list and enter CNM01690. In the results page, click on the gene stable ID CNM01690 to navigate to the 'Gene' tab.



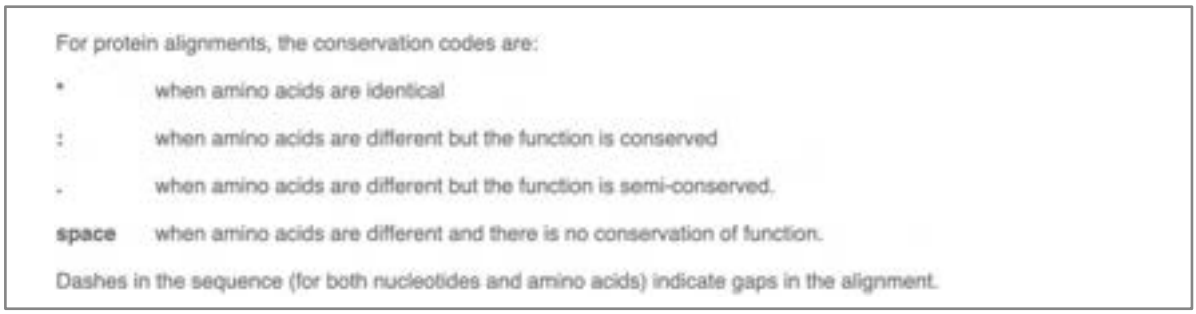
(a) In the left-hand panel under 'Gene-based displays', click on External references. Yes, the this gene has a UniProtKB-Gene Ontology annotation. The database ID for the UniProtKB-Gene Ontology annotation is Q5K7P9.

(b) Go to Fungal Compara: Orthologues in the left-hand panel. Click on the Hide ⊖ button for the 'Summary of orthologues of this gene' table. In the 'Selected orthologues' table, use the search bar in the top right-hand corner to search for *Zymoseptoria tritici*. Click on View Sequence Alignments and in the pop-up menu select View Protein Alignment.

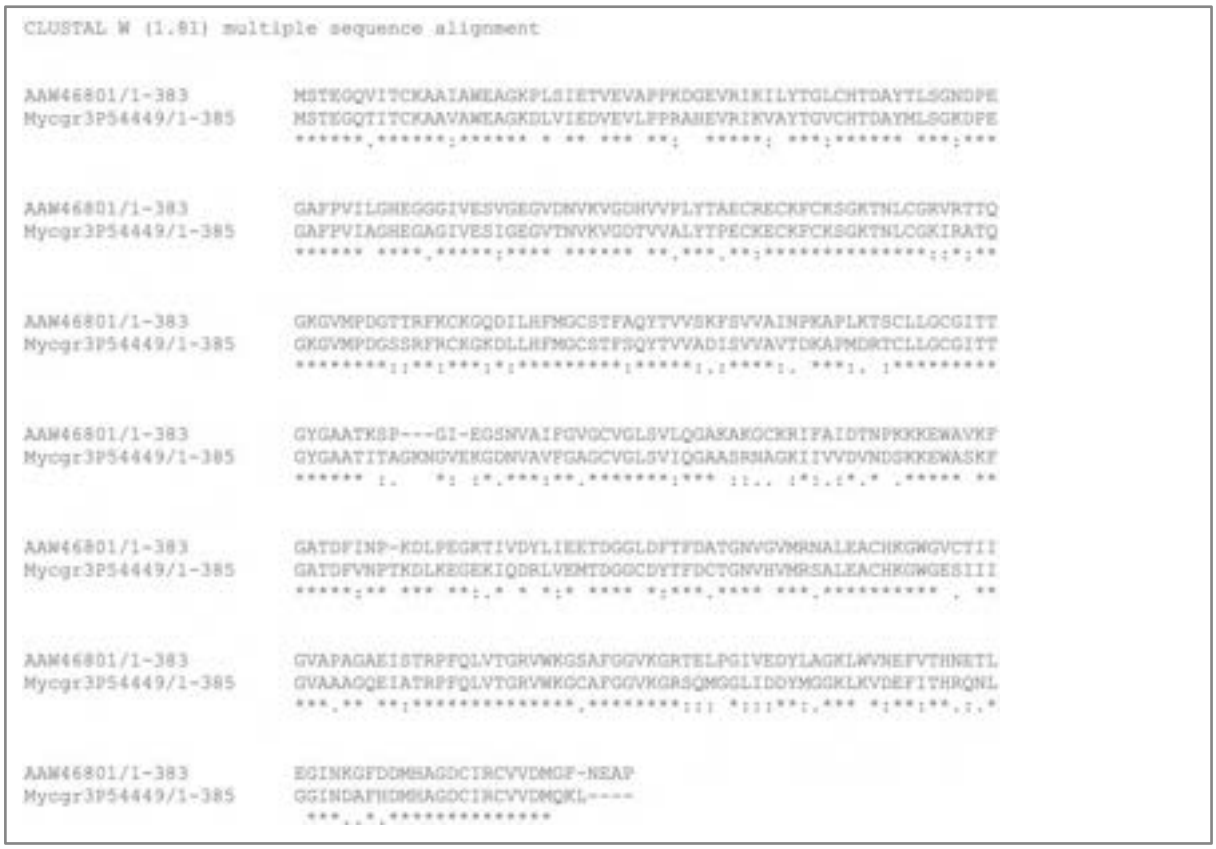
(c) You can find a description of the different symbols by clicking the question mark icon ❓ next to 'Orthologue alignment'. This opens the corresponding help page in a new tab.



In the help page, we can find a description of the conservation codes:



Looking at the ClustalW alignment and referring to the conservation codes, we can see that the N-terminus is more highly conserved than the C-terminus as there is a gap in the alignment in the C-terminus:



You can read more about Clustal alignments in the '[The Clustal Omega Multiple Alignment Package]' publication by Sievers and Higgins (2020).

Additional Exercise 2: Mushroom Genes

We're going to take a look at the gene CC1G_05700 in *Coprinopsis cinerea* okayama7#130.

From the 'Gene' tab, click to view the Gene tree. At the bottom of the image click to collapse all the nodes at the taxonomic rank of Class.

(a) What do you notice about the types of fungi shown in the gene tree?
(b) Does this match with what you would expect from the gene description? (*Hint: Agaricomycetes class belongs to the Basidiomycota phylum*)
(c) Based on the protein alignment shown at the right, can you predict which end of the gene/protein is most conserved?
(d) Click to view the Orthologues page. In the Selected orthologues table, find the entry for the species *Amanita thiersii* and click to view a protein alignment. Does this support your conclusion about the conserved region of the gene/protein?

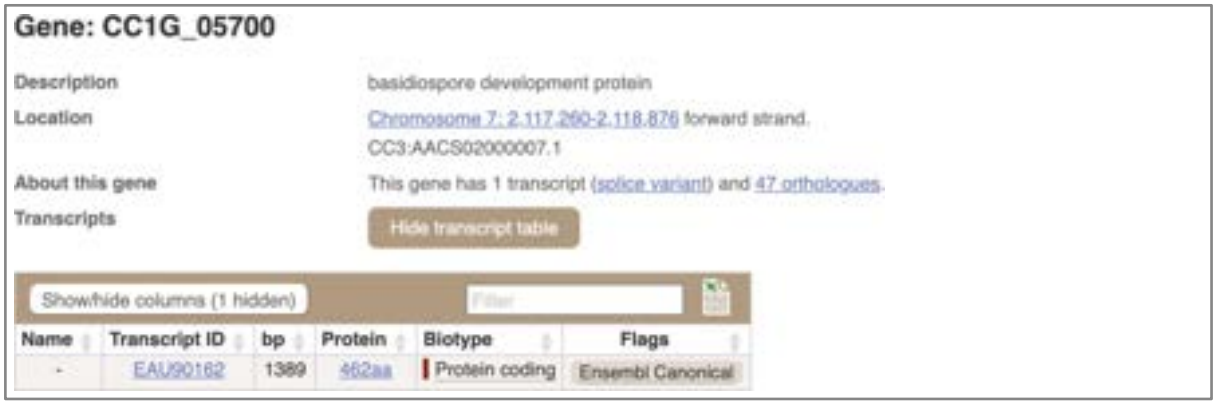
Additional Exercise 2 Answer: Mushroom Genes

Go to fungi.ensembl.org in your browser. In the species-specific search box, select *Coprinopsis cinerea* okayama7#130 from the drop-down list and enter CC1G_05700.

Click on the gene stable ID CC1G_05700 to open the 'Gene' tab. In the left-hand panel, click on Fungal Compara: Gene tree. Scroll to the bottom of the gene tree and collapse all the nodes at the taxonomic rank Class under 'View options'.

(a) All fungi shown in the gene tree are Agaricomycetes:

(b) The gene description is as follows:



The class Agaricomycetes belongs to the phylum Basidiomycota, therefore we would expect the gene encoding the basidiospore development protein to be conserved across Agaricomycetes species.

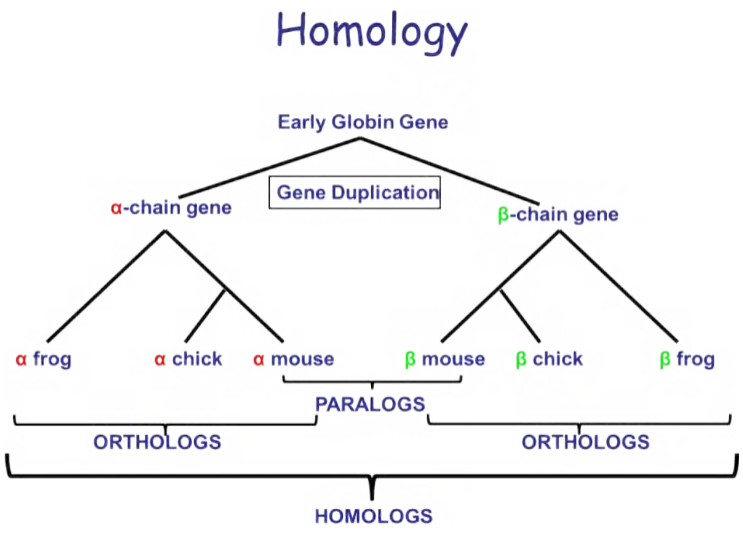
(c) Dark green regions in the alignment indicate highly conserved sequences (see ‘Collapsed Alignments’ legend):

(d) Go to Fungal Compara: Orthologues in the left-hand panel. Click on the Hide ⊖ button for the 'Summary of orthologues of this gene' table. In the 'Selected orthologues' table, use the search bar in the top right-hand corner to search for *Amanita thiersii*. Click on View Sequence Alignments and in the pop-up menu select View Protein Alignment.

FungiDB & OrthoMCL: Orthology and Phyletic Patterns

Learning objectives:

- Run searches in OrthoMCL.
- Run phyletic pattern searches using checkboxes or an expression.
- Combine searches using the strategy system.
- Explore individual ortholog group pages.
- Explore the group cluster graphs.



About OrthoMCL

OrthoMCL is a genome-scale database that groups orthologous protein sequences across the tree of life. An orthogroup contains genes descended from a common ancestor by a process of duplication and speciation (see figure above), so a single orthogroup may contain both genes across different species with similar function and paralogs within a single species.
Each protein in every OrthoMCL species is assigned to precisely one ortholog group (e.g. OG6_162879). Importantly, proteins within a single OrthoMCL group have been shown to display a high degree of functional conservation (e.g., a group's proteins have consistent EC numbers) (Li et al. 2003). Orthology is important in predicting the function of the rapidly increasing number of newly identified proteins produced by genome sequencing and the automated discovery of protein sequences (Glover et al. 2019). Within VEuPathDB, orthology can be used to transform a list of genes from one species into their closest equivalents in another species.

OrthoMCL contains two sets of genomes. A **Core** set of 150 genomes have been chosen as well annotated reference organisms that broadly represent the major branches of the tree of life. The OrthoMCL algorithm uses BLAST to calculate pairwise distances among all proteins in the 150 core genomes, normalizes the scores for sequence length and evolutionary distance, then uses MCL clustering (Dongen 2000; www.micans.org/mcl) to create orthogroups of similar proteins. All of the non-core VEuPathDB species (pathogens, hosts, and vectors) have been added as **Peripheral** organisms, in some cases including multiple strains and genome assemblies for the same species. All proteins from the Peripheral organisms are assigned to the most similar Core cluster by best BLAST score, but proteins that do not match any Core protein with an e-value better than $1e^{-5}$ are set aside as **Residuals**.

Pairwise BLAST distances among all Residual proteins are computed and used for a second round of MCL clustering to create Residual groups (e.g. OG6r20_100305)

The OrthoMCL website offers the ability to explore orthogroups by taxonomy, number of proteins or species, sequence similarity, EC numbers, PFam domains, and text search of gene descriptions. Users can use the Ortholog Group or Protein queries in the grey Search box to the left or the Searches menu in the header bar, or just type a search term in the 'Site search' box above which will result in a list of proteins and groups to explore. In addition, users can use a VEuPathDB Galaxy workflow to map their own set of proteins (e.g. protein sequences derived from a genome sequence of an organism) to OrthoMCL groups. See the Assign Proteins to Groups page.

For more information, see the About OrthoMCL and OrthoMCL FAQ pages.

Examining OrthoMCL output on gene record pages in FungiDB

- Go to the FungiDB gene record page for CGB_L0350W, a hypothetical protein in *Cryptococcus gattii* .

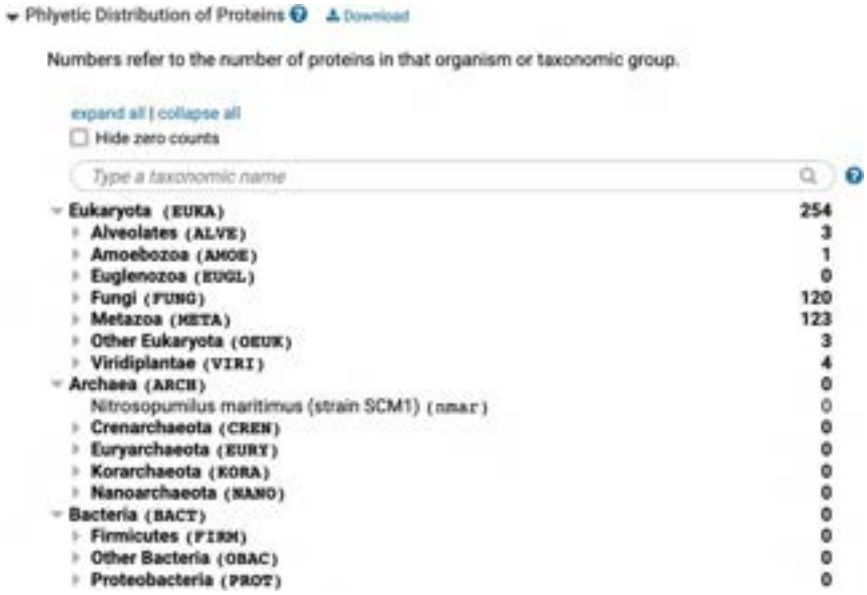
    a. What is the function of this gene? How can you infer its function?
        i. Click on the “Orthology and Synteny” link in the Contents menu on the left. Does this gene have orthologs in other *Cryptococcus* species?

    b. Examine evidence in the “Function prediction” section.
    c. What about other organisms outside fungi? (Hint: click on the Ortholog Group OG6_106189).
    d. The OrthoMCL group page is divided into 5 sections:
        1. Phyletic distribution
        2. Group summary
        3. List of proteins
        4. PFam domains
        5. Cluster graph

- Is this gene found in both Ascomycetes and Basidomycetes?

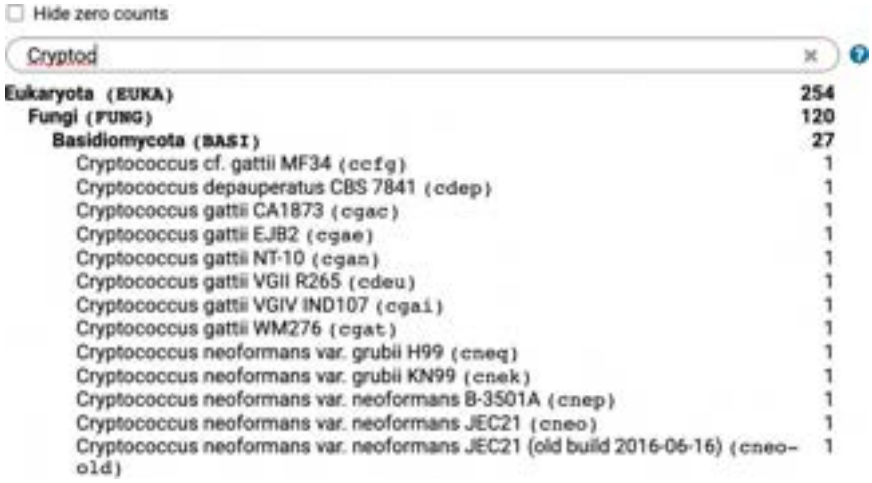
- Does this protein have othologs in Archaea and Bacteria (Hint: uncheck the box for “Hide zero counts”?

Phyletic distribution: Numbers refer to the number of proteins in that organism or taxonomic group. In order to see organisms and taxonomic groups without proteins in this ortholog group, uncheck 'Hide zero counts.'

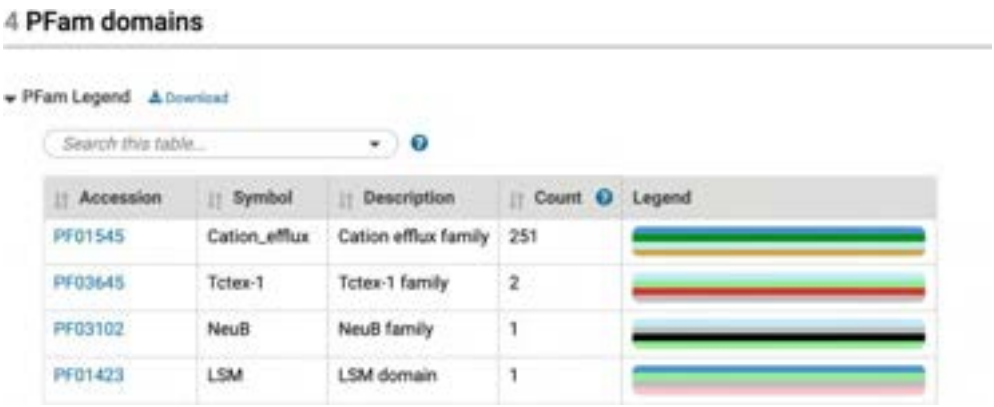


Group summary breaks down summary by protein types: A core protein is from one of the 150 core species that were initially used to form 'core' groups. A peripheral protein is from a peripheral species whose entire proteome was mapped into the 'core' groups.
Peripheral proteins that do not map into a 'core' group are placed into residuals groups.

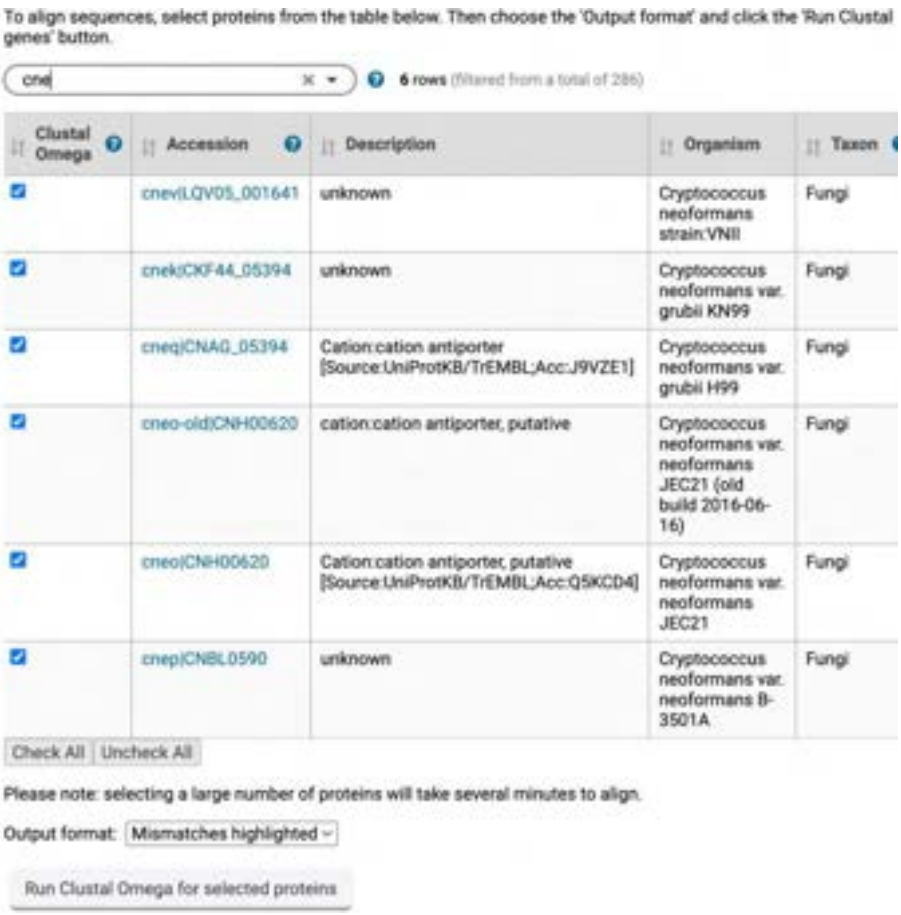
- **Do all *Cryptococcus* species currently integrated in FungiDB contain this protein?**



- **What is the most common PFAM domain associated with the proteins in this group?**



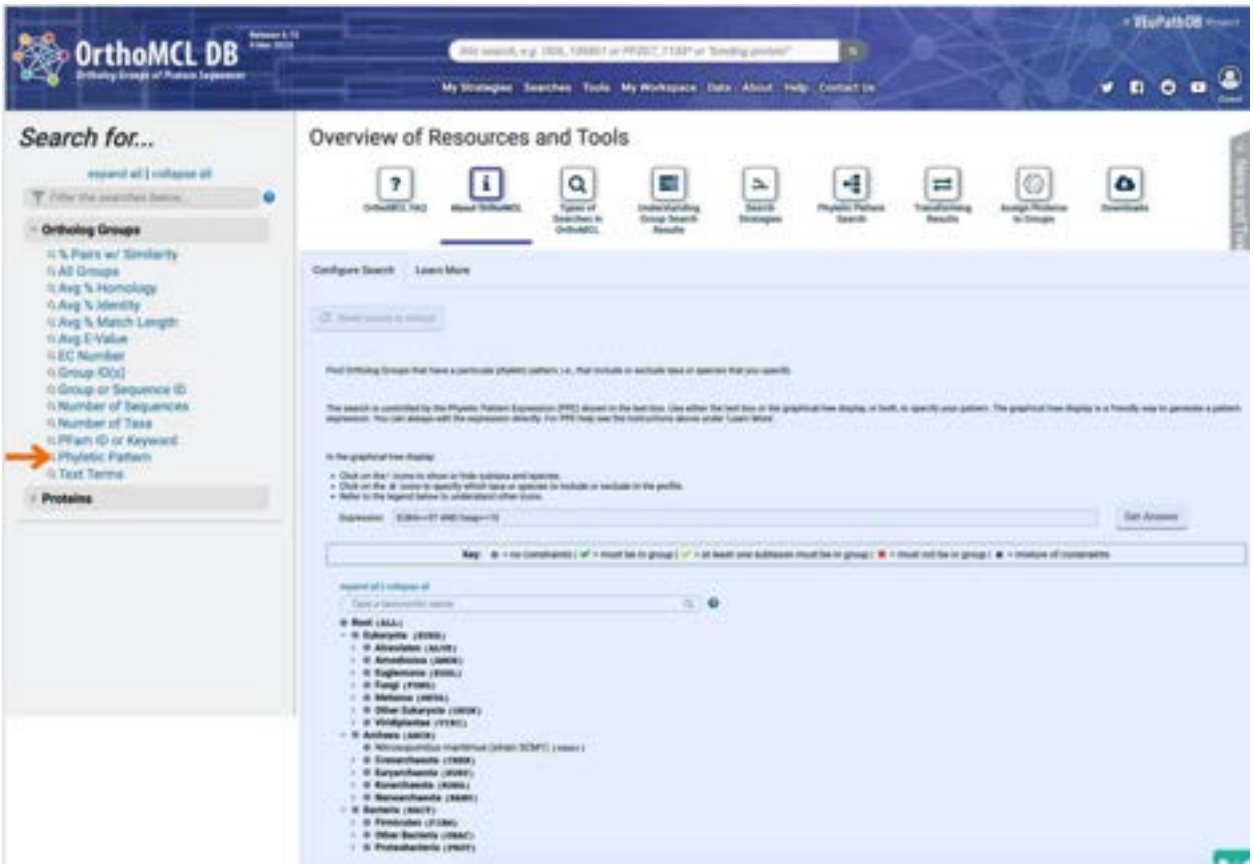
- **How can you create protein alignments for *Cryptococcus* genes?**
(Hint: Open List of All Proteins" section and use the "Search this table" filter to limit the alignment to "Cryptococcus", check all boxes, then hit "Run Clustal Omega for selected proteins" button at the bottom of this section).



Using the Phyletic Pattern search in OrthoMCL

The “Phyletic Pattern” search is an ortholog group search – look under the ortholog groups category and explore the available searches.

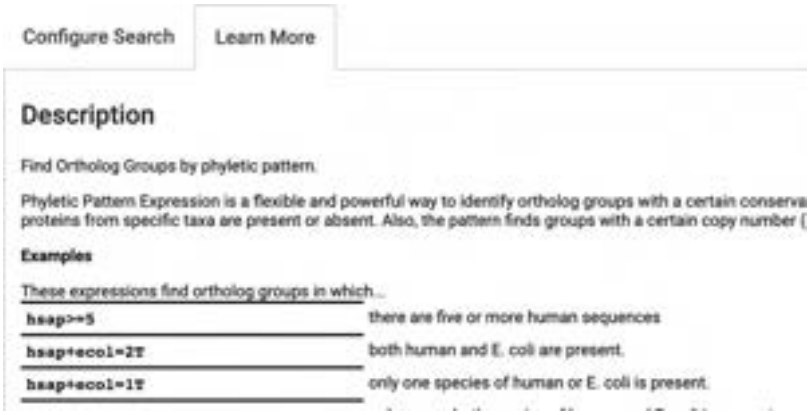
- Find the “Phyletic Pattern” search.



There are two ways to specify a phyletic pattern:

1. Using the expression box.
   - **Run the default search for EUKA>=5T AND hsap>=10**.

   - Use the “Learn More” tab to decipher the expression used above.



2. Using the selectable tree menu.
   You can click on the circle next to the taxon you want to include or exclude it from the search.

- **Using the "Phyletic pattern" search, identify how many eukaryotic protein groups do not contain orthologs from bacteria and archaea.**

  Hint: leave EUKA class with no constraints.

Strategy URL: https://orthomcl.org/orthomcl/app/workspace/strategies/import/eebc49abcff1d99f

- **Find all groups that contain orthologs from <u>at least one species</u> of *Ascomycota fungi* (1T) but <u>not from</u> bacteria, archaea or metazoan (0T).**

- **Examine your results and learn how to interpret the graphical representation for each group.**
  Scroll to the right of the results table examine graphical representation of the results. You can hover over each graph to learn more about phyletic distribution for each class.

Strategy URL: https://orthomcl.org/orthomcl/app/workspace/strategies/import/555afd9c529d4927

- **Revise your search to find groups that:**
  - <u>do not</u> contain orthologs from Alveolates, Amoebozoa, archaea, bacteria and Ascomycetes.
  - contain <u>at least one ortholog group</u> from *Mucor circinelloides* f. *circinelloides* 1006PhL (mcic) **AND** *Mucor circinelloides* f. *lusitanicus* CBS 277.49 (mcir).

Hint: You cannot answer this question by using the check boxes alone. For Mucor, use the expression field to finish the parameter set up manually.

If you are getting frustrated trying to figure this one out, you have a right to be! If your results look different, hover over the search step and click to revise the parameter search. The cool thing about OrthoMCL is that has an added feature that allows you to enter an expression to define the phyletic pattern. This option provides additional flexibility.
Can you figure out what expression to use to answer this question? (hint: start by assigning the "do not contain" parameter (x) using check boxes to Alveolates, Amebozoa, archaea, bacteria and Ascomycetes. Next, use the expression window to add "AND" followed by specific criteria for Mucor spp. Use the learn more tab for more information.

Strategy URL: https://orthomcl.org/orthomcl/app/workspace/strategies/import/88e60b823cb2c959

If you ran a search using just check boxes, the search will be configured to look for groups that:

- do not contain orthologs from Alveolates, Amoebozoa, archaea, bacteria and Ascomycetes.
- contain ortholog groups from both *Mucor circinelloides* f. *circinelloides* 1006PhL (mcic) **AND** *Mucor circinelloides* f. *lusitanicus* CBS 277.49 must be present

Strategy URL: https://orthomcl.org/orthomcl/app/workspace/strategies/326574153/430551723

Useful information:

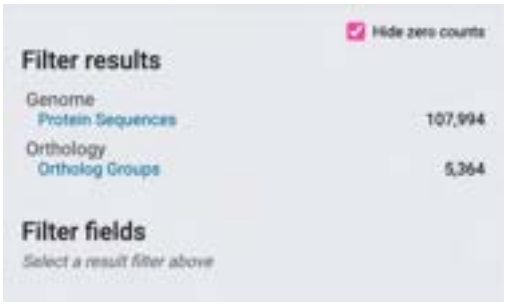
All VEuPathDB genomics sites (e.g., FungiDB) have an integrated phyletic pattern search that uses OrthoMCL to return lists of genes. For example, you use the "Orthology Phylogenetic Profile" search to identify genes in your organism of interest that are restricted in their profile. For example, you frequently want to identify genes that are conserved among organisms in your genus of interest but not present in the host as these genes may make good drug targets or vaccine candidates.

Combining searches in OrthoMCL

- **Find all fungal proteins that are likely to be phosphatases and that do not have orthologs outside of fungal kingdom.**

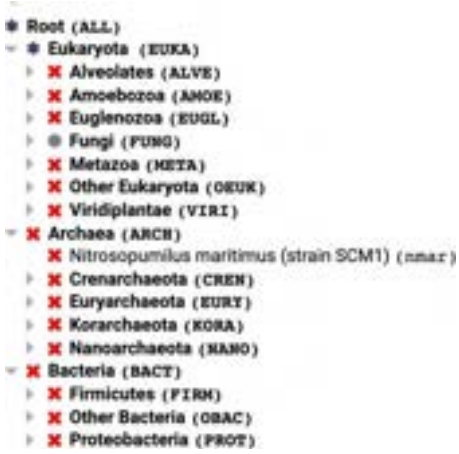
a. Use the site search to look for *phosphatase* (use asterisks to find any combination of the word “phosphatase”).

How many protein sequences were identified? How many ortholog groups did you identify?

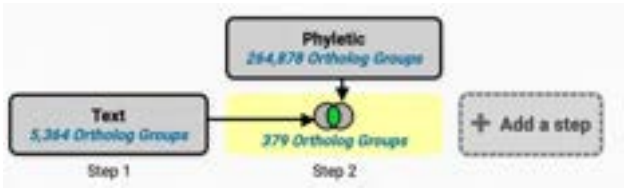


b. Display the ortholog groups containing the word phosphatase and export the resuts as a search strategy.

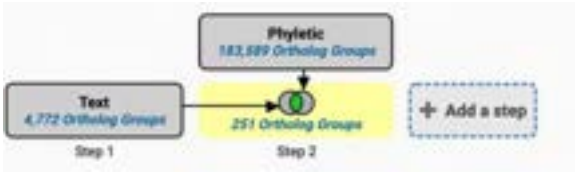
c. Add a step and run a phyletic pattern search for groups that contain any fungi proteins but do not contain any other organism outside fungi. (Hint: make sure everything has a red **x** on it except for fungi, which should be a grey circle (no constraints)). How would this search be different if you used a green check instead of the grey circle for Fungi?



How many groups did the search return?

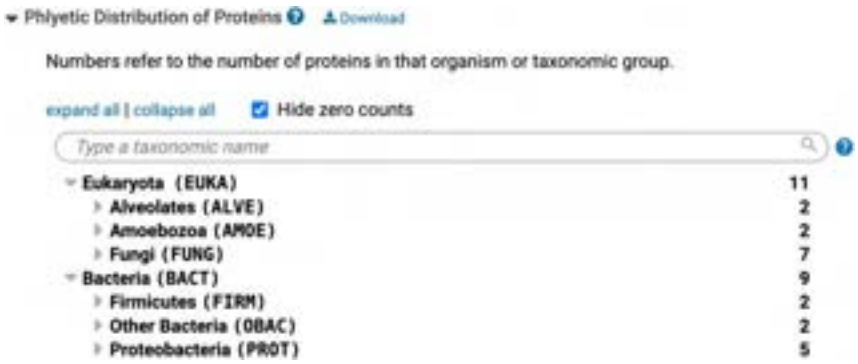


Strategy URL: https://orthomcl.org/orthomcl/app/workspace/strategies/326574223/430551843



Exploring a specific OrthoMCL group - examining the cluster graph.

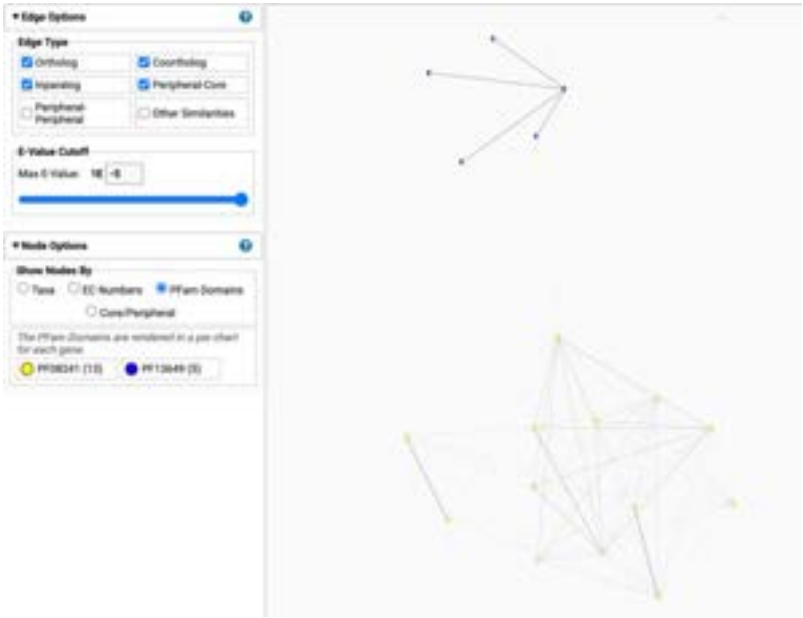
- Visit the OrthoMCL record page for the group **OG6_129371**
- Examine the phyletic distribution tree. What taxa does this group contain?



- Examine the cluster graph for this group (it can be accessed at the bottom of the page)

You can interact with the cluster graph. For example, move the slider to the left to increase to remove less significant edges connections between proteins. Can you identify subclusters? Click on the nodes in the graph – notice how the organism is updated on the right.

On the left of the page in the *Node Options* panel, click on PFam Domains to see which proteins have PFam domains. Is there a pattern to the subclusters?



In the *Node Options* panel, you can click on *Core/Peripheral* to observe which proteins were derived from Core species and which proteins were derived from Peripheral species. Proteins from Core species were used in the initial OrthoMCL algorithm to form Core ortholog groups. Proteins from Peripheral species were mapped into these Core groups by sequence similarity (determined by BLAST score).

What is Galaxy?

Galaxy is an open-source, web-based platform for data analysis. It adheres to the FAIR principles of data sharing and re-use and eliminates the need for command-line scripting, enabling users to conduct, replicate, and disseminate comprehensive large-scale data analyses. In collaboration with Globus [1], the VEuPathDB project has established its own instance of Galaxy.

VEuPathDB Galaxy, accessible at https://fungidb.org/fungidb/app/galaxy-orientation, provides users with pre-loaded genomes, pre-configured workflows, and a suite of tools for private data analysis and visualization. Additionally, a tailored selection of tools facilitates the export of Galaxy results to private workspaces within VEuPathDB sites, accessible via the "My Workspace > My data sets" section. These datasets within the workspace can be explored using the FungiDB interface and tools, seamlessly integrating with public data housed in FungiDB. Accessing VEuPathDB Galaxy necessitates an account with FungiDB/VEuPathDB, which is freely available and applicable across all VEuPathDB genomics sites.

It's important to note that the VEuPathDB Galaxy instance isn't designed for long-term data storage, with datasets automatically purged after 60 days. To retain data, users are advised to download their analysis results locally and subsequently delete and purge files to create space for future analyses.
The Galaxy project offers extensive learning materials that can be accessed here: https://wiki.galaxyproject.org/Learn#Galaxy_101

Important:

- The Galaxy module consists of RNA-Seq and SNP analysis modules.
- All Galaxy exercises will be conducted using the workshop instance of Galaxy (link provided below).
- This a group exercise module. Group activities within Galaxy will be organized into teams of four individuals. Only one person per group should deploy workflows within the workshop Galaxy environment. After the workflow is completed, everyone will get a copy of the workflow.

RNA-Seq analysis, Part I

Learning objectives:

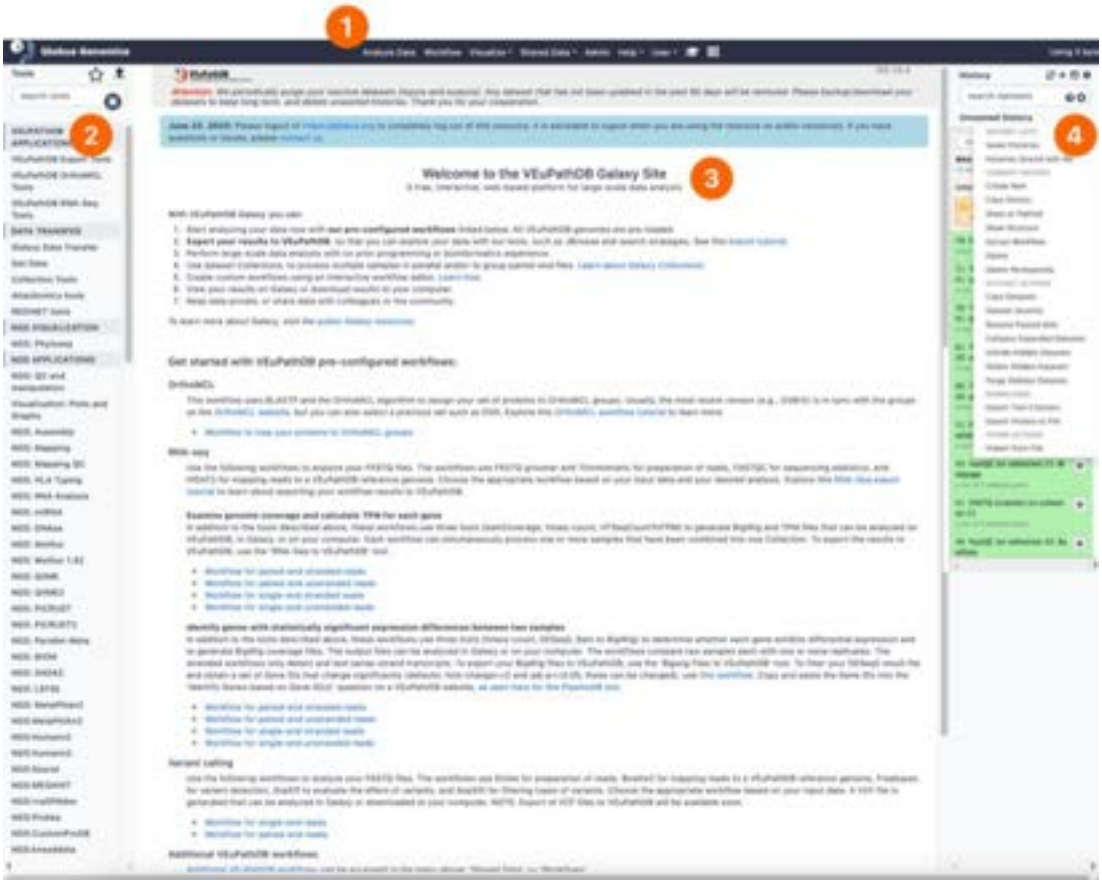
- Become familiar with the VEuPathDB Galaxy workspace.
- Upload raw data into Galaxy workspace and run a pre-configured SNP workflow

During this exercise, we will obtain raw sequence files from the "shared history" section in the workshop instance of VEuPathDB Galaxy. Subsequently, we will process these files using a pre- configured RNA-Seq workflow for paired or single-end reads. This workflow will entail aligning the data to a reference genome, computing gene expression, and interpreting the data.

The anatomy of the VEuPathDB Galaxy landing page.

The workspace comprises four major components:

1. The top menu, which governs the main interface, offers access to the landing page, shared data, public and private workflows, and additional features.
2. The left panel contains a list of available tools, with VEuPathDB export tools featured at the top.
3. The main welcome (landing) page serves as an interactive interface and houses pre- configured workflows, workflow editors, and more.
4. The right panel provides access to histories, and options to delete and purge datasets.



Note: Don’t see Galaxy tools needed for your research? – Let us know by sending an email to help@fungidb.org

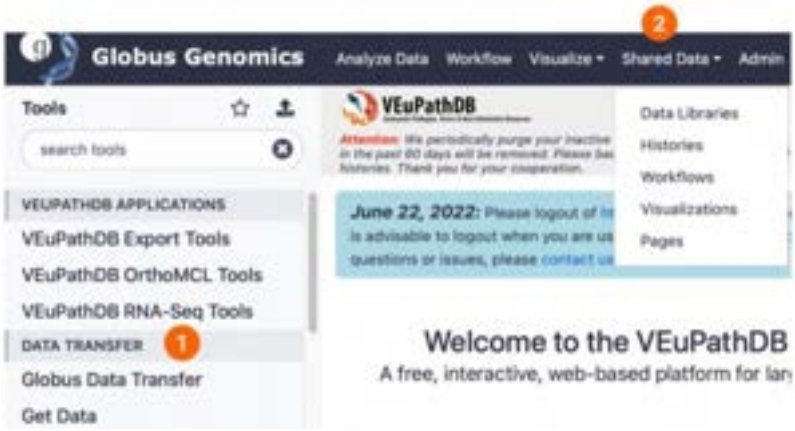
Important:

- If you do not have an account with VEuPathDB/FungiDB, please create one now.

- **Access the workshop instance of VEuPathDB Galaxy.**

1. Click on the following URL to begin: https://veupathdb1.globusgenomics.org/
2. On the next page, choose the “VEuPathDB” option and click on the ‘Continue’ button.
3. If you are not already logged into VEuPathDB, you will be prompted to do so.
4. Click ‘Continue’ on the next page (no need to link an existing account).
5. Select “non-profit” and agree to the Terms of Service. Click ‘Continue’.
6. When asked for grant permissions to use this Galaxy instance. Click ‘Allow’.

There are multiple ways to important data into your Galaxy workspace. For example, you can transfer data via tools located under the “Data Transfer” section in menu on the left (1). You can also transfer data from the “Shared Data” section in the main menu (2).

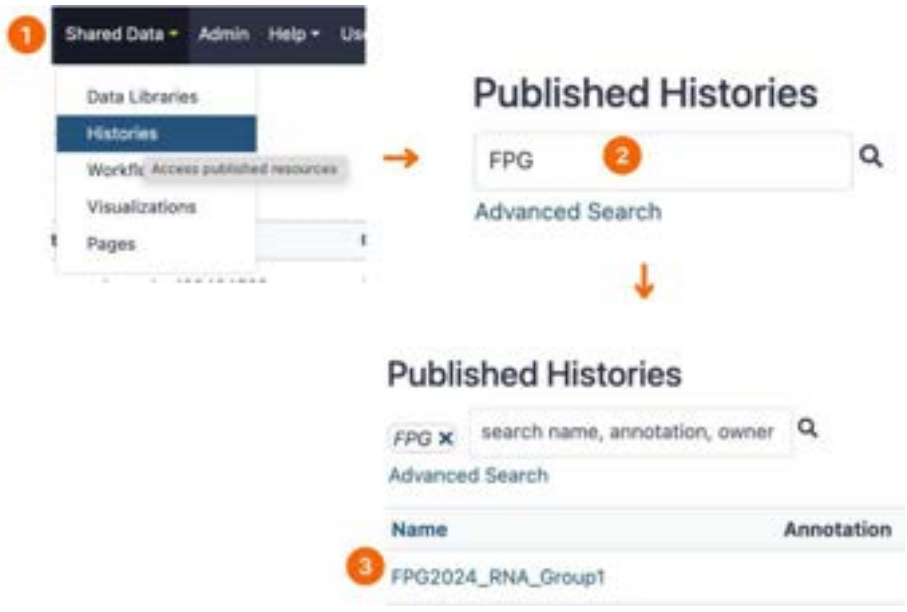


Important:

- In this exercise, we will use the ‘Shared data’ menu to access pre-loaded raw data.
- Only one person per each group should import data files and deploy an SNP workflow. Note: Everyone will get a chance to practice data analysis in the NGS Part 2 module.
- For group assignments, see below.

- **Import data for your SNP workflow via the Shared histories option.**

1. From the top menu, select the ‘Shared Data > Histories’ option.
2. Filter all public workflows on “FPG”.
3. Click on the history link that corresponds to your group number (e.g., FPG2024_RNA_Group1) to import the data into the Galaxy workspace.



Group assignments.

Group 1 *Candida auris.* Analyze transcriptomes from cells grown under high concentrations of tunicamycin. Control: no drug. Single-read data.

Comparison	No drug vs Tunicamycin
History name for download (in Galaxy)	FPG2024_RNA_Group1
Ref genome (in Galaxy)	FungiDB-37_CaurisB8441_Genome

Reference: PMID: n/a BioProject: PRJEB60034

Group 2 *Rhizopus delemar.* Analyze transcriptomes from germinated spores. Control: 0 h. Paired-end data.

Comparison	0 h vs 6 h
History name for download (in Galaxy)	FPG2024_RNA_Group2
Ref genome (in Galaxy)	FungiDB-29_RdelemarRA99-880_Genome

Reference: PMID: PRJNA472797 BioProject: PRJNA472797

Group 3 *Candida parapsilopsis.* Analyze transcriptomes from cells grown under planktonic and biofilm-inducing conditions. Control: planktonic. Paired-end data.

Comparison	Planktonic vs Biofilm
History name for download (in Galaxy)	FPG2024_RNA_Group3
Ref genome (in Galaxy)	FungiDB-42_CparapsilosisCDC317_Genome

Reference: PMID: 25233198 BioProject: PRJNA246482

Group 4 *Coccidioides posadasii*. Analyze transcriptomes from mycelia (non-pathogenic stage) and spherules (pathogenic stage). Single read data.

Comparison	Mycelia vs Spherules
History name for download (in Galaxy)	FPG2024_RNA_Group4
Ref genome (in Galaxy)	FungiDB-61_CposadasiiSilveira2022_Genome

Reference: PMID: 22911737 BioProject: PRJNA169242

Group 5 *Fusarium graminearum*. Analyze spore and mycelial transcriptomes. Paired-end data.

Comparison	Spores vs Mycelia
History name for download (in Galaxy)	FPG2024_RNA_Group5
Ref genome (in Galaxy)	FungiDB-31_FgraminearumPH-1_Genome

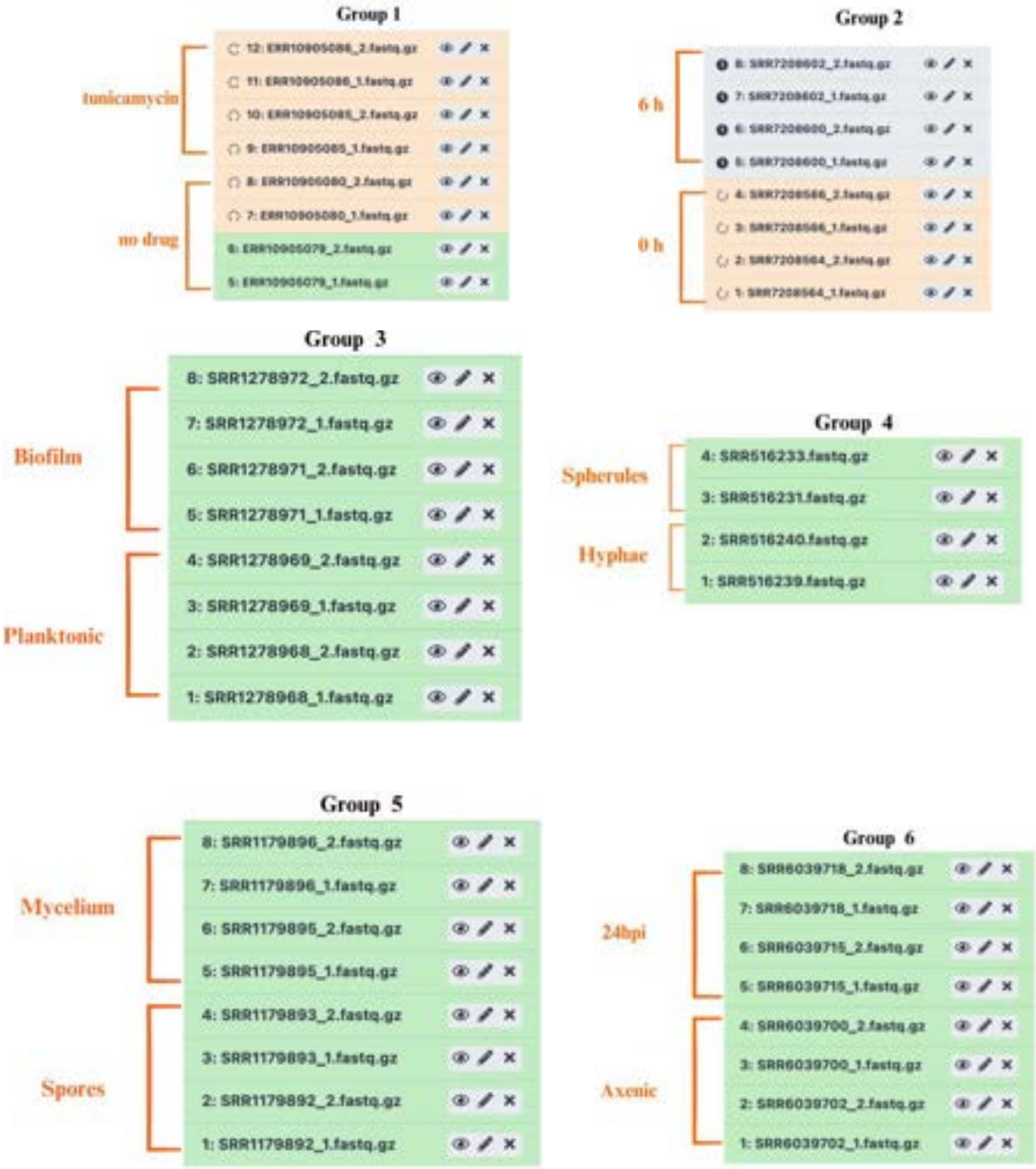
Reference: PMID: 24625133 BioProject: PRJNA239711

Group 6 *Ustilago maydis*. Analyze transcriptomes from plant-associated development samples (axenic culture vs 24 hours post infection (hpi)). Paired-end data.

Comparison	axenic vs 24hpi
History name for download (in Galaxy)	FPG2024_RNA_Group6
Ref genome (in Galaxy)	FungiDB-51_Umaydis521_Genome

Reference: PMID: 33653886 BioProject: PRJNA407369

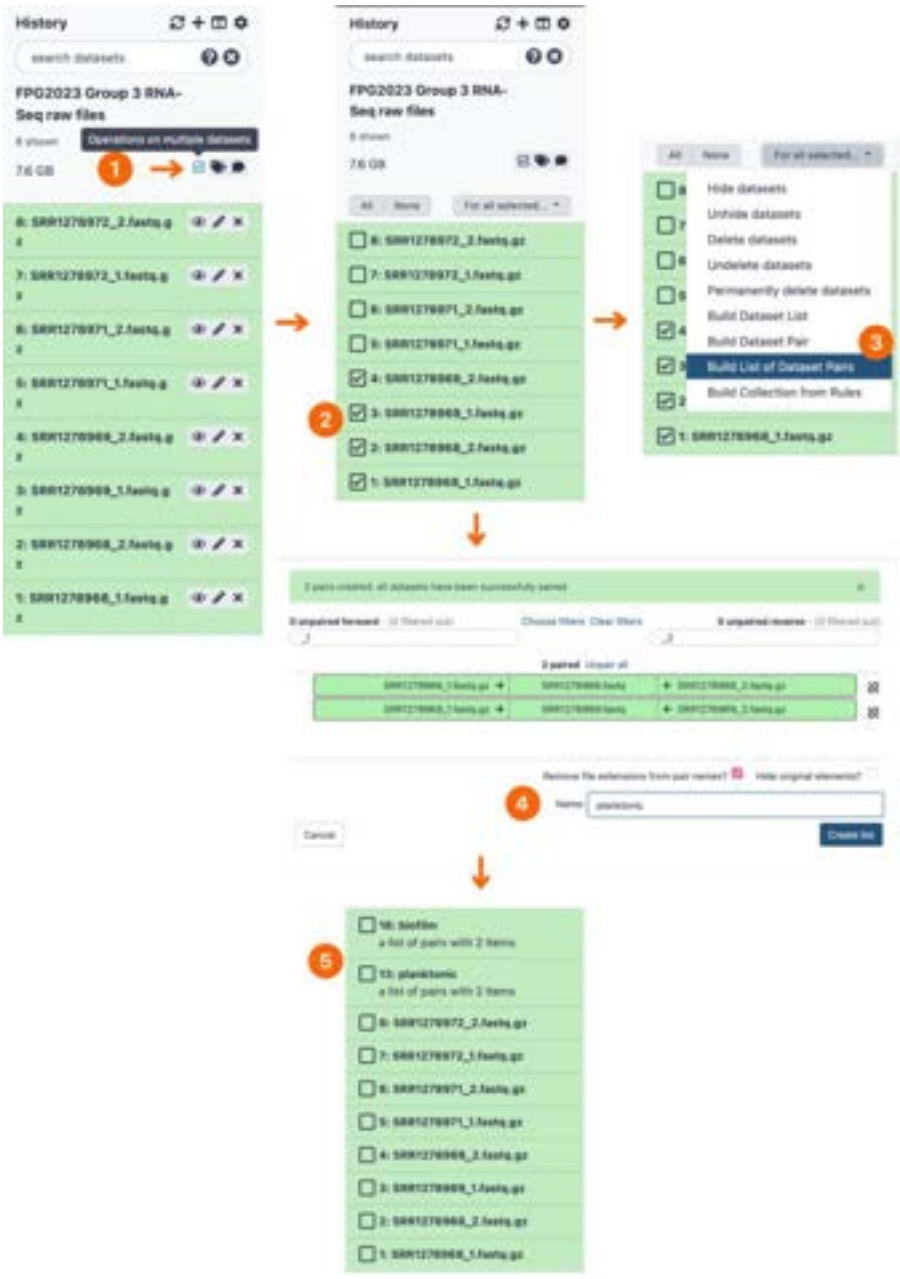
Guide to RNA-Seq histories and file organisation.



Each dataset contains two replicates. For datasets with multiple samples (e.g., containing biological replicates), it is useful to organize them into “Collections” (e.g., spore and mycelia). Organizing samples with replicates into collections also reduces the complexity of Galaxy workflows.

- **Organize samples with replicates into collections:**

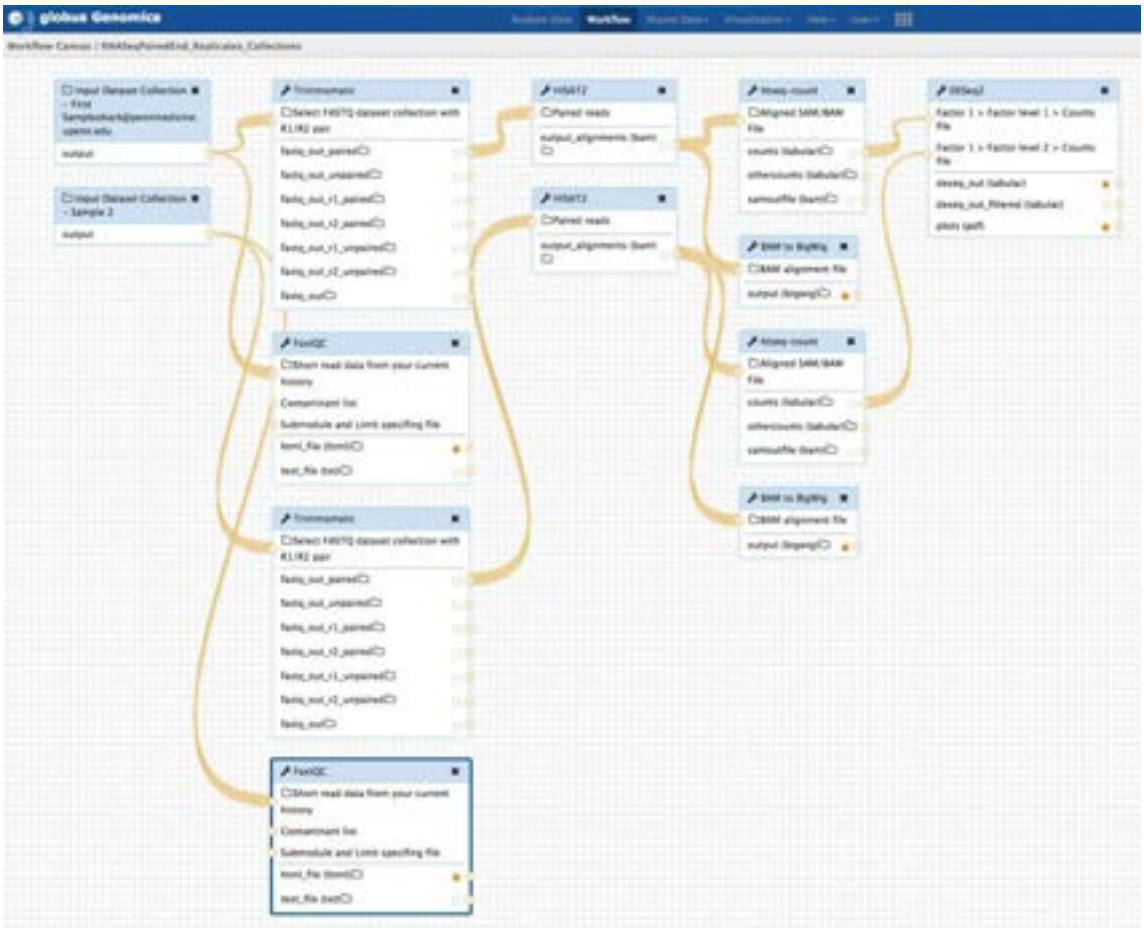
1. Click on the checkbox function “operation on multiple datasets”.
2. Select samples that belong to the same condition (control samples will appear at the bottom, see file mapping notes for each group below).
3. Click on “For all selected” and choose “Build List of Dataset Pairs”.
4. Name the sample (e.g. planktonic) and click “Create List”. Note: Usually, the correct pairs are auto-selected.
5. Repeat for the comparator sample. You should end up with 2 datasets (e.g., planktonic and biofilm).



Running a workflow in Galaxy

You can create your own workflows in Galaxy using the tools from the menu on the left. For this exercise, we will use a preconfigured workflow that consists of the following steps:

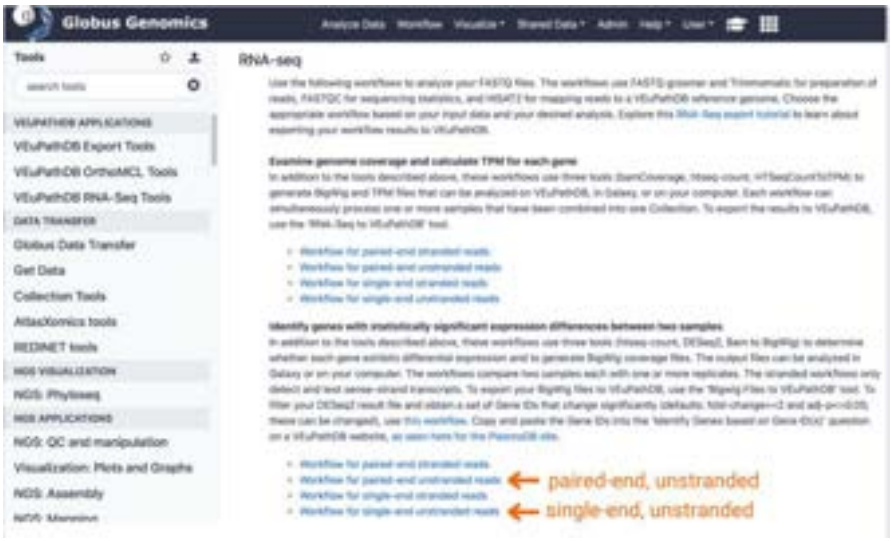
1. Input: raw data, dataset collections.
2. FASTQC: analyse for quality, generate read quality reports.
3. Trimmomatic: trims the reads based on their quality scores and adaptor sequences.
4. HISAT2: align reads to a reference and generate coverage plots.
5. HTSeq: estimate abundance (read counts per gene), generate coverage plots for JBrowse (BAM to BigWig).
6. DESeq2: differential expression of genes between samples.



- **Deploy a pre-configured workflow.**

To do this, navigate to the Galaxy home page and select the workflow appropriate for your dataset:

  - For paired-read datasets choose “Workflow for paired-end unstranded reads”.
  - For single read data, choose “Workflow for single-end unstranded reads”.



- **Configure an RNA-Seq workflow.**

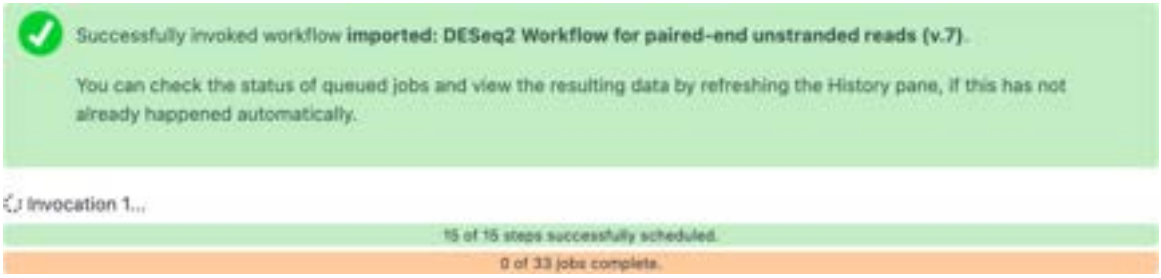
There are multiple steps in the workflow, but you do not need to configure all of them. For this exercise, you will need to configure the following:

1. Input dataset collection 1 (e.g., planktonic).
2. Input dataset collection 2 (e.g., biofilm).
3. Both HISAT2 steps (requires reference genome – refer to the group assignments section above for this info).
4. Both htseq-count steps (requires reference genome – refer to the group assignments section above for this info).
5. DESeq2 (requires reference genome – refer to the group assignments section above for this info).

Make sure to set the correct reference genomes for HISAT2, htseq-count, and DESeq2 steps. It is critical that you select the correct genome that matches the experimental organism for your samples:

Name you factor levels. This helps keep everything organized and name properly in the workflow. Each factor level is typically the name of the condition, like “mycelia” or “spore”.

a. Once you are sure everything is configured correctly, click on “Run Workflow” at the top.



The steps will start running in the history section on the right. Grey means they are waiting to start. Yellow means they are running. Green means they have completed. Red means there was an error in the step.

How to work with Galaxy editor (optional)

You can create your own workflows. An interactive workflow editor allows you to add and configure tools.

1. Navigate to the “Shared Data” menu.
2. Click on “Workflows”.
3. Left-click on the “FPG2023 workflow editor practice” work to “import”
4. Once the workflow is important in your workspace, left-click and select “edit.

Once you are in the workflow editor:

5. Delete the Trimmomatic - HISAT2 connection.
6. Re-establish the connection by linking the “Trimmomatic on input dataset(s): paired (input) step to the “Paired reads” option in the HISTAS2.

7. Delete HISAT2 step completely by clicking on the “x” in the top right corner and use the tools menu on the left to insert it back.

Note: Sometimes, you may be unable to re-establish a connection. When this happens, take a look at the tool documentation notes in the right panel, and check your selection for single-read or paired-end setting in particular (paired-end setting must be selected if you are dealing with reverse and forward reads).

Now that you have learned the principles of workflow editing, you can either practice saving the workflow by clicking on the wheel at the far top corner or simply existing the workflow editor without saving.

Variant Calling analysis, Part I.

Learning objectives:

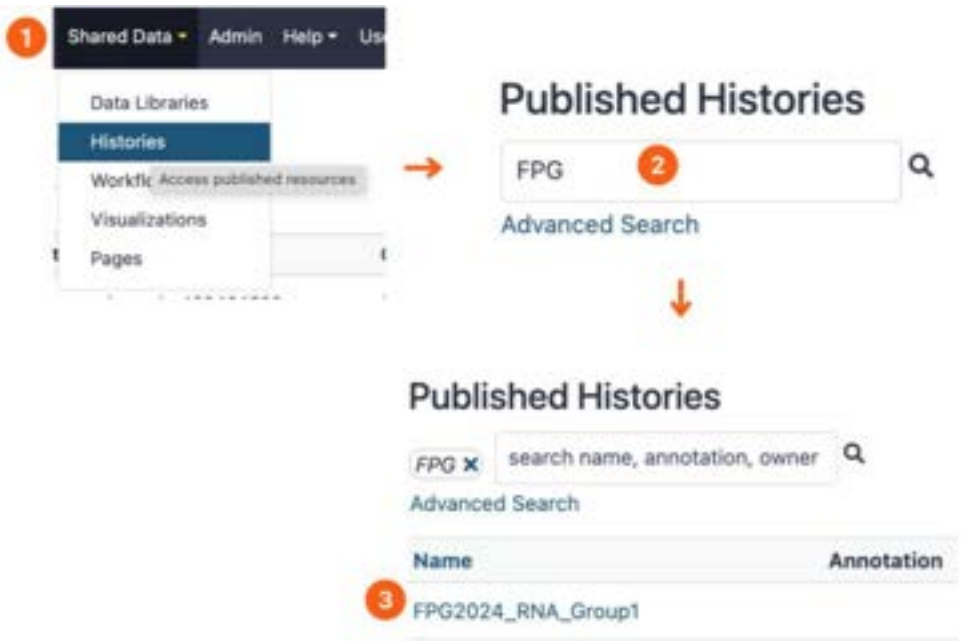
- Become familiar with the VEuPathDB Galaxy workspace.
- Upload raw data into Galaxy workspace and run a pre-configured SNP workflow

Important:

- In this exercise, we will use the 'Shared data' menu to access pre-loaded raw data.
- Only one person per each group should import data files and deploy a SNP workflow. Note: Everyone will get a chance to practice data analysis in the NGS Part 2 module.
- For group assignments, see below.

- **Import data for your SNP workflow via the Shared histories option.**

1. From the top menu, select 'Shared Data > Histories' option.
2. Filter all public workflows on "FPG2024"..
3. Click on the history link that correspond to your group number (e.g., FPG2024_SNP_Group1) to import the data into the Galaxy workspace.



Group assignments.

Group 1 *Aspergillus fumigatus*. AFIS2503 clinical isolate from pleural fluid of a patient. Paired-end data.

History name	FPG2024_SNP_Group1
Ref genome (in Galaxy)	FungiDB-29_AfumigatusAf293_Genome

Group 2 *Aspergillus fumigatus*. AFIS1415 clinical isolate from pleural fluid of a patient. Paired-end data.

History name	FPG2024_SNP_Group2
Ref genome (in Galaxy)	FungiDB-29_AfumigatusAf293_Genome

Group 3 *Zymoseptoria tritici*. ST16CH_1A27 isolate collected from common wheat (Triticum aestivum) in Eschikon, Switzerland.
Paired-end data.

History name	FPG2024_SNP_Group3
Ref genome (in Galaxy)	FungiDB-34_ZtriticilPO323_Genome

Group 4 *Zymoseptoria tritici*. ORE15_Mad_G1isolate collected from common wheat (Triticum aestivum) in Oregon, USA.
Paired-end data.

History name	FPG2024_SNP_Group4
Ref genome (in Galaxy)	FungiDB-34_ZtriticilPO323_Genome

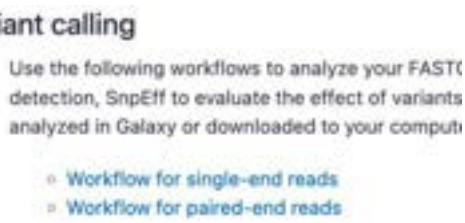
Group 5 *Candida auris*. VPCI-F37-B-2021 isolate collected from an apple surface in India. Paired-end data.

History name	FPG2024_SNP_Group5
Ref genome (in Galaxy)	FungiDB-37_CaurisB8441_Genome

Group 6 *Candida auris*. VPCI-F1-A-2020 isolate collected from an apple surface in India. Paired-end data.

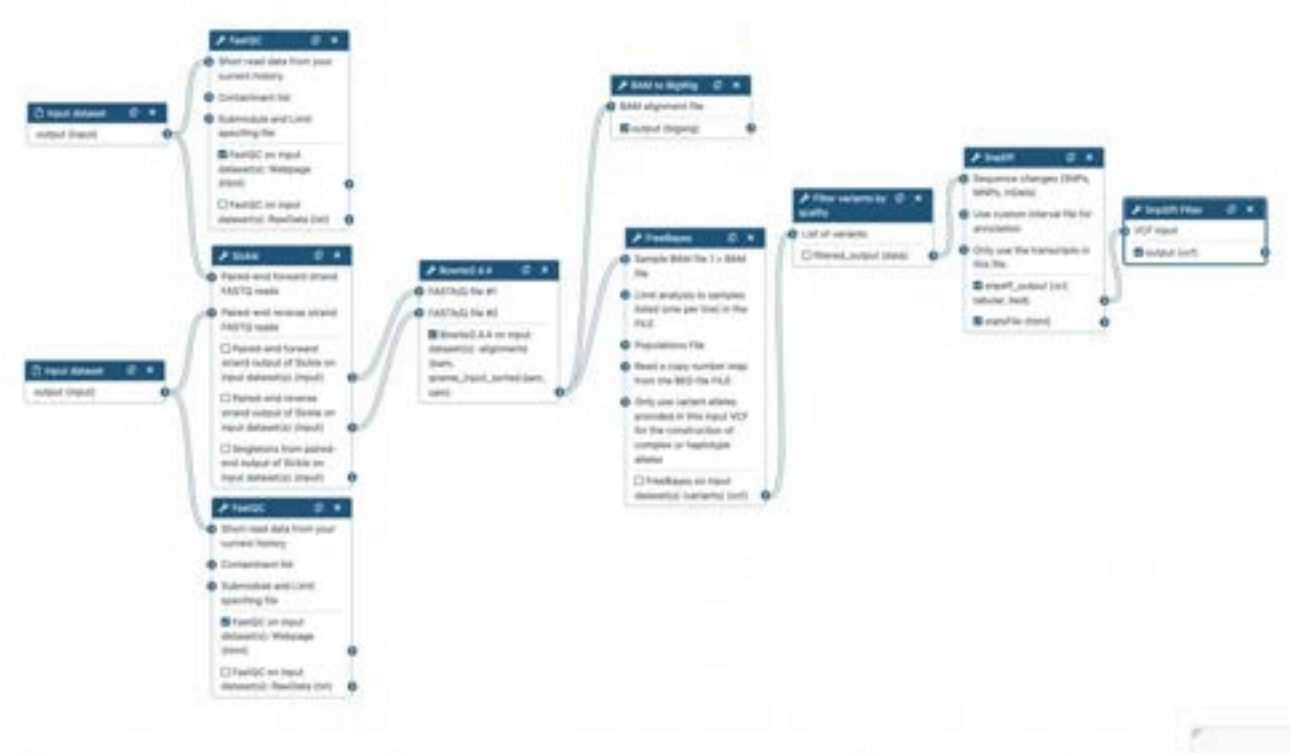
History name	FPG2024_SNP_Group6
Ref genome (in Galaxy)	FungiDB-37_CaurisB8441_Genome

Once the data files have been transferred into your galaxy history, you will need to choose a workflow appropriate for your data (paired or single -read).



The pre-configured workflows follow these steps:

- Determine quality of the reads in your files and generates FASTQC reports.
- Trim reads based on their quality scores.
- Align reads to a reference genome using Bowtie2 and generating coverage plots.
- Sort alignments with respect to their chromosomal positions.
- Detect variants using FreeBayes.
- Filter SNP candidates.
- Analyze and annotate of variants, and calculation of the effects via SnpEff.



- **Define workflow parameters.**

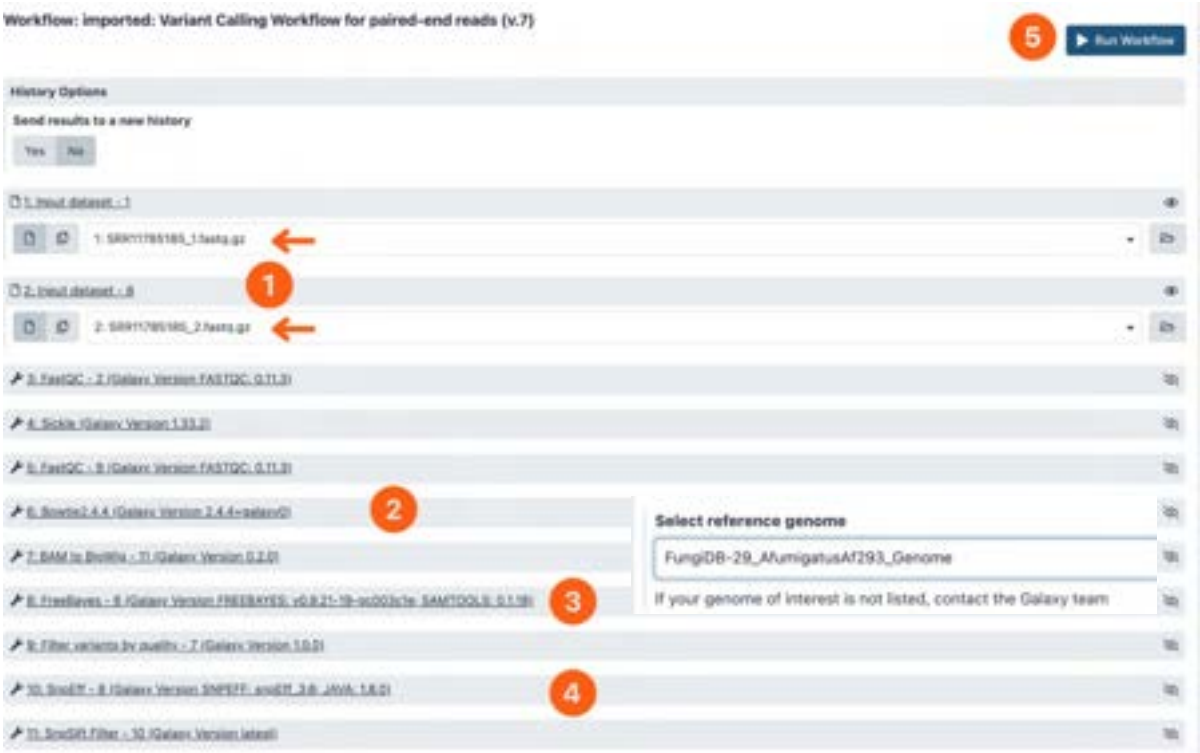
    1. For paired-end data, make sure that the input steps are set to the *xxxx_1.fastq.gz* and *xxxx_2.fastq.gz* (Default will have the same file selected for both input files). Hint: for single read data, you will have only one file.

    2. Select reference genome for Bowtie2
    Hint: or reference genome infromatio, see group assignment table above).

    3. Repeat genome selection for FreeBayes.

    4. Select the same genome for SnpEff.

    5. Click Run Workflow.



References

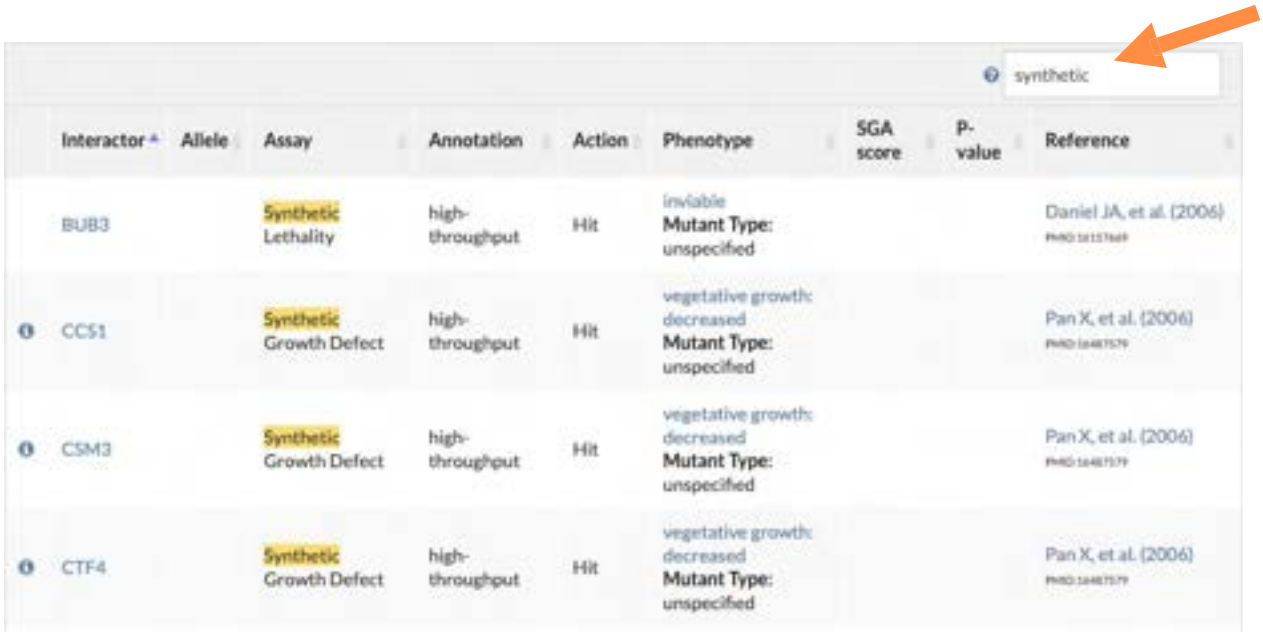
1. Foster I. 2011. Globus online: accelerating and democratizing science through cloud-based services. IEEE Internet Comput. 15(3):70–73. doi:10.1109/MIC.2011.64.

Using SGD GO Slim Mapper and Interaction Data to Predict Gene Function

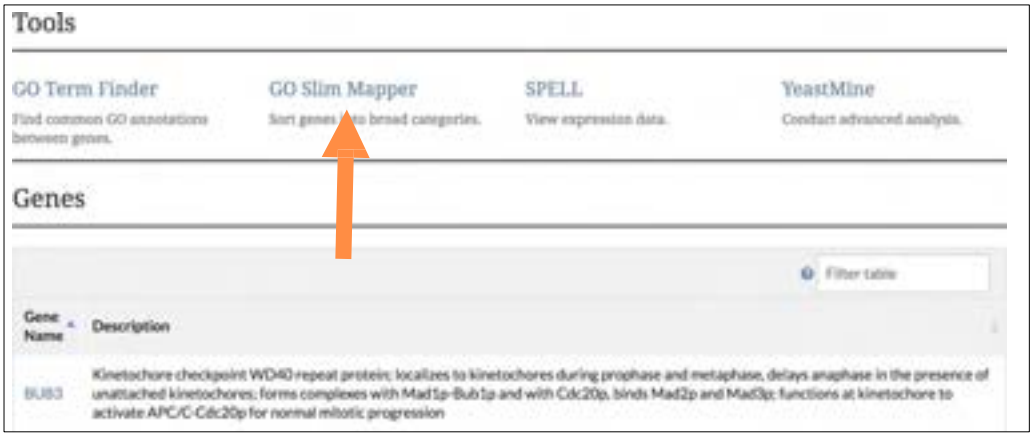
The Gene Ontology (GO) is structured in a hierarchy, such that granular terms (“perinuclear space”) are connected and further down the hierarchy than their related broader terms (“nucleus”). However, for many purposes, such as reporting the upregulated cellular functions of a transcriptomics experiment, is very useful to focus on the broad, high-level part of the GO. For example, if you were interested in which of your upregulated genes are involved in DNA replication, it would be useful to map genes that have been annotated to specific terms (e.g. “synthesis of RNA primer involved in nuclear cell cycle DNA replication”) to more general terms (e.g. “DNA replication”).

The **Gene Ontology (GO) Slim Mapper** at SGD maps granular GO annotations of a group of genes to more general terms and/or bins them into broad categories, i.e., “**GO Slim**” terms.
Using GO Slim Mapper, predict what biological processes an uncharacterized gene may be involved in based on its genetic interactions.

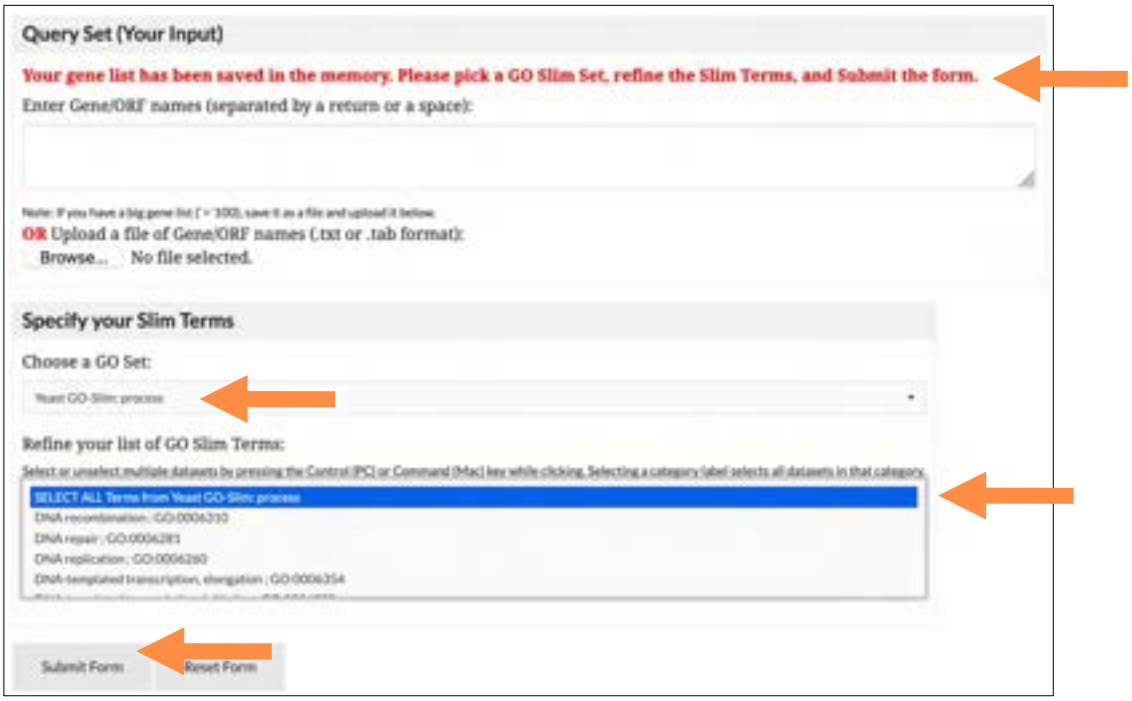
- From the SGD home page (www.yeastgenome.org), go to the Locus Summary page for the uncharacterized gene **YLR287C.**
- Select **Genetic Interactions** tab. Here, we are interested in finding genes that have a genetic interaction with YLR287C, as the function of these genes may provide hints about the function of YLR287C.
- Search for “synthetic” in the **Genetic Interactions** table. This will filter the table for genes that, when knocked out in combination with YLR287C, elicit some sort of synthetic growth defect, haploinsufficiency, lethality, etc. These harsh phenotypes may suggest clues about related functions to YLR287C.



- Find and click on the **Analyze** button at the bottom of the Annotation table. This will import the table you filtered to a page where you can send the genes to other SGD tools.
- On the next page that lists the YLR287C interactors, select **GO Slim Mapper**.

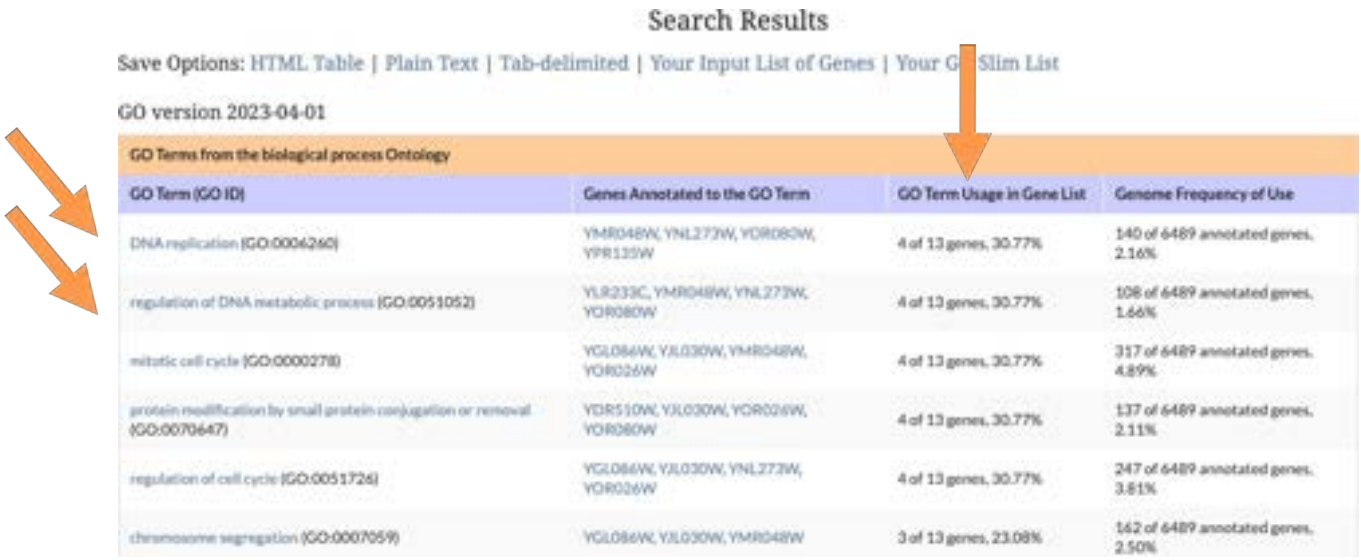


- The GO Slim Mapper has three steps (plus one optional step) in which you can specify your query. The Query Set (Your Input) box has been preloaded in memory with the list of genes you imported from the table.



- Choose a **GO Set** by selecting **Yeast GO-Slim: Process** from the pull-down.
- Highlight **SELECT ALL Terms from Yeast GO-Slim: Process.**
- Click the **Submit Form** button to use the default settings or go further down to customize your query.

- Results appear in a table with four columns:
    a. GO Slim terms picked by GO Slim Mapper
    b. Genes from your list that are annotated to that term, hyperlinked to their Locus Summary pages.
    c. GO Term Usage in Gene List (cluster frequency), the number and percentage of genes in your list annotated to each term.
    d. Genome frequency of use, the number and percentage of all genes in the genome annotated to each term.
- You can also download the results in a tab-delimited file.



Search Results

Save Options: HTML Table | Plain Text | Tab-delimited | Your Input List of Genes | Your G Slim List

GO version 2023-04-01

GO Terms from the biological process Ontology			
GO Term (GO ID)	Genes Annotated to the GO Term	GO Term Usage in Gene List	Genome Frequency of Use
DNA replication (GO:0006260)	YMR048W, YNL273W, YOR080W, YPR135W	4 of 13 genes, 30.77%	140 of 6489 annotated genes, 2.16%
regulation of DNA metabolic process (GO:0051052)	YLR233C, YMR048W, YNL273W, YOR080W	4 of 13 genes, 30.77%	108 of 6489 annotated genes, 1.66%
mitotic cell cycle (GO:0000278)	YGL086W, YJL030W, YMR048W, YOR026W	4 of 13 genes, 30.77%	317 of 6489 annotated genes, 4.89%
protein modification by small protein conjugation or removal (GO:0070647)	YDR510W, YJL030W, YOR026W, YOR080W	4 of 13 genes, 30.77%	137 of 6489 annotated genes, 2.11%
regulation of cell cycle (GO:0051726)	YGL086W, YJL030W, YNL273W, YOR026W	4 of 13 genes, 30.77%	247 of 6489 annotated genes, 3.81%
chromosome segregation (GO:0007059)	YGL086W, YJL030W, YMR048W	3 of 13 genes, 23.08%	162 of 6489 annotated genes, 2.50%

- Based on the results, what biological processes might YLR287C be involved in?

GO Enrichment, Phenotype Data at CGD

The Gene Ontology (GO) provides a common language to describe aspects of a gene product's biology. GO Terms are standardized phrases, arranged in a hierarchy, that describe a gene product's **molecular function** ("protein kinase activity"), **biological process** ("gluconeogenesis"), and **cellular component** ("cytoplasm"). Together, molecular function, biological process, and cellular component are the three ontologies of GO that describe a gene product's function, the processes that function is involved in, and the location where the function is performed.

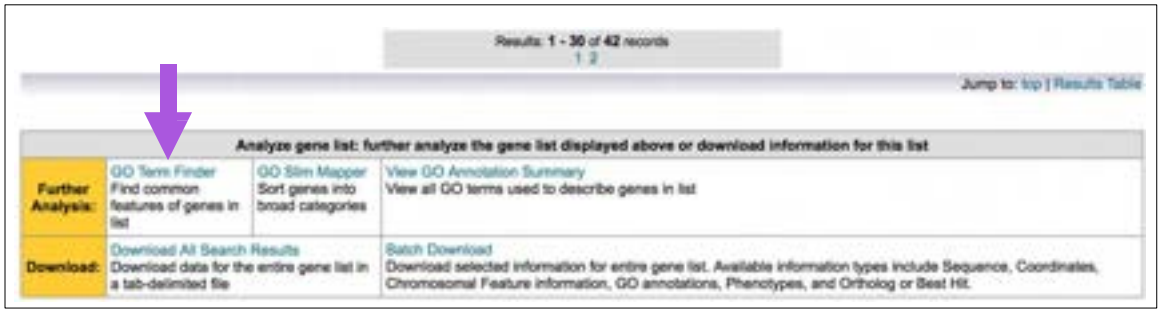
GO Term Finder takes a list of genes and identifies what GO terms are significant for the list. It is a powerful way to interpret the results of omics experiments or any situation where determining common functions and roles are important. For example, GO Term Finder can take a list of upregulated genes from an RNA-Seq experiment and determine what biological processes are significant for the set of genes, providing an idea of what processes are being upregulated in the cell.

In this exercise, we will attempt to uncover what processes are important for hygromycin B tolerance in *C. albicans.* To do so, we will use the CGD GO Term Finder to find shared biological processes for a set of genes whose mutation lowers resistance to hygromycin B.

- From the CGD home page (www.candidagenome.org), go to the Locus Summary page for the hygromycin B-sensitivity gene PMT6. Enter **PMT6** into the **search our site** box and click **GO**. On the next page, under ***Candida albicans* Search Results**, click on hyperlinked **1 Gene names (gene name/alias/ORF name)**.

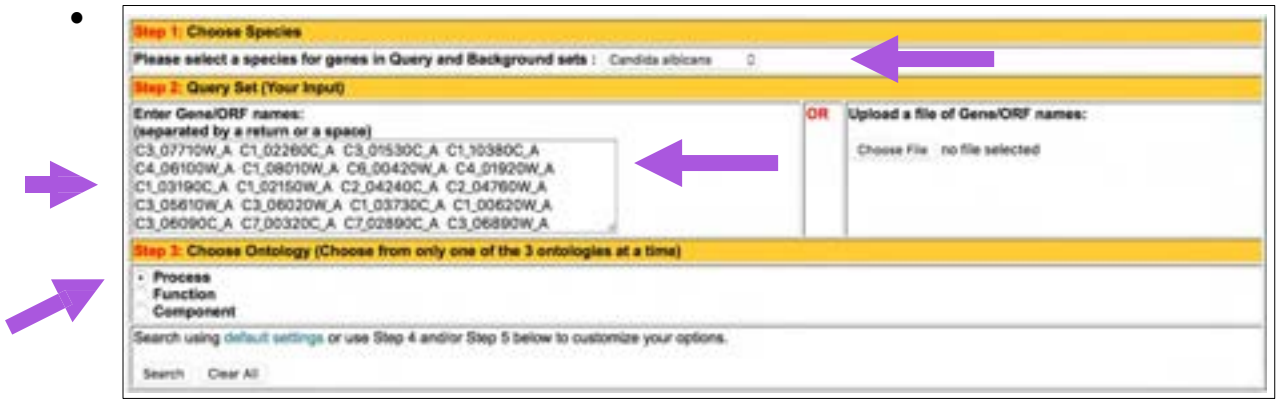
- From the PMT6 Locus Summary page, find other genes involved in hygromycin B sensitivity: scroll down to the **Mutant Phenotype** section and click on **resistance to Hygromycin B: decreased**

- On the **Phenotype Search Results** page, click on **Jump to: Analyze Gene List** above the table on the right (or simply scroll down to the bottom of the page). Click on **GO Term Finder** link.



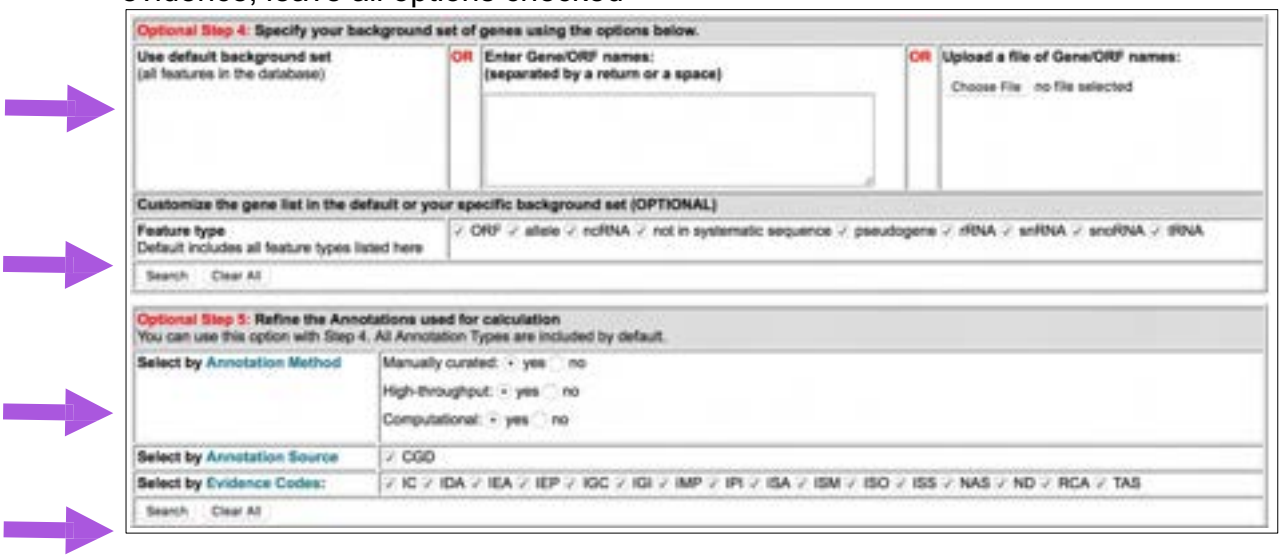
  - With your own list of genes, you can access GO Term Finder from any CGD page by opening **GO** menu in the banner on top and clicking on **GO Term Finder**. Or you use this URL: http://www.candidagenome.org/cgi-bin/GO/goTermFinder

- The **CGD Gene Ontology Term Finder** has five steps (two optional) to specify your query. First, make sure that **Candida albicans** is selected as your species.
- Your input genes should be already entered. Alternatively, copy and paste your own list of genes into the text box (note: the more genes processed, the longer it takes).Choose **Process** as the ontology. Click the **Search** button to use the default settings.
-

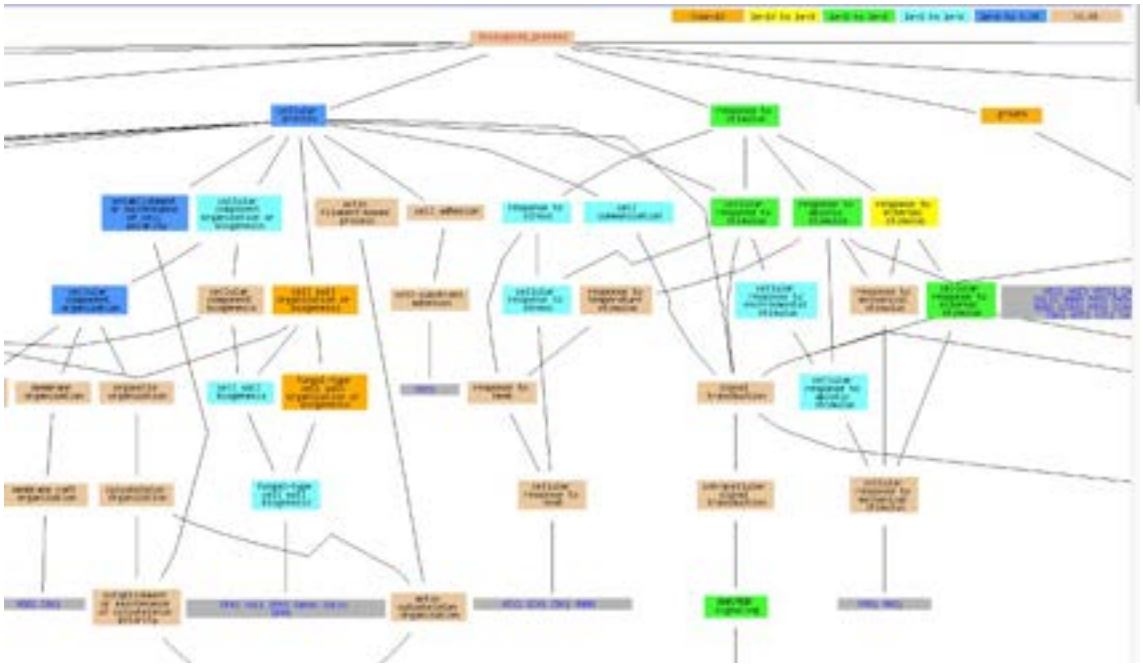


You can further customize your query in the next steps down the page:

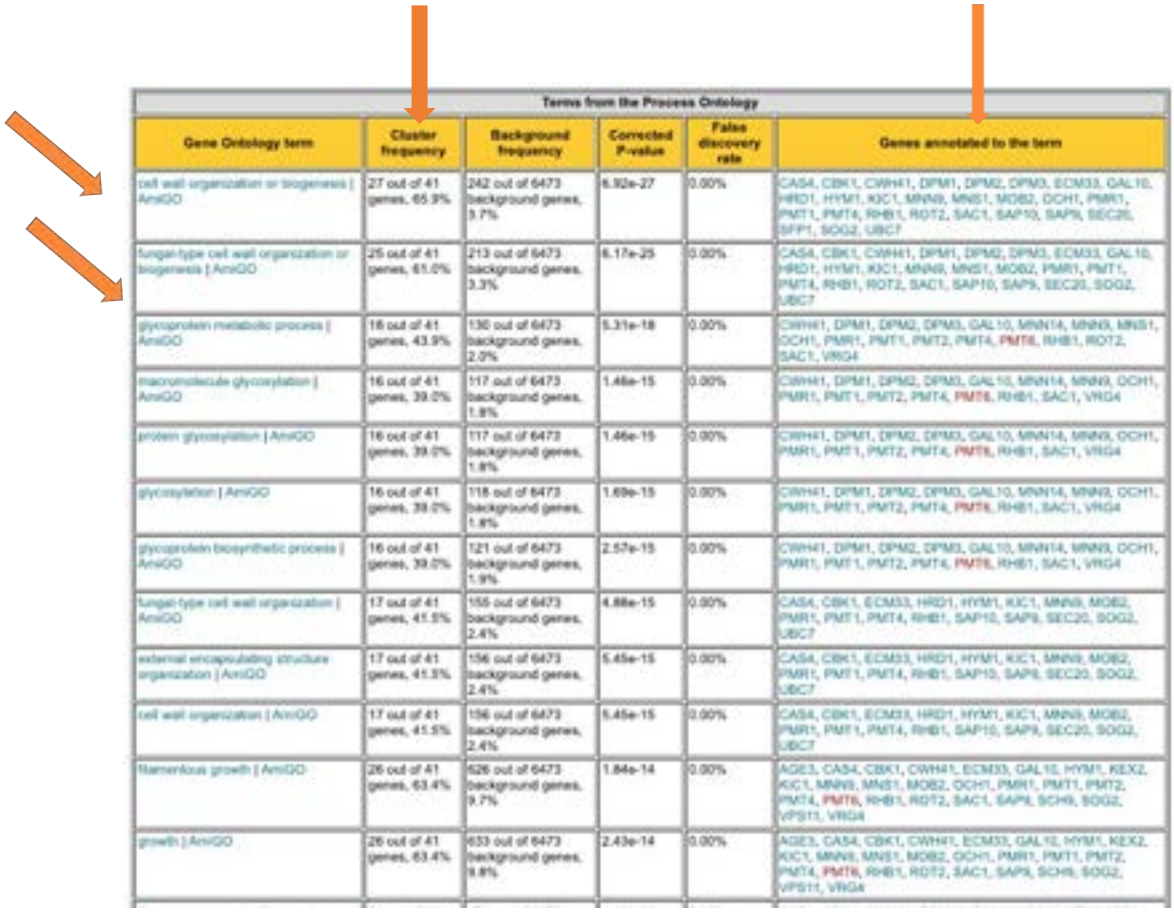
- Optional Step 4 allows submitting a custom background set; use default set, all *C. albicans* genes in CGD
- Step 4 also allows restricting the search to specific feature types; use default settings
- Optional Step 5 allows selection of annotation methods, sources and evidence; leave all options checked



- Click **Search**. The input is checked and any genes that are not recognized as valid for the selected *Candida* species are rejected; click on **Proceed** in the following window.
- The results page displays the significant shared GO terms (or their parents) in both graphic and table form, within the set of genes associated with hygromycin B sensitivity entered on the previous page:
  - The graph shows the GO tree that includes terms used directly or indirectly in annotations for the genes in your list.
  - The terms are color-coded to indicate their statistical significance (p-value score), where the terms in **orange** have the highest likelihood of sharing meaningful relationships for the genes in your list.
  - Genes associated with the GO terms are shown in gray boxes, with links to their respective Locus Summary pages.



- The table below the graph lists each significant GO term, the number of times the GO term is used to annotate genes in the list, and the number of times that the term is used to annotate genes in the background set (all genes in *C. albicans* genome)



Terms from the Process Ontology					
Gene Ontology term	Cluster frequency	Background frequency	Corrected P-value	False discovery rate	Genes annotated to the term
cell wall organization or biogenesis | AmiGO	27 out of 41 genes, 65.9%	242 out of 6473 background genes, 3.7%	6.92e-27	0.00%	CAS4, CBK1, CWH41, DPM1, DPM2, DPM3, ECM33, GAL10, HRD1, HYM1, KIC1, MNN9, MNS1, MOB2, OCH1, PMR1, PMT1, PMT4, RHB1, ROT2, SAC1, SAP10, SAP9, SEC20, SFP1, SOG2, UBC7
fungal-type cell wall organization or biogenesis | AmiGO	25 out of 41 genes, 61.0%	213 out of 6473 background genes, 3.3%	6.17e-25	0.00%	CAS4, CBK1, CWH41, DPM1, DPM2, DPM3, ECM33, GAL10, HRD1, HYM1, KIC1, MNN9, MNS1, MOB2, PMR1, PMT1, PMT4, RHB1, ROT2, SAC1, SAP10, SAP9, SEC20, SOG2, UBC7
glycoprotein metabolic process | AmiGO	18 out of 41 genes, 43.9%	130 out of 6473 background genes, 2.0%	5.31e-18	0.00%	CWH41, DPM1, DPM2, DPM3, GAL10, MNN14, MNN9, MNS1, OCH1, PMR1, PMT1, PMT2, PMT4, PMT6, RHB1, ROT2, SAC1, VRG4
macromolecule glycosylation | AmiGO	16 out of 41 genes, 39.0%	117 out of 6473 background genes, 1.8%	1.48e-15	0.00%	CWH41, DPM1, DPM2, DPM3, GAL10, MNN14, MNN9, OCH1, PMR1, PMT1, PMT2, PMT4, PMT6, RHB1, SAC1, VRG4
protein glycosylation | AmiGO	16 out of 41 genes, 39.0%	117 out of 6473 background genes, 1.8%	1.46e-15	0.00%	CWH41, DPM1, DPM2, DPM3, GAL10, MNN14, MNN9, OCH1, PMR1, PMT1, PMT2, PMT4, PMT6, RHB1, SAC1, VRG4
glycosylation | AmiGO	16 out of 41 genes, 39.0%	118 out of 6473 background genes, 1.8%	1.69e-15	0.00%	CWH41, DPM1, DPM2, DPM3, GAL10, MNN14, MNN9, OCH1, PMR1, PMT1, PMT2, PMT4, PMT6, RHB1, SAC1, VRG4
glycoprotein biosynthetic process | AmiGO	16 out of 41 genes, 39.0%	121 out of 6473 background genes, 1.9%	2.57e-15	0.00%	CWH41, DPM1, DPM2, DPM3, GAL10, MNN14, MNN9, OCH1, PMR1, PMT1, PMT2, PMT4, PMT6, RHB1, SAC1, VRG4
fungal-type cell wall organization | AmiGO	17 out of 41 genes, 41.5%	155 out of 6473 background genes, 2.4%	4.88e-15	0.00%	CAS4, CBK1, ECM33, HRD1, HYM1, KIC1, MNN9, MOB2, PMR1, PMT1, PMT4, RHB1, SAP10, SAP9, SEC20, SOG2, UBC7
external encapsulating structure organization | AmiGO	17 out of 41 genes, 41.5%	156 out of 6473 background genes, 2.4%	5.45e-15	0.00%	CAS4, CBK1, ECM33, HRD1, HYM1, KIC1, MNN9, MOB2, PMR1, PMT1, PMT4, RHB1, SAP10, SAP9, SEC20, SOG2, UBC7
cell wall organization | AmiGO	17 out of 41 genes, 41.5%	156 out of 6473 background genes, 2.4%	5.45e-15	0.00%	CAS4, CBK1, ECM33, HRD1, HYM1, KIC1, MNN9, MOB2, PMR1, PMT1, PMT4, RHB1, SAP10, SAP9, SEC20, SOG2, UBC7
filamentous growth | AmiGO	26 out of 41 genes, 63.4%	626 out of 6473 background genes, 9.7%	1.84e-14	0.00%	AGE3, CAS4, CBK1, CWH41, ECM33, GAL10, HYM1, KEX2, KIC1, MNN9, MNS1, MOB2, OCH1, PMR1, PMT1, PMT2, PMT4, PMT6, RHB1, ROT2, SAC1, SAP9, SCH9, SOG2, VPS11, VRG4
growth | AmiGO	26 out of 41 genes, 63.4%	633 out of 6473 background genes, 9.8%	2.43e-14	0.00%	AGE3, CAS4, CBK1, CWH41, ECM33, GAL10, HYM1, KEX2, KIC1, MNN9, MNS1, MOB2, OCH1, PMR1, PMT1, PMT2, PMT4, PMT6, RHB1, ROT2, SAC1, SAP9, SCH9, SOG2, VPS11, VRG4

- Additional columns list the p-value, the false discovery rate (FDR), and a list of all the genes annotated, either directly or indirectly, to the term. FDR is an estimate of the percent chance that a particular GO term might actually be a false positive. It represents the fraction of the nodes with p-values as good or better than the node with this FDR that would be expected to be false positives.

- Explore the table. Based on the results, what biological processes are important for resisting the antibiotic action of hygromycin B in *C. albicans* cells?

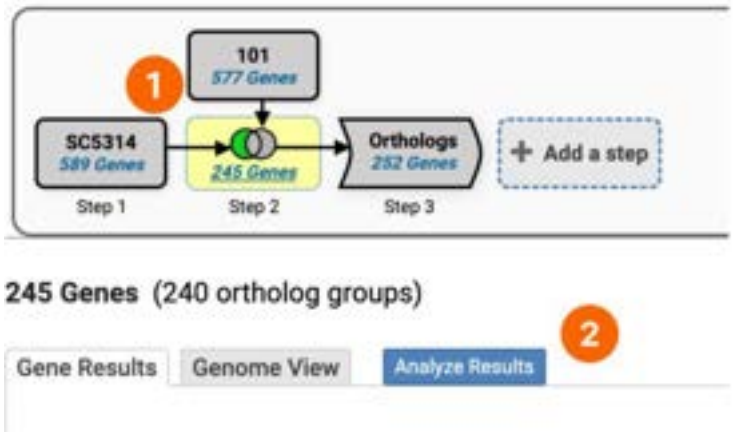
FungiDB: Performing GO Enrichment analysis

Learning objectives:

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

- **Perform enrichment analysis on *C. albicans* SC5314 gene upregulated when the pathogen is exposed to mucosal surfaces.**
  - Use a search strategy created in the 'Transcriptomics & Proteomics'. Strategy URL: https://fungidb.org/fungidb/app/workspace/strategies/import/802d9f2b606fc1fa

1. Click on Step 2 to highlight upregulated genes in *C. albicans* SC5314 only.
2. Click on the "Analyze Results" tab for enrichment analysis options.



The enrichment analysis tools can be accessed under the blue Analyze Results tab. They include Gene Ontology, Metabolic Pathway, and Word Enrichment tools. The three types of analysis apply Fisher's Exact test to evaluate ontology terms, overrepresented 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 the Benjamini-Hochberg false discovery rate and Bonferroni methods.

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

GO enrichment analysis can be performed on the following ontology groups:

- Molecular function,
- Biological processes,
- Cellular component.

Other parameters limit users' analysis to 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 curation. The default P-value is set to 0.05 but can be adjusted manually.

When the GO Slim option is chosen, 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) using default selection criteria.

The results table includes several additional statistical measurements:

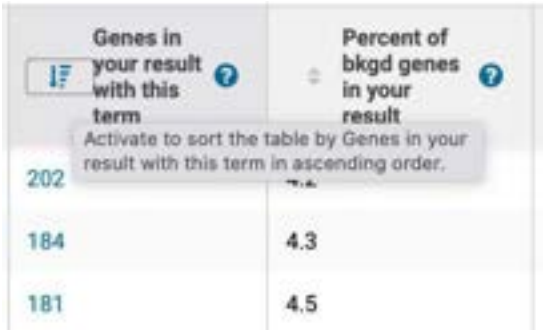
- **Fold enrichment** - The ratio of the proportion of genes in the list of interest with a specific GO term over the proportion of genes in the background with that term.
    - **Odds ratio—Determines whether the odds of the GO term appearing in the list of interest are the same as those** for the background list.
- **P-value** - Assumptions under a null hypothesis, the probability of getting a result that is equal to 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 Revigo results by clicking 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 represents 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/)

Optional exercise. Creating queries across FungiDB and SGD.

Use case: During a genetic screen in *Lomentospora prolificans*, you identified several exciting genes, including jhhlp_004726, a hypothetical protein. Use FungiDB and SGD records to learn more about this gene.

1. Navigate to jhhlp_004726 in FungiDB and examine available records.

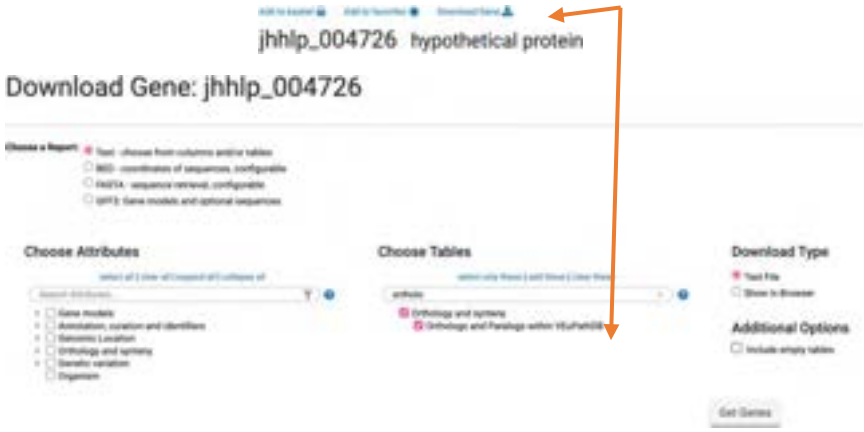
https://fungidb.org/fungidb/app/record/gene/jhhlp_004726

- 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 gene record page's Protein features and properties section.

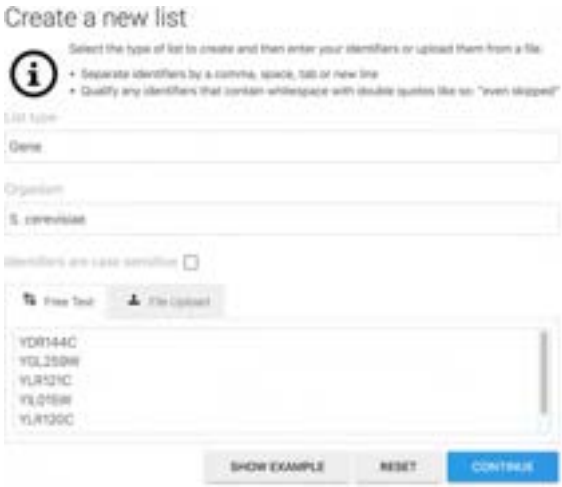
2. Export orthologs of this gene and carry over *S. cerevisiae* gene IDs into SGD.

- Click on the Download gene link at the top of the gene record page and select the option to export orthologs, as shown below.



The exported text file can be opened with Excel.

- Sort genes on the [Organism].
- Copy GeneIDs for *S. cerevisiae* (e.g., YDR144C).
- Navigate to the SGD gene lists search to create a new upload.
- Paste *S. cerevisiae* orthologs for jhhlp_004726 in the form: https://www.yeastgenome.org/locus/YDR144C.



- Give your list a name such as 'Yeast orthologs 1' and save it.

- Click on the GeneIDs to examine *S. cerevisiae* genes and answer the following questions:

    - 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?

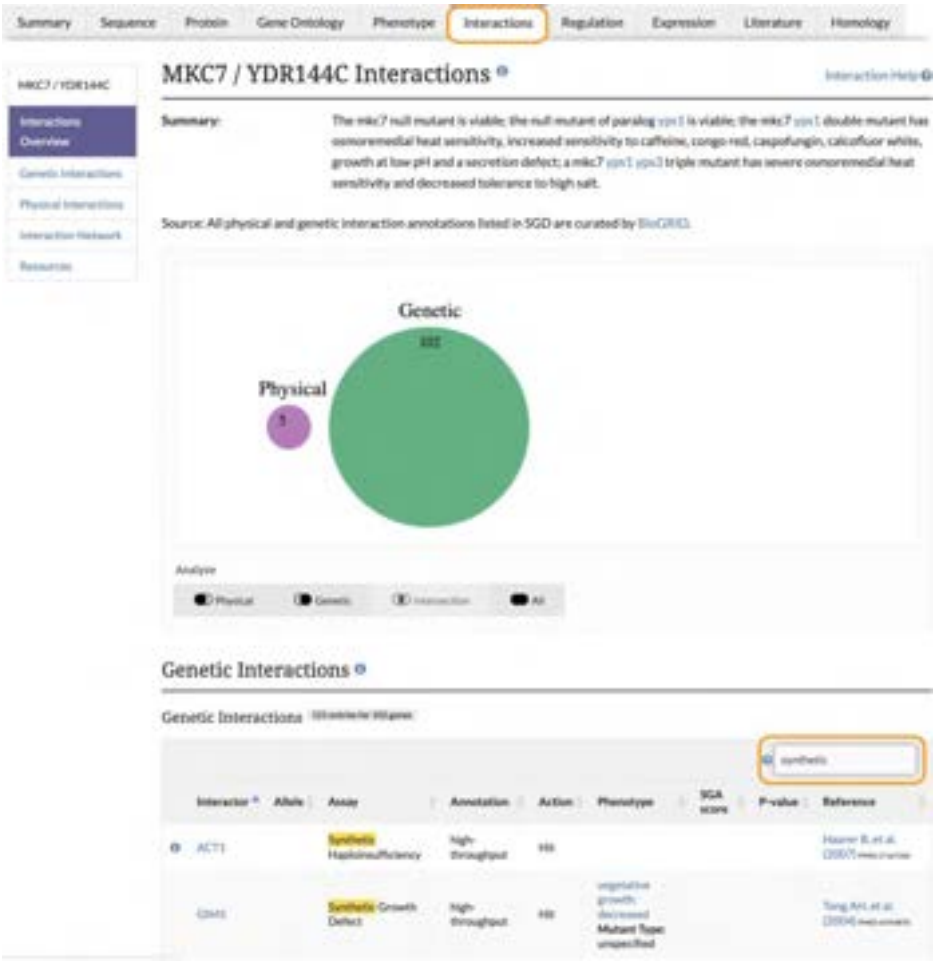
  Hint: see the **GO Annotation** section under the 'Data' on the locus page.

3. Find known genetic interactions for MKC7.

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 an unexpected phenotype, given the phenotypes of the single mutants.

- In SGD, find the MKC7 locus page and navigate to the **Interactions** section on the left, listed in the Quick Links panel near the top. The interactions are divided into physical and genetic interactions, as shown in the tables below the summary.

- Filter the **Genetic Interactions** table on "synthetic." This will show only the genetic interactions that produce some sort of synthetic growth defect, haploinsufficiency, or lethality.



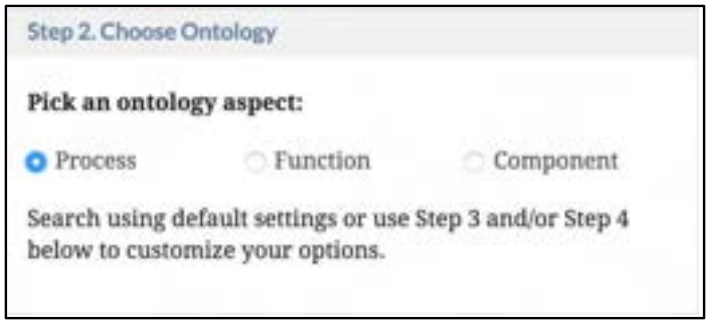
- Click on the **Download** button, which is located under the results table, and save this gene list. *Rename the file to **synthetic.txt***.

*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*.

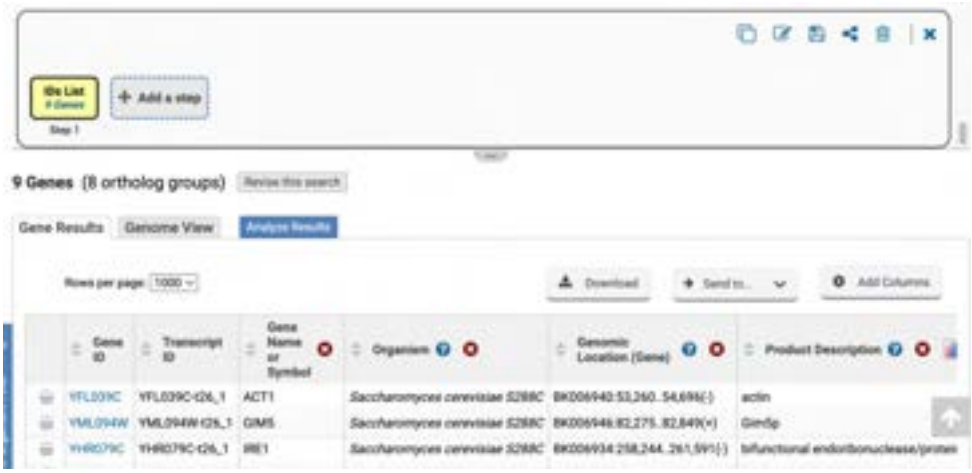
- Open this file in Excel and copy the Gene IDs in the **Interactor Systematic Name** column (not including the header)

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 Gn	high-through
MKC7	YDR144C	IRE1	YHR079C	Genetic	Synthetic Gn	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 Gn	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 Gn	manually cur
MKC7	YDR144C	YPS1	YLR120C	Genetic	Synthetic Let	manually cur
MKC7	YDR144C	YPS3	YLR121C	Genetic	Synthetic Let	manually cur

- Revisit FungiDB and initiate the List of IDs search query

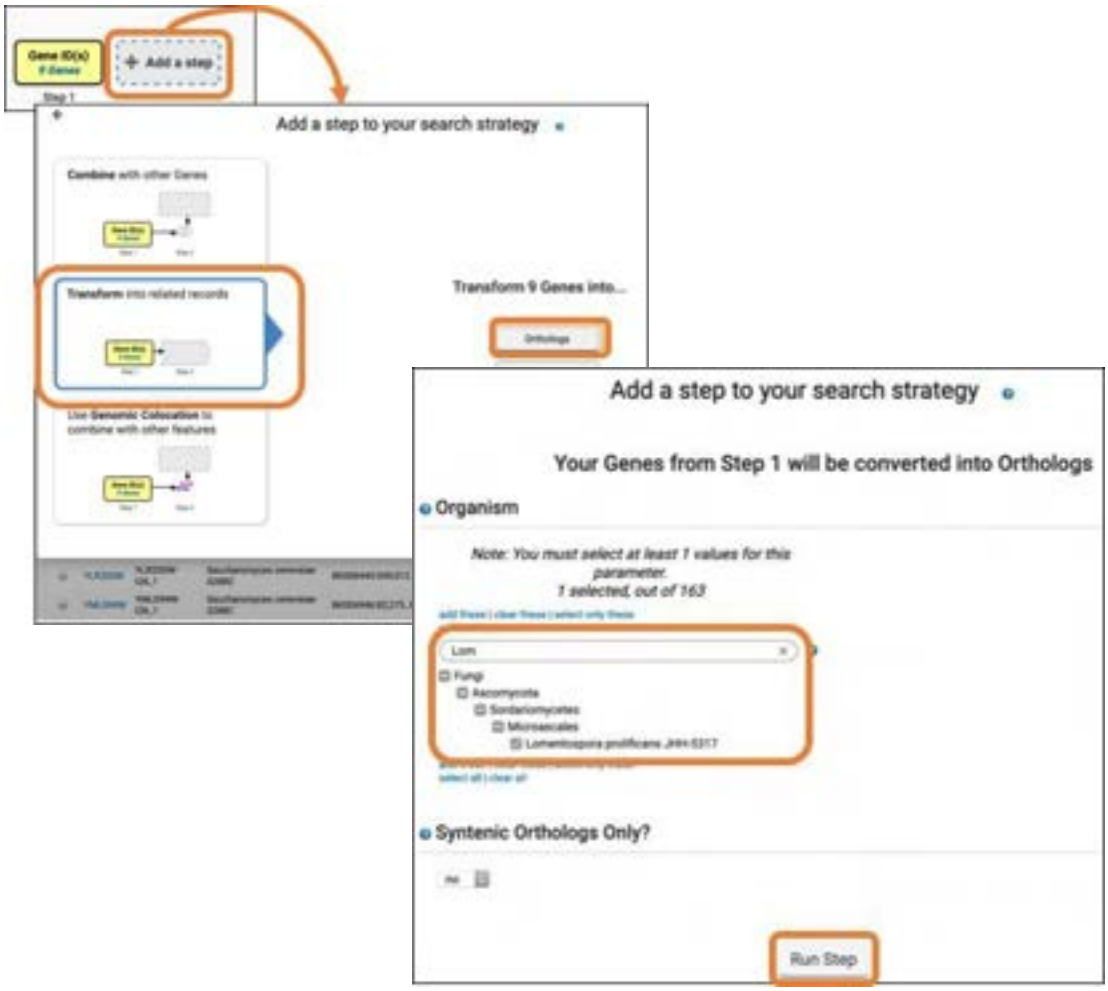
The query can be deployed from the “Searches” menu at the top of the “Search for Genes” section on the main page.

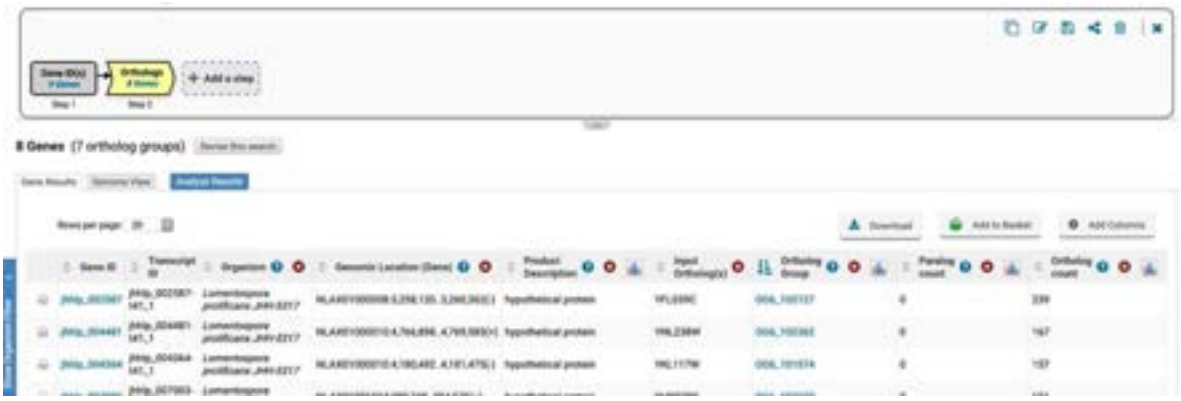
- Paste the list of Gene IDs with the “synthetic” genetic interactions with MKC7 into the FungiDB query and click the **Get Answer** button.



- Find orthologs in *Lomentospora prolificans*.

Click 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 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 defences. 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 varies 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/

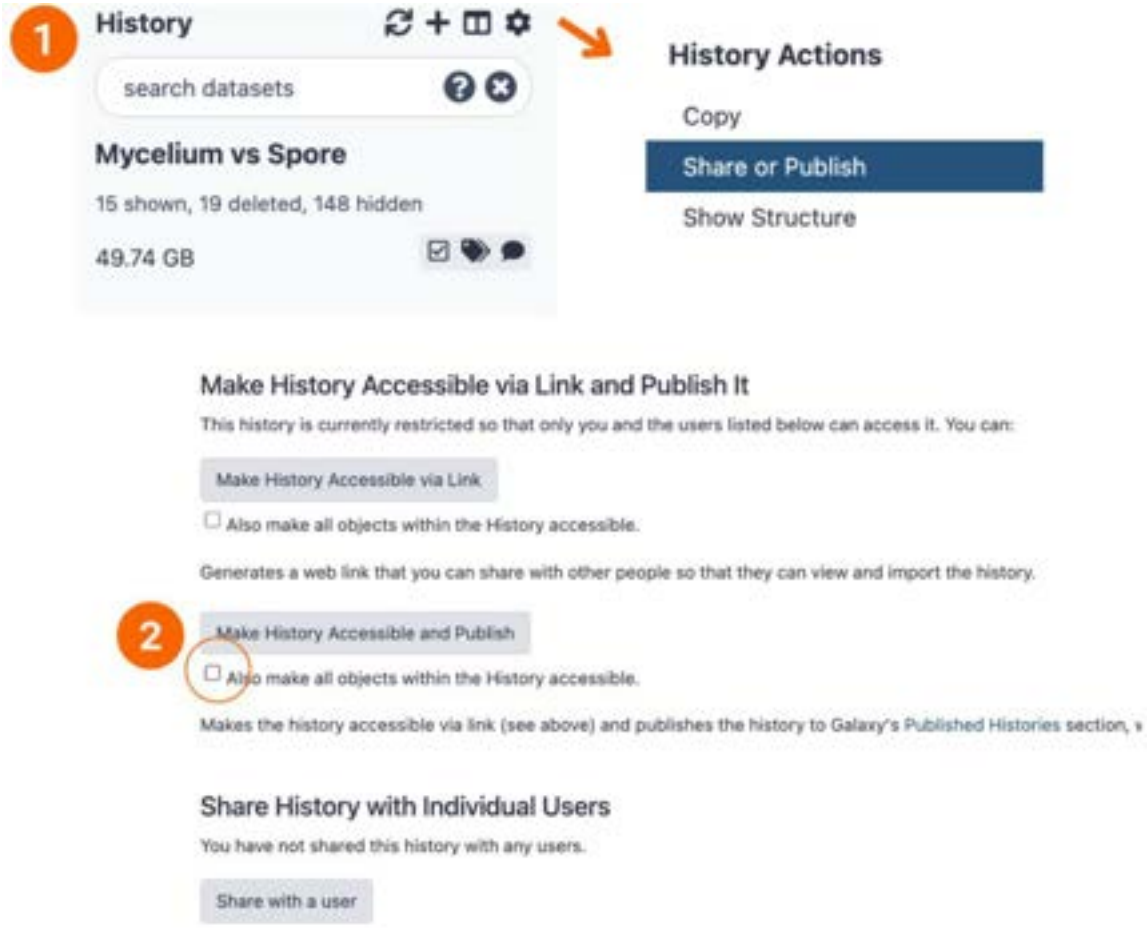
False discovery rates and P value correction:
http://brainder.org/2011/09/05/fdr-corrected-fdr-adjusted-p-values/

RNA sequence data analysis via Galaxy, Part 2

Learning objectives:

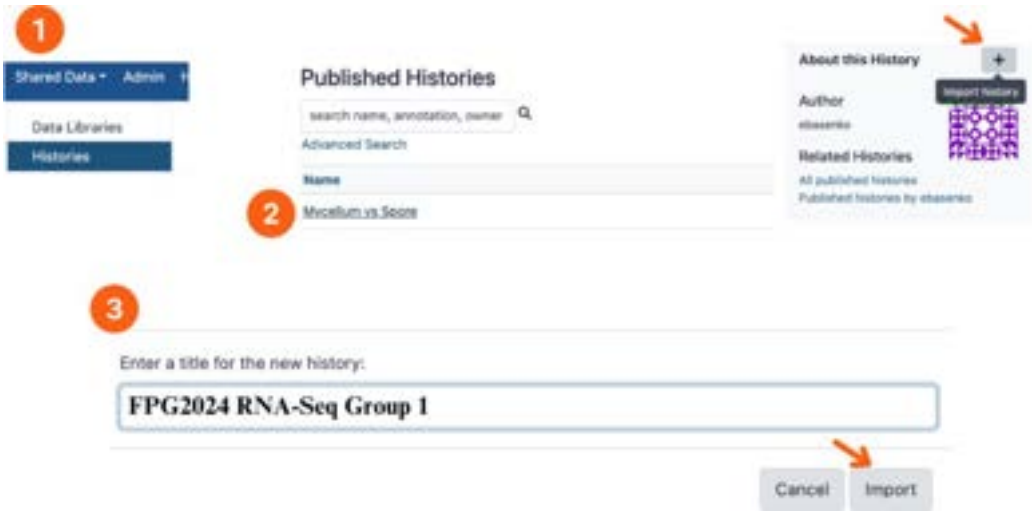
- Examine RNA-Seq analysis workflow and outputs.
- Import data from Galaxy to FungiDB My Workspace.
- Analyze the results using the FungiDB interface and tools.

- **Sharing workflow histories with others.**
    1. Ensure your history has a useful name (e.g., Mycelium vs Spore, RNA Group3, etc.) and click the history action menu icon.
    2. Select the “Make History Accessible and Publish” option and check to ensure all objects within History are accessible.



- **Importing workflow histories and output files into your own Galaxy workspace.**

1. Click on “Shared Data” at the top and select “Histories”.
2. Click on the history shared by your colleague, click on the plus icon on the far right and choose to import the history.
3. You can give it a descriptive name or leave it as is.



If everything worked out, you should see a list of completed workflow steps highlighted in green. The workflow generates many output files, but not all are visible. You can explore all the hidden files by clicking on the word “hidden” (orange circle)—this will reveal all hidden files.


Many more output files are available to explore
Differential expression data on the two collection
Coverage data in BigWig format
FastQC results (one per each file submitted)
Mycelium vs Spore
16 shown, 18 deleted, 148 hidden
49.74 GB
94: DESeq2 plots on data 88, data 86, and others
93: DESeq2 result file on data 88, data 86, and others
90: BAM to BigWig on collection 72
a list with 2 items
75: BAM to BigWig on collection 69
a list with 2 items
39: FastQC on collection 18: Webpage
a list of pairs with 2 items
24: FastQC on collection 13: Webpage
a list of pairs with 2 items
18: mycelium
a list of pairs with 2 items
13: spores
a list of pairs with 2 items
8: SRR1179896_2.fastq.gz
7: SRR1179896_1.fastq.gz
6: SRR1179895_2.fast

- **Explore the FastQC results.**

  To do this, find the step called “FastQC on collection ##: Webpage.” Click on the name. This will open the FastQ pairs. Click on one of them, then click on the view data icon (OBJ) on either forward or reverse. Note that each FastQ file will have its own FastQC results.

Explore the differential expression results.

We will explore two output files:

A. **DESeq2 Plots** – you can view these directly in Galaxy by clicking the view icon. These plots give you an idea about the quality of the experiment. The link above includes a detailed description of the graphs.

B. **DESeq2 results file—This table contains the actual differential expression results. While these can be viewed within Galaxy, it will be more beneficial to download this table and open it in Excel so you can sort the** results.

The tabular file contains seven columns:

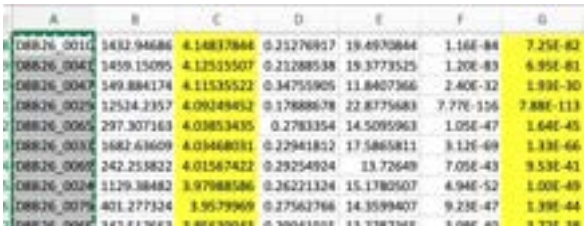
COLUMN	DESCRIPTION
1	Gene Identifiers
2	mean normalized counts, averaged over all samples from both conditions
3	the logarithm (to basis 2) of the fold change (See the note in inputs section)
4	standard error estimate for the log2 fold change estimate
5	Wald statistic
6	p value for the statistical significance of this change
7	p value adjusted for multiple testing with the Benjamini-Hochberg procedure which controls false discovery rate (FDR)

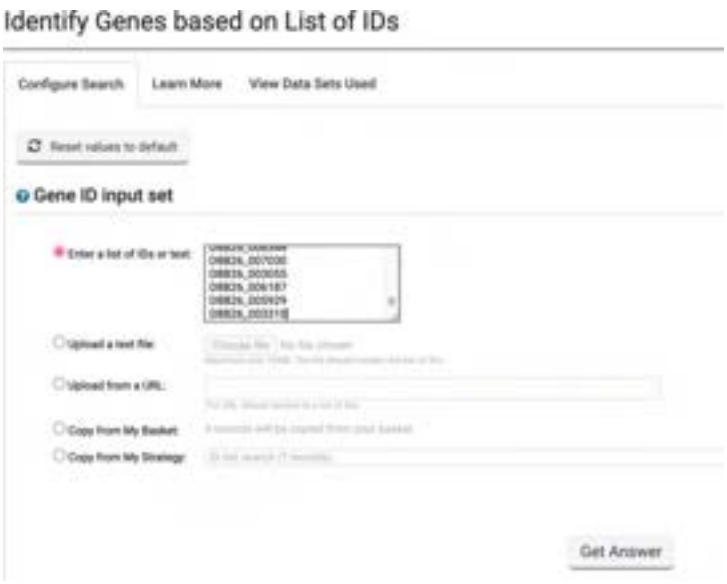
- **Download DESeq2 results (tabular format) by clicking the floppy disk save icon.**

*** **Important**: the file name ends with the extension ". tabular " change this to .txt and then open the file in Excel.

- **Explore the results in Excel.**
    1. Sort them based on the log2 fold change – column 3.

2. Pick a list of upregulated gene IDs from column 3 with a good corrected P value (column 7) and load them into FungiDB using the “List of IDs” search.





3. Next, analyze the results with GO or metabolic enrichment tools. Note that you can do the same for down-regulated genes.

Exporting data to VEuPathDB/FungiDB

The VEuPathDB RNA-Seq export tool provides a mechanism to export your RNASeq results (TPM values) and BigWig RNASeq coverage files. The advantage of doing this is that it allows you to search the TPM data using the RNASeq search in VEuPathDB and view the BigWig files in the genome browser.

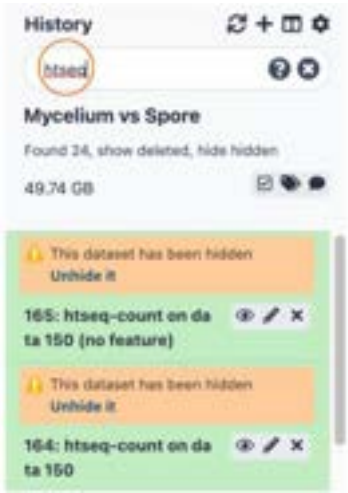
However, to use this feature, you need to generate TPM values for genes in your datasets and organize your results into two collections, one for the TPMs and one for the BigWigs.

- **Create a Dataset List with "htseq-count on data" files.**

1. **Reveal hidden files**.
   Click on the link at the top of your history that says "## hidden". This will show all hidden files.

2. **Search for htseq-count files.**

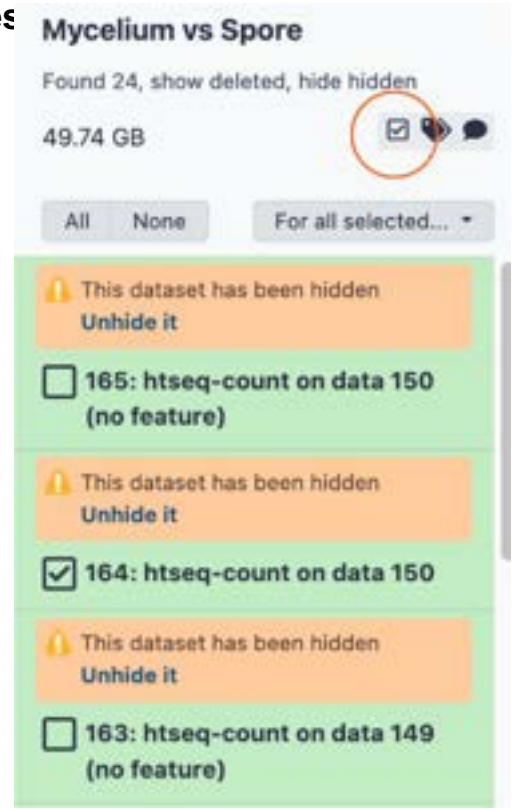
   Use the search datasets box at the top of your history to find any file in your history with the word "the-count". To do this, type "htseq" and click the "Enter" key on your keyboard.



3. Select “htseq-count on data” files

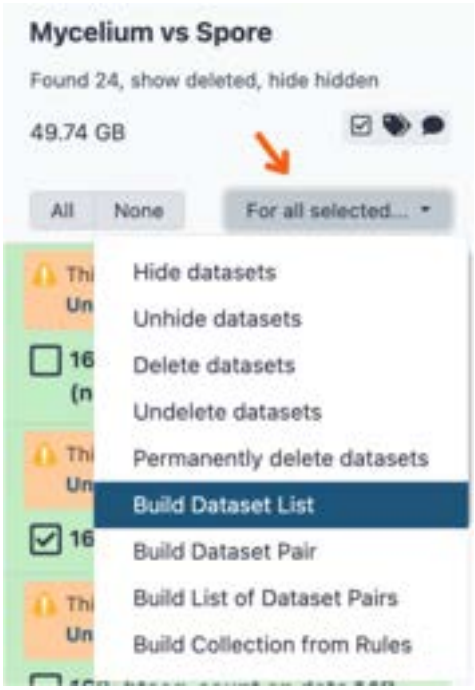
Click on the “operation on multiple datasets” tool and select the individual htseq-count files. These should look something like this: **htseq-count on data** xx. Do not select “no feature” or “..on collection” files.

Note: if you are comparing two conditions, each done in duplicate, you should have selected four files.



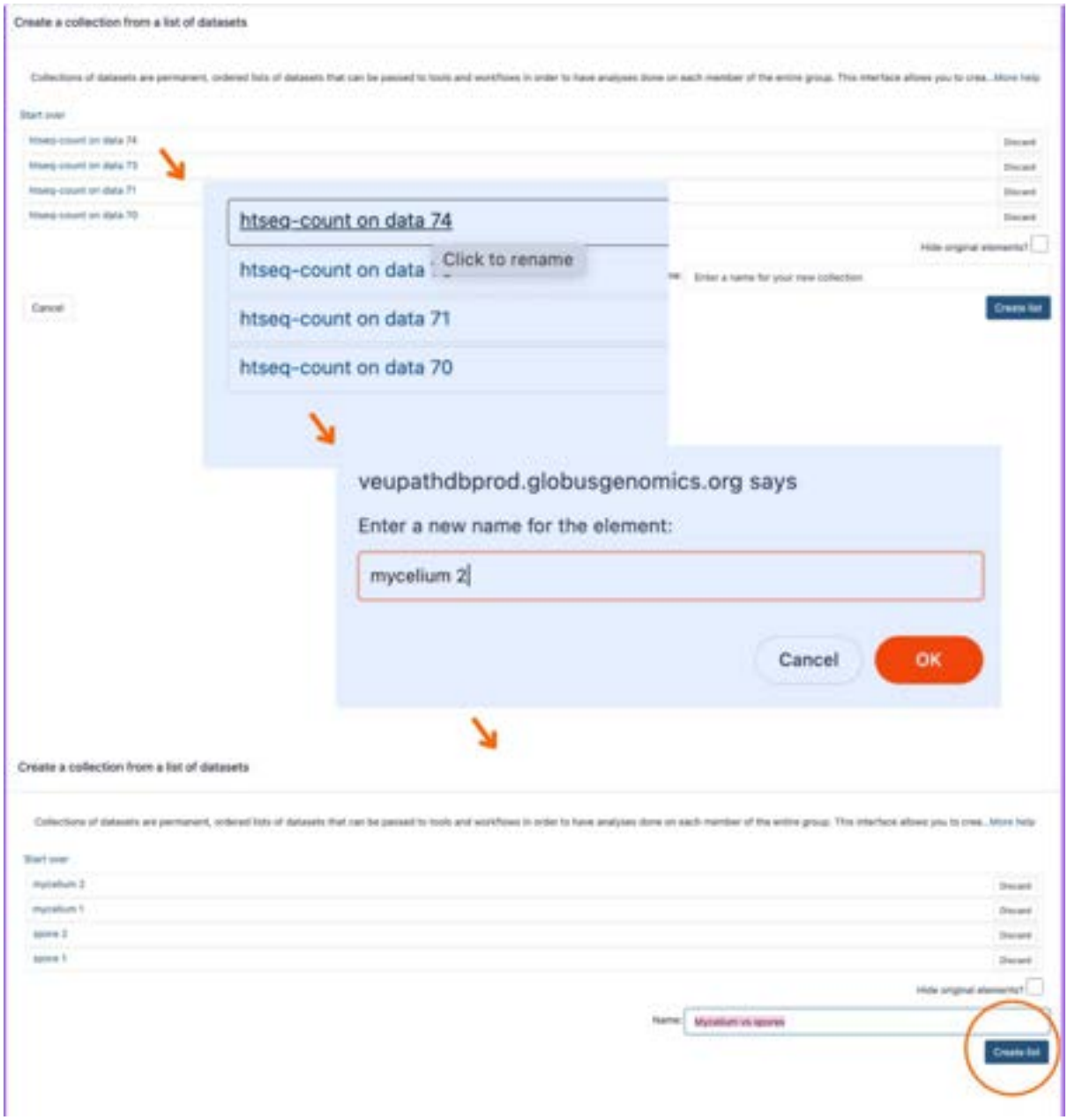
4. “Build dataset list”.

Click the “For all selected” button and choose the “Build dataset list” option.



5. **Rename each htseq-count sample, give the collection a name and create a dataset list.**

Note: the htseq-count files will be in the same order as the raw files loaded into the history. For more info, use the “Guide to FPG2023 RNA-Seq histories and file organization” in Part 1.



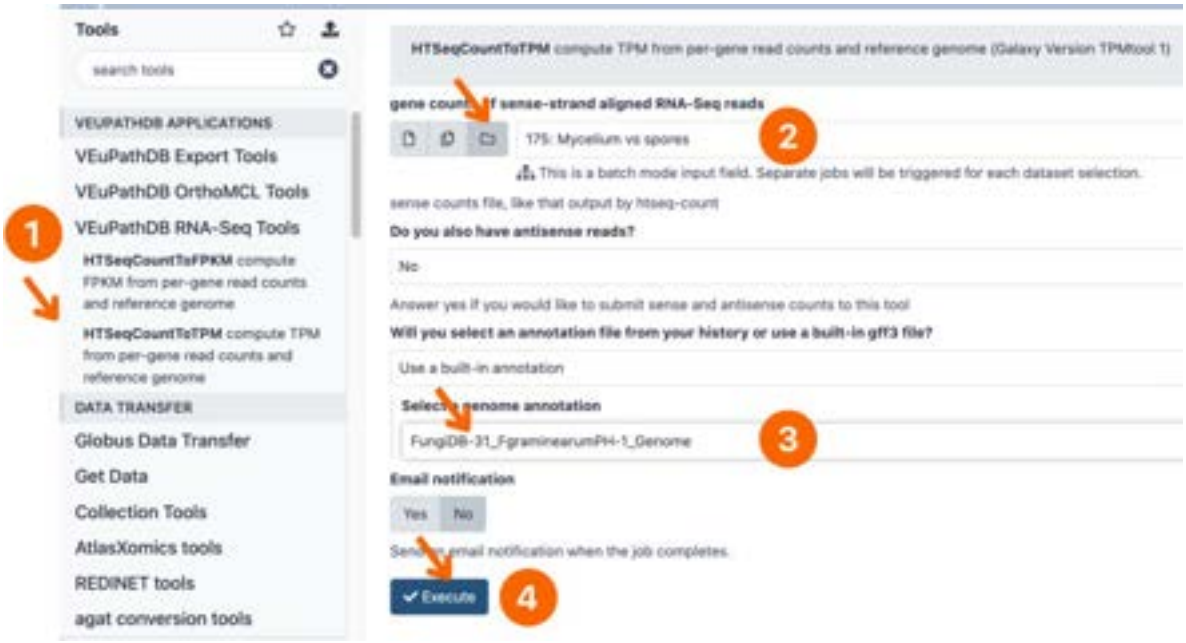
- **Create a Dataset List with “BAM to BigWig on data" files.**

Use the tutorial for htseq-count files to create a dataset list with BigWig files. Do not use “BAM to BigWig on collection” files.

Now that your count and bigwig files are nice and organized, the next step is to convert the counts into TPMs.

- **Use the HTSeqCountToTPM tool to convert counts to TPM.**

    1. Select the HTSeqCountToTPM tool (under the VEupathDB RNAseq tools in the left menu).
    2. Make sure the list of count files is selected.
    3. Select the reference organism.
    4. Click on the “Execute” button.

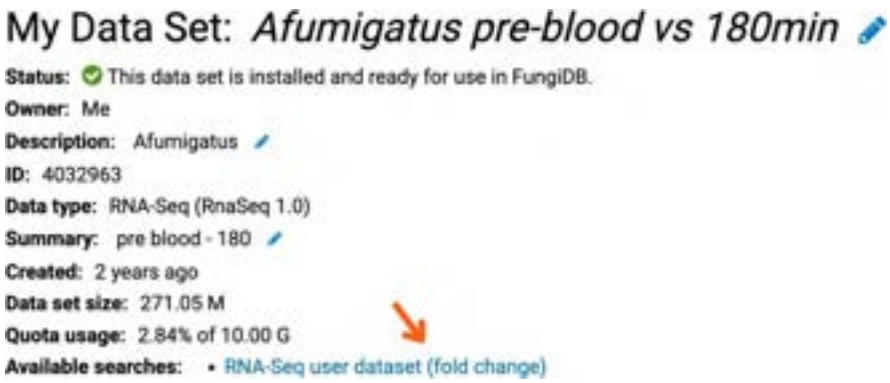


- **Export TPM counts and BigWig data to VEuPathDB/FungiDB workspace.**

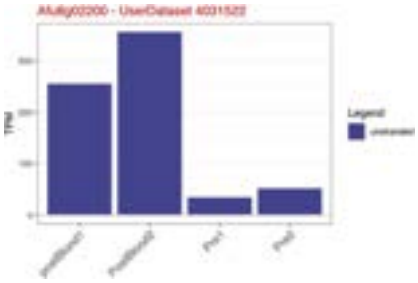
1. Click on “VEuPathDB Export Tools” > “RNA-Seq to VEuPathDB”
2. Enter a Data Set name.
3. Choose, if not already selected, the correct BigWig collection.
4. Choose, if not already selected, the correct TPM collection.
5. Provide a data set summary.
6. Provide a data set description and click on the “Execute” button.

- **Explore your data in FungiDB.**
  1. Click on the “My Workspace” link in the grey menu bar. Then select “My data sets” from the list.

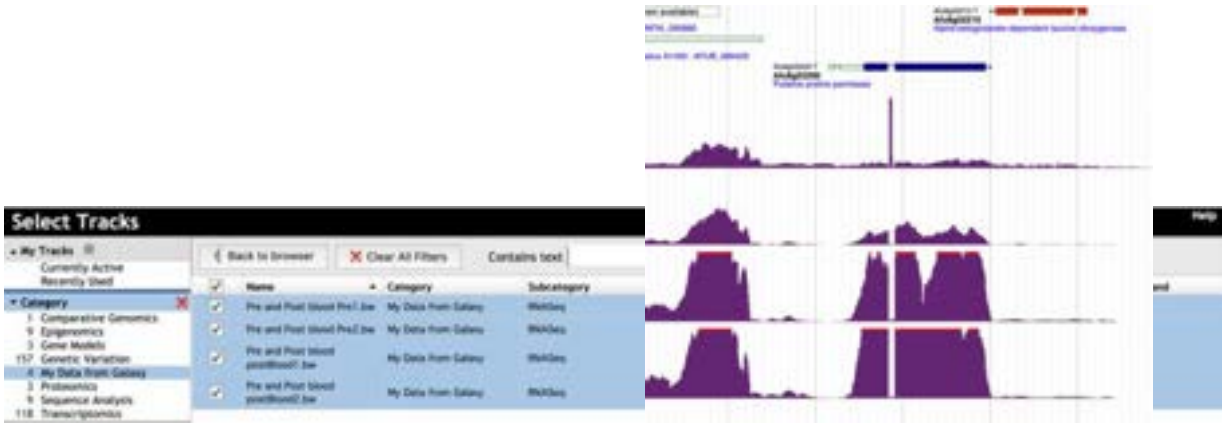
  2. Explore the RNA-Seq dataset via the fold-change search in FungiDB.



Note that custom graphs are generated for your data in the results table so you can easily visualize the results for each gene.



  3. Explore the coverage plots in the genome browser.

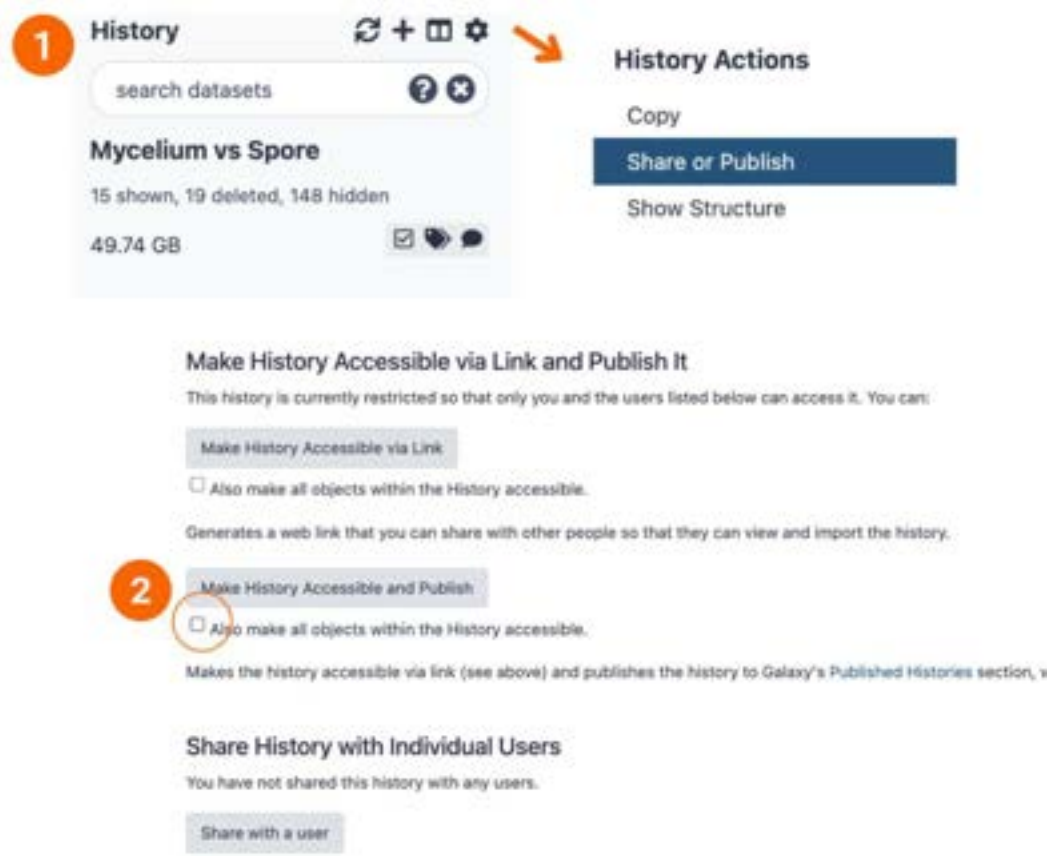


Variant Calling analysis, Part 2: Analyzing results (Group Exercise)

Learning objectives:

- Share and publish your workflow histories.
- Examine the outputs.
- View VCF files in JBrowse.
- Examine the filtered VFC file, extract Gene IDs, and create a Venny diagram.

- **Share workflow histories with others.**
  1. Make sure your history has a useful name (e.g., Group3 SNPs, etc.) and click on the history action menu icon.
  2. Select the “Make History Accessible and Publish” option and check to ensure all objects within History are accessible.

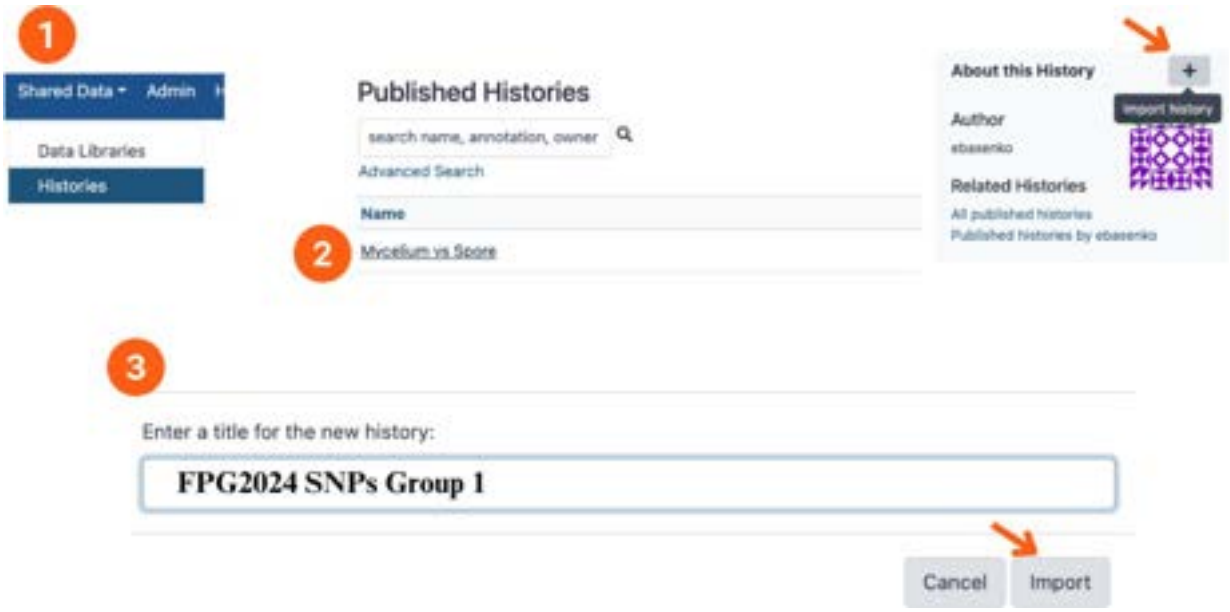


- **Importing workflow histories and output files into your own Galaxy workspace.**

  1. Click on “Shared Data” at the top and select “Histories”.
  2. Click on the history shared by your colleague, click on the plus icon on

the far right and choose to import the history.

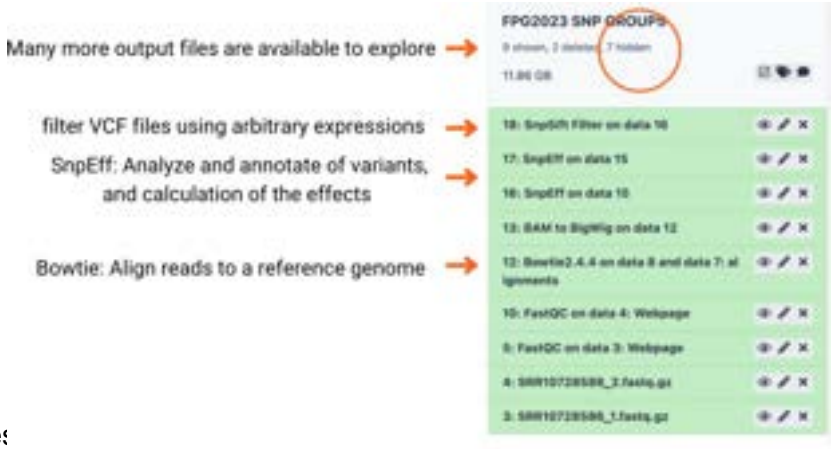
3. You can give it a descriptive name or leave it as is.



If everything worked out, you should see a list of completed workflow steps highlighted in green. The workflow generates many output files; however, not all of them are visible. You can explore all the hidden files by clicking on the word “hidden” (orange circle)—this will reveal all hidden files.

The Variant calling workflow has three major components: (1) mapping of raw reads to the reference genome, (2) calling variants, and (3) annotating variants. This workflow can be used to call single nucleotide polymorphisms, insertions and deletions (also defined as indels), and multiple nucleotide polymorphisms.

We used Bowtie2 to align and map sequences to a reference genome in this workflow. Once they are aligned, it may be worth checking the quality of this process because misalignments lead to false SNP calls.
SAM or BAM files provide this information, and you can find these files to export in the hidden workflow steps.



After reads have been aligned, they are sorted based on the chromosomal position. The tool we are using is called Sort and belongs to the SAMtools suite. The sorted file is an input for downstream FreeBaye variants.

Analysis and annotation of the genomic variants are carried out by the SnpEff tool. SnpEff is a variant annotation and effect prediction tool. It annotates and predicts the effects of variants on genes (such as amino acid changes). It uses reference genomes to annotate genomic variants based on their location and predicts SNP coding effects. The genomic location features are intronic regions, 5’ and 3’ UTRs, and upstream, downstream, splice site and intergenic regions. SNP coding effects are categorized based on the impact of the amino acid

change. They are classified into synonymous and non-synonymous, gain or loss of start codons, gain or loss of stop codons, and frameshifts.

The SnpSift tool annotates, filters, and manipulates genomic annotated variants. Once you have annotated your files using SnpEff, you can use SnpSift to help you filter large genomic datasets (e.g. sort on high or moderate-impact SNPs, etc.).

- **Examine your resul**

1. Click on the *hidden* files link in the history panel to reveal all workflow output files.
2. Examine the output files.
3. What does the tool FASTQC do?
4. What about Sickle?

The output of Sickle is used by a program called Bowtie2. Bowtie generates a file called a BAM file. You will likely hear of file formats called SAM or BAM when dealing with sequence alignment files. SAM stands for Sequence Alignment/Map format, and BAM is the binary version of a SAM file.

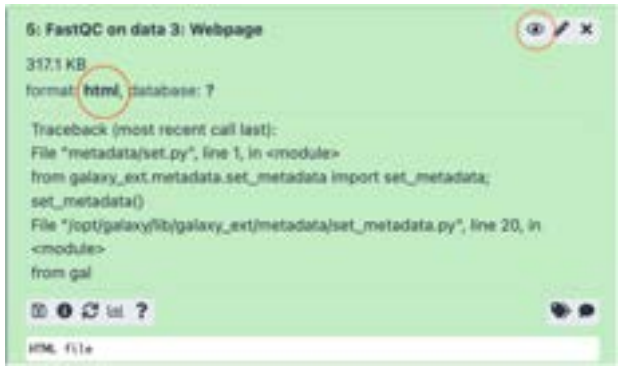
Many downstream analysis programs that use BAM files require a sorted BAM file. This allows for more efficient analysis.

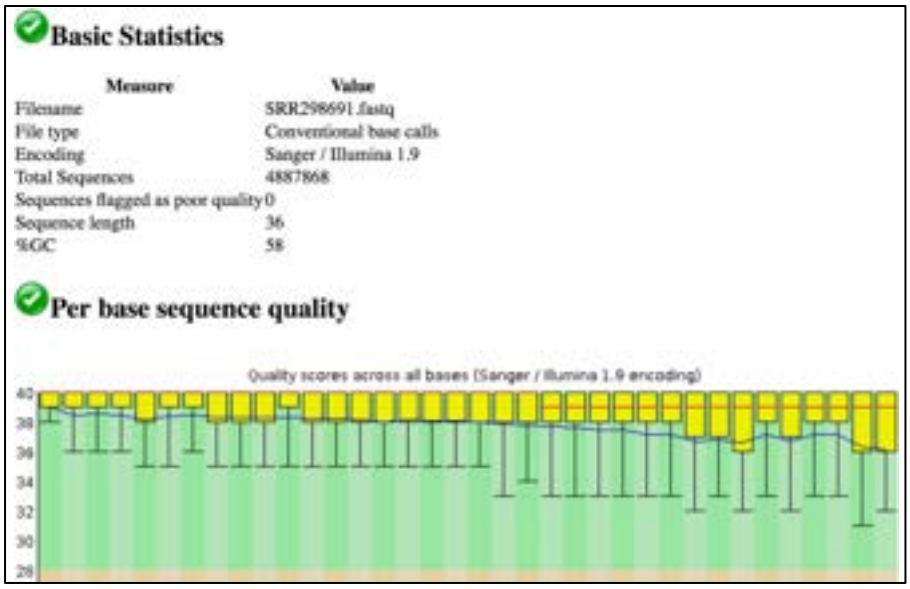
The sorted BAM file is the input for a program called FreeBayes. This program is a Bayesian genetic variant detector designed to find small polymorphisms, specifically SNPs (single-nucleotide polymorphisms), indels (insertions and deletions), MNPs (multi-nucleotide polymorphisms), and complex events (composite insertion and substitution events) smaller than the length of a short-read sequencing alignment. The output for many variant callers is a file called a VCF file. VCF stands for variant interchange format.

5. Examine the VCF file in your results (click the *eye* icon to view its contents). Detailed information about VCF file content is available here: https://samtools.github.io/hts-specs/VCFv4.2.pdf.

- **Examine sequence quality based on FastQC quality scores.**

FastQC provides an easy-to-navigate visual representation of sequencing data quality and distribution of nucleotides per read position. What does the report tell you about the quality?





- **Examine SnpEff summaries (HTML)**

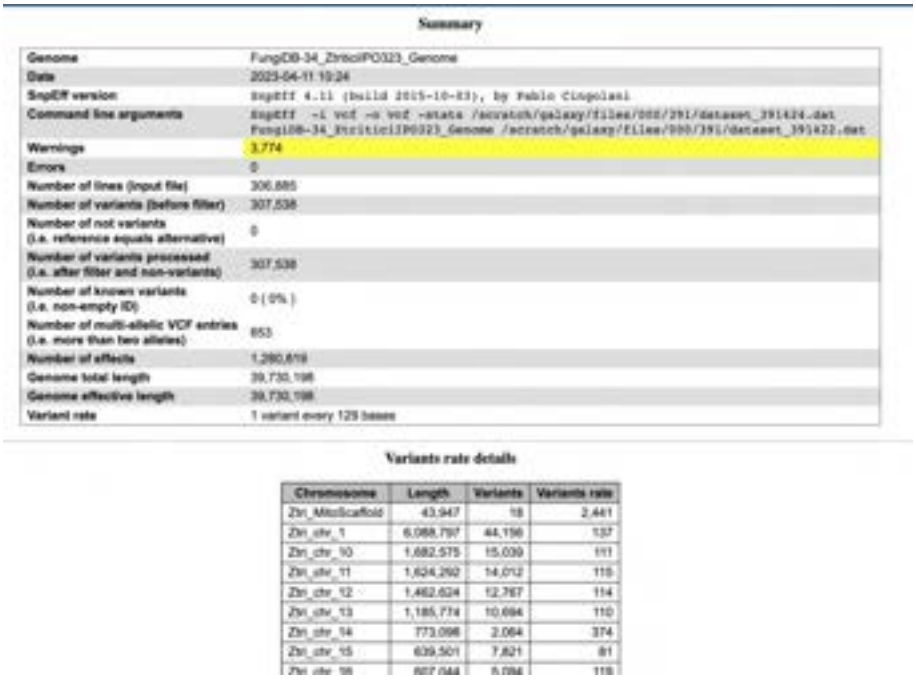
  - Click on the *View data icon* (eye) in the SnpEff output file with the HTML format.

This will open the HTML file in Galaxy for your review.

The header contains a summary and information about the run, and it has several major components:

The Summary includes warnings about possible genomic annotation errors or inconsistencies identified in the reference genome. If there are many, use caution when interpreting results and examine associated GFF files for any issues (e.g., missing feature values in GFF files, incomplete gene sequences, more than one stop codon per gene, *etc.*). Other components:

- Number of lines (input file) - number of lines in the vcf file
- Number of non-variants: 0—some packages report non-variant observations for nt positions between the reference genome and the vcf file generated.
- Number of known variants and multi-allelic VCF entries - if you work with a model organism where some variants were given an accession number (most commonly in



Summary

Genome	FungiDB-34_ZtritiIPO323_Genome
Date	2023-04-11 10:24
SnpEff version	SnpEff 4.11 (build 2015-10-03), by Pablo Cingolani
Command line arguments	SnpEff -i vcf -o vcf -stats /scratch/galaxy/files/000/391/dataset_391424.dat FungiDB-34_ZtritiIPO323_Genome /scratch/galaxy/files/000/391/dataset_391422.dat
Warnings	3,774
Errors	0
Number of lines (input file)	306,885
Number of variants (before filter)	307,538
Number of not variants (i.e. reference equals alternative)	0
Number of variants processed (i.e. after filter and non-variants)	307,538
Number of known variants (i.e. non-empty ID)	0 (0%)
Number of multi-allelic VCF entries (i.e. more than two alleles)	653
Number of effects	1,280,619
Genome total length	39,730,198
Genome effective length	39,730,198
Variant rate	1 variant every 129 bases

Variants rate details

Chromosome	Length	Variants	Variants rate
Ztri_MitoScaffold	43,947	18	2,441
Ztri_chr_1	6,088,797	44,156	137
Ztri_chr_10	1,682,575	15,039	111
Ztri_chr_11	1,624,292	14,012	115
Ztri_chr_12	1,462,624	12,787	114
Ztri_chr_13	1,185,774	10,684	110
Ztri_chr_14	773,098	2,064	374
Ztri_chr_15	639,501	7,821	81

mice and human projects) any recognised variants will be listed here
- Number of effects - SNP effects summary by type and regions
- Genome total length - number of bp in the reference genome
- Genome effective length - how many nucleotides can be mapped back to the genome
- Variant rate - higher frequency of variants before samples can indicate selective pressure

Summary statistics for variant types

Here is an example of variant calls and what they mean in terms of nucleotide changes:

Number variantss by type

Type	Total
SNP	114,034
MNP	12,864
INS	6,907
DEL	7,304
MIXED	2,180
INTERVAL	0
Total	143,289

Type	What is means	Example
SNP	Single-Nucleotide Polymorphism	Reference = 'A', Sample = 'C'
Ins	Insertion	Reference = 'A', Sample = 'AGT'
Del	Deletion	Reference = 'AC', Sample = 'C'
MNP	Multiple-nucleotide polymorphism	Reference = 'ATA', Sample = 'GTC'
MIXED	Multiple-nucleotide and an InDel	Reference = 'ATA', Sample = 'GTCAGT'

Statistics for the variant effects and impacts:

- **High impact** commonly refers to frameshift or new stop codon detections, as those changes will profoundly affect gene function.

- **Modifier SNPs** can affect promoter function, while low and moderate SNPs are most commonly identified inside genes and are either non-coding or non-synonymous SNPs.

- Base changes summary. SnpEff HTML files provide a breakdown of SNPs across gene features:

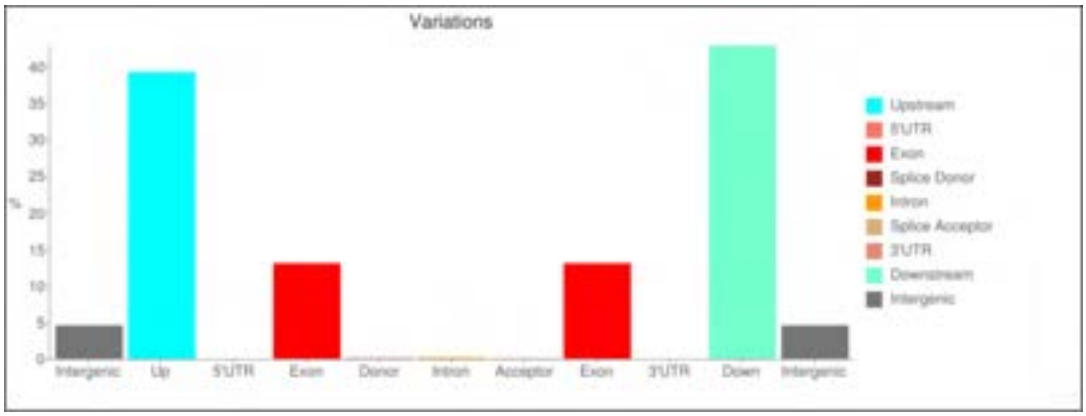
Number of effects by impact

Type (alphabetical order)	Count	Percent
HIGH	1,857	0.145%
LOW	87,874	6.861%
MODERATE	41,970	3.277%
MODIFIER	1,149,118	89.717%

Number of effects by functional class

Type (alphabetical order)	Count	Percent
MISSENSE	29,331	28.472%
NONSENSE	370	0.359%
SILENT	73,317	71.169%

Type (alphabetical order)	Count	Percent
DOWNSTREAM	321,858	40.292%
EXON	67,505	8.451%
INTERGENIC	74,749	9.358%
INTRON	1,064	0.133%
NONE	1	0%
SPLICE_SITE_ACCEPTOR	5	0.001%
SPLICE_SITE_DONOR	4	0.001%
SPLICE_SITE_REGION	176	0.022%
TRANSCRIPT	12	0.002%
UPSTREAM	333,432	41.741%



Additionally, you may see several SNPs being reported in several classes: missense variant + splice region variant. This means that some SNPs found within certain splice sites also contain a missense variant. SNPs in the splice sequences may affect intron splicing and lead to readthrough.

- The quality of reads is indicated in Phred's scale and is a good indicator of the quality of your datasets and results. Quality scores are usually represented by a bar graph where count = number of SNPs and X axis is quality score (a higher score means better p-values and high confidence in the results)

- Base changes: Reflects the frequency of base changes (purine-purine, purine- pyrimidine, pyrimidine-purine, pyrimidine-pyrimidine).

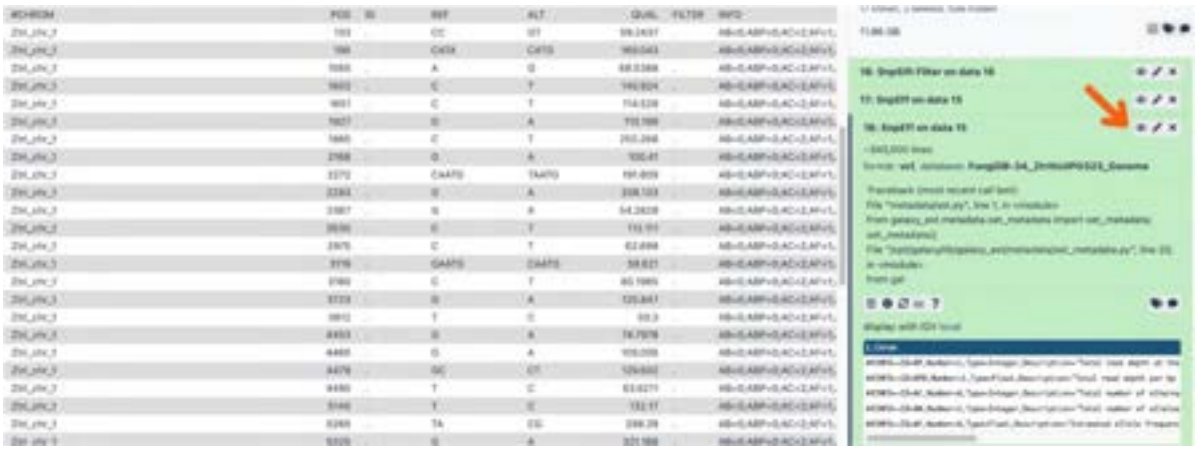
- Transition and transversion ratio help to identify if you may have a selective pressure on specific alleles (a high ratio suggests that genes may be under selective pressure).

- Allele frequency statistics report the frequency of alleles and help identify potential sequencing artefacts due to the PCR enrichment step (generation of heterozygous counts in a haploid organism).

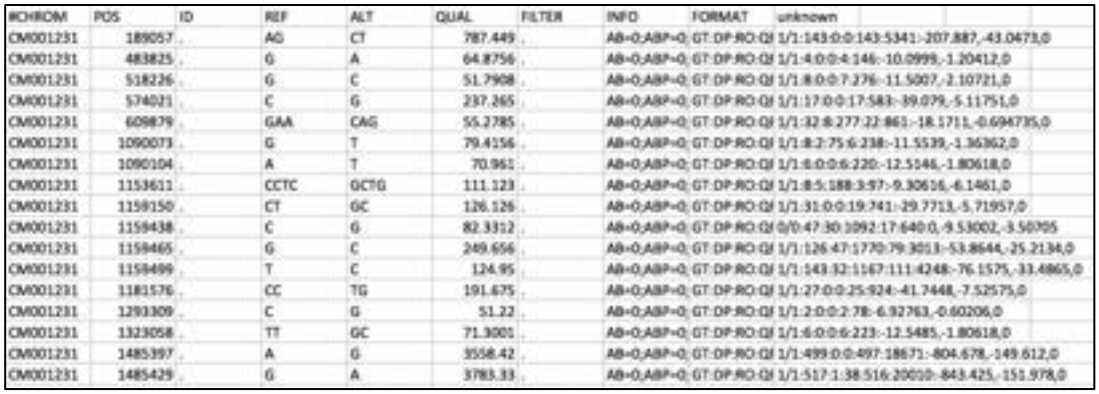
The vcf file generated by SnpEff contains information about SNPs and their genomic location. Post-processing of SNP data is usually required to make sense of thousands of SNPs and to decide which ones have biological and functional importance. Data processing can help you extract SNP distribution, parse associated data, including GeneIDs, protein-coding annotations, and effects in sequence ontology terms such as missense or synonymous variants, stop codon gain, etc., and link changes to the genome model. SnpSift is among other programs that are often in SNP data post-processing. It can be installed and run locally to manipulate vcf files. Alternatively, you can visualize vcf files in Artemis (additional steps are required to format the data).

Examining SNP information.

You can view the SNP information by clicking the "eye" icon within the SnpEff vcf file.



The vcf file generated by SnpEff contains information about SNPs and their genomic location. Here is an example of a file opened in Excel:



#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	unknown
CM001231	189057	.	AG	CT	787.449	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:143:0:0:143:5341:-207.887,-43.0473,0
CM001231	483825	.	G	A	64.8756	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:4:0:0:4:146:-10.0999,-1.20412,0
CM001231	518226	.	G	C	51.7908	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:8:0:0:7.276:-11.5007,-2.10721,0
CM001231	574021	.	C	G	237.265	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:17:0:0:17:583:-39.079,-5.11751,0
CM001231	609879	.	GAA	CAG	55.2785	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:32:8:277:22:861:-18.1711,-0.694735,0
CM001231	1090073	.	G	T	79.4156	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:8:2:75:6:238:-11.5539,-1.36362,0
CM001231	1090104	.	A	T	70.961	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:6:0:0:6:220:-12.5546,-1.80618,0
CM001231	1153611	.	CCTC	GCTG	111.123	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:8:5:188:3:97:-9.30616,-6.1461,0
CM001231	1159150	.	CT	GC	126.126	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:31:0:0:19:741:-29.7713,-5.71957,0
CM001231	1159438	.	C	G	82.3312	.	AB=0;ABP=0;	GT:DP:RO:QI	0/0:47:30:1092:17:640:0,-9.53002,-3.50705
CM001231	1159465	.	G	C	249.656	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:126:47:1770:79:3013:-53.8644,-25.2134,0
CM001231	1159499	.	T	C	124.95	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:143:32:1167:111:4248:-76.1575,-33.4865,0
CM001231	1181576	.	CC	TG	191.675	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:27:0:0:25:924:-41.7448,-7.52575,0
CM001231	1293309	.	C	G	51.22	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:2:0:0:2:78:-6.92763,-0.60206,0
CM001231	1323058	.	TT	GC	71.3001	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:6:0:0:6:223:-12.5485,-1.80618,0
CM001231	1485397	.	A	G	3558.42	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:499:0:0:497:18671:-804.678,-149.612,0
CM001231	1485429	.	G	A	3783.33	.	AB=0;ABP=0;	GT:DP:RO:QI	1/1:517:1:38:516:20010:-843.425,-151.978,0

Filtering VCF file data.

VCF files contain a lot of data about variants and their positions. SnpEff generates various analyses/summaries of VCF files (including GeneIDs that overlap variant positions). However, it is often necessary to filter VCF files further to obtain helpful information for your specific question. For example, you may want to filter out SNP positions that have an impact on the coding sequence.

One tool that can be used is called SnpSift Filter (look at the last step of the pipeline you just ran). This tool allows you to write complex expressions to filter a VCF file. Your workflow is set up to use an expression that filters VCF files on moderate and high-impact SNPs (this setting can be adjusted manually in the workflow editor). Here is the exact expression used:

(((ANN[*].IMPACT has 'HIGH') | (ANN[*].IMPACT has 'MODERATE')) & ((na FILTER) | (FILTER = 'PASS')))

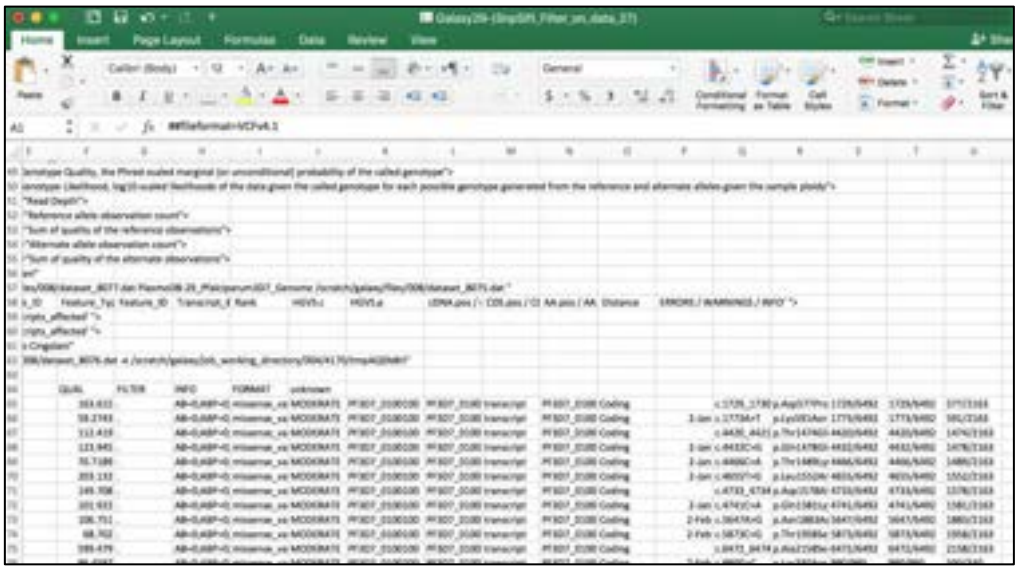
- **Extract the filtered VCF file (SnpSift output) and convert it into an Excel document.**

For this exercise, two groups will share data SnpSift outputs: group 1 and 2, group 3 and 4, and group 5 and 6. File manipulations should be performed on both SnpSift vcf files.

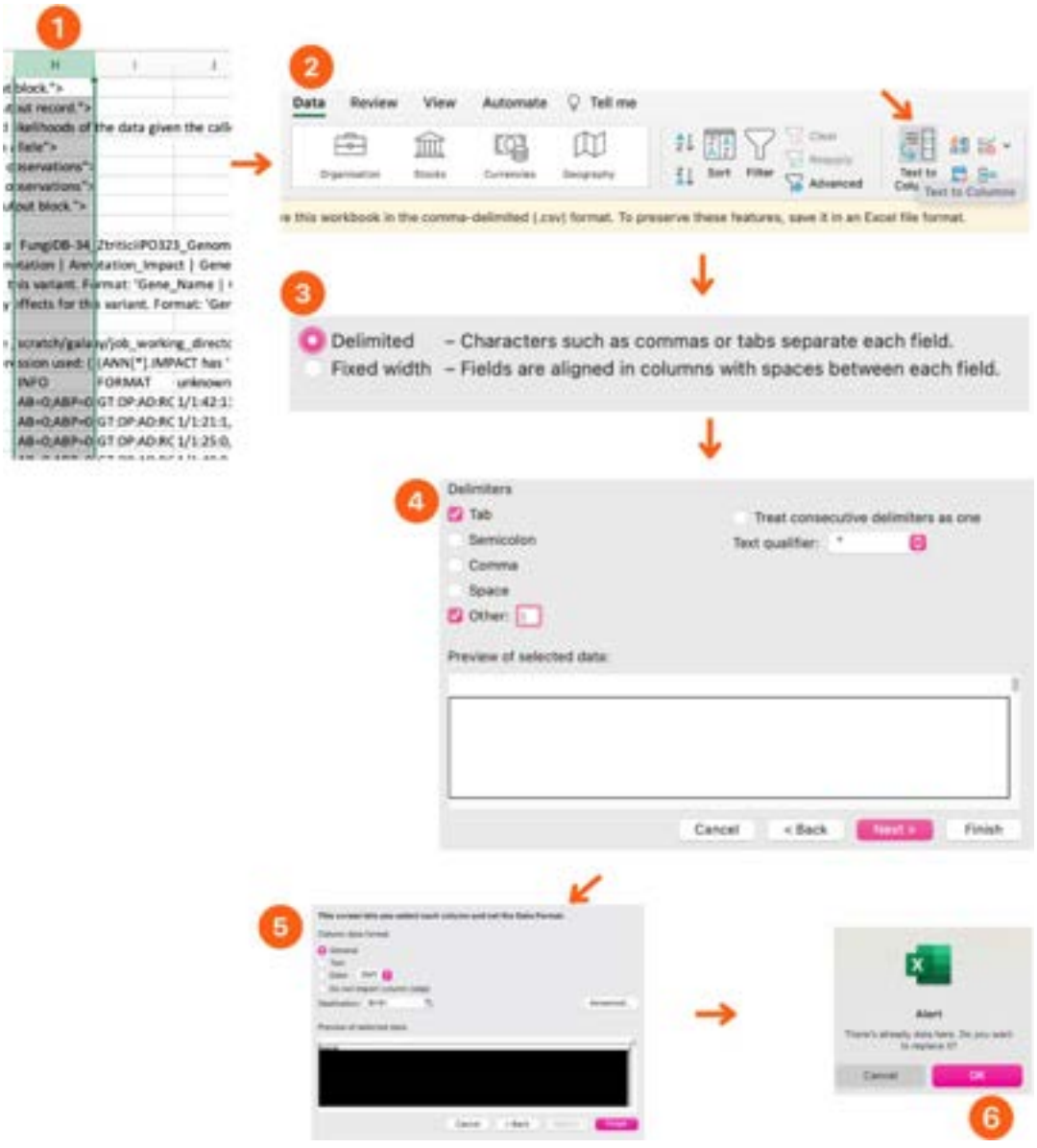
Look at the filtered vcf file in Galaxy. Notice that the Gene IDs are buried in the file, but the file has some structure, meaning you can extract them programmatically or using a program like Excel.

Here are some steps you can take to extract Gene IDs from two VCF files and compare them to identify genes that are common or distinguish the two files.

1. Download the SnpSift Filter output by clicking on the save icon.
2. Right-click and open this file with Excel.

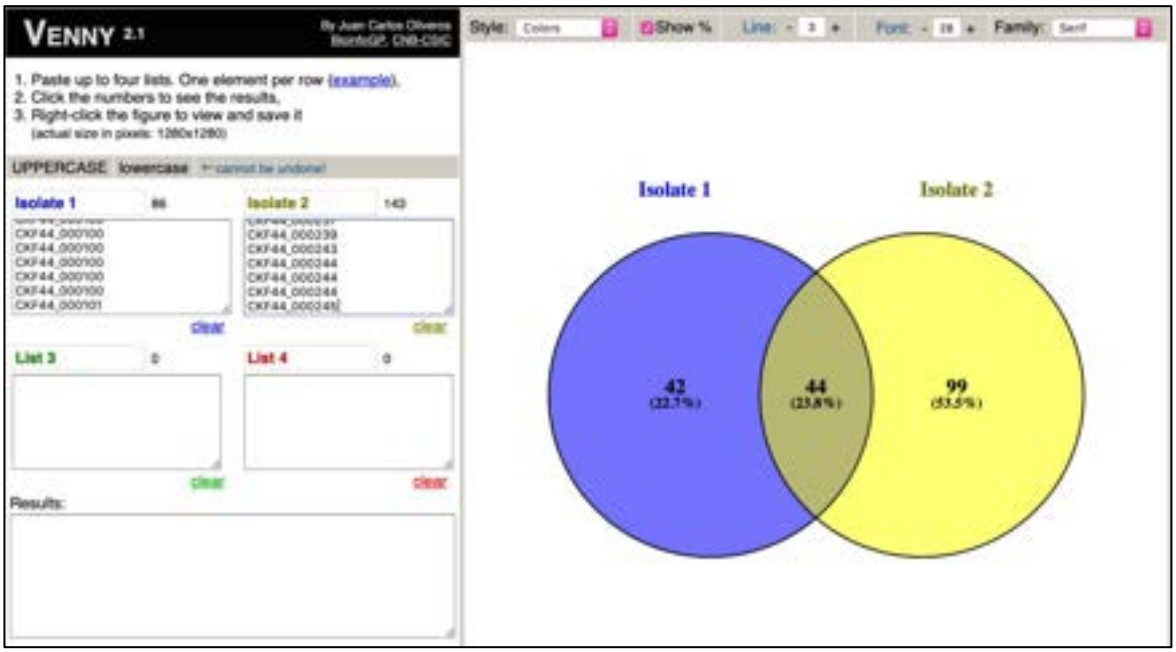


- **Manipulate Excel file to display SNP info in columns.**
  1. Select the “INFO” column.
  2. Navigate to the “Data” tab in Excel and choose “Text to Columns”.
  3. Use the “Delimited” option.
  4. Set delimiters to the “Tab” and “ | ” in the “Other” and click “Next”
  5. Leave other criteria at default and click on the “Finish” button.
  6. Click “OK” on the Alert pop-up.



Now, you can look for Gene IDs of interest in the Excel file. For example, if this is a known drug-resistant line, you can sort and examine SNPs based on their characteristics.

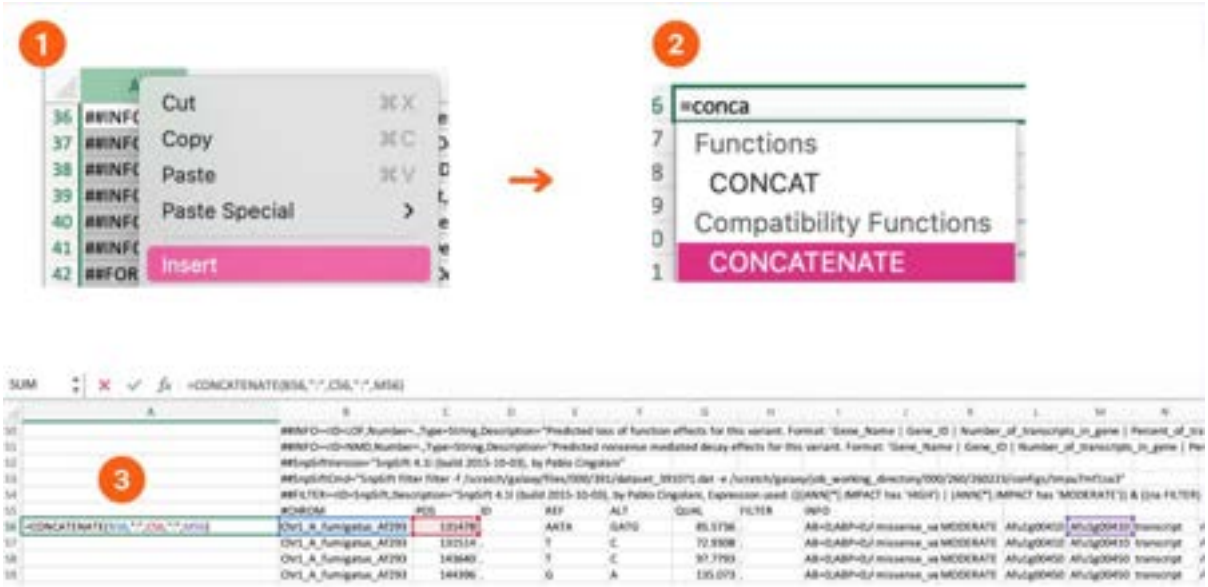
If comparing two or more strains, you may want to extract gene IDs from all VCF files and identify common signatures across isolates or strains. For this type of analysis, you can use http://bioinfogp.cnb.csic.es/tools/venny/ to generate a Venn diagram:



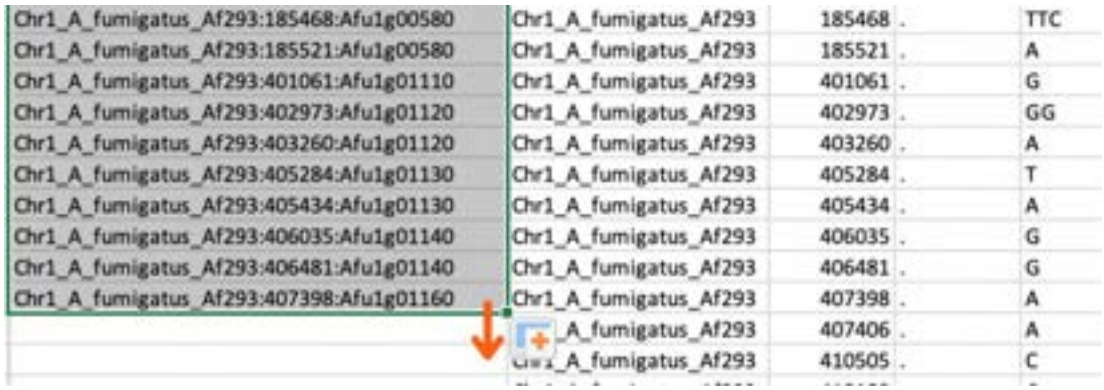
The screenshot above shows a comparison between lists of GeneIDs. Is it possible to miss some important polymorphisms using this method? Of course, the answer is yes☺
For example, it is quite possible that a gene with an SNP in the WT and an SNP in the mutant that will be at the intersection of the two gene lists contains different SNPs—you will miss this by doing the above steps. Below is a description of steps you can take to create a list of unique IDs for SNPs. This list of unique IDs can then be used in Venny.

- **Analyze your data in Venny.**

1. Start with the same Excel files that you opened in the above section. Insert an empty column before the data.
2. Deploy the concatenate function in Excel.
3. Create a unique ID for SNPs by combining information from multiple columns to create something that looks like this:
   chromosome:position:geneID
   To do this, you will use the concatenate function in Excel:
   =concatenate(cell#1,":",cell#2,":",cell#3) Cell#1
   = cell with chromosome number
   Cell#2 = cell with position Cell#3
   = cell with GeneID



You should get unique SNP IDs that look like this (for example): CP022321.1:15259:CKF44_000003. Copy this function for other entries:



4. Copy these newly generated unique IDs into List 1 and Lis2 on Venny http://bioinfogp.cnb.csic.es/tools/venny/ and examine the data.

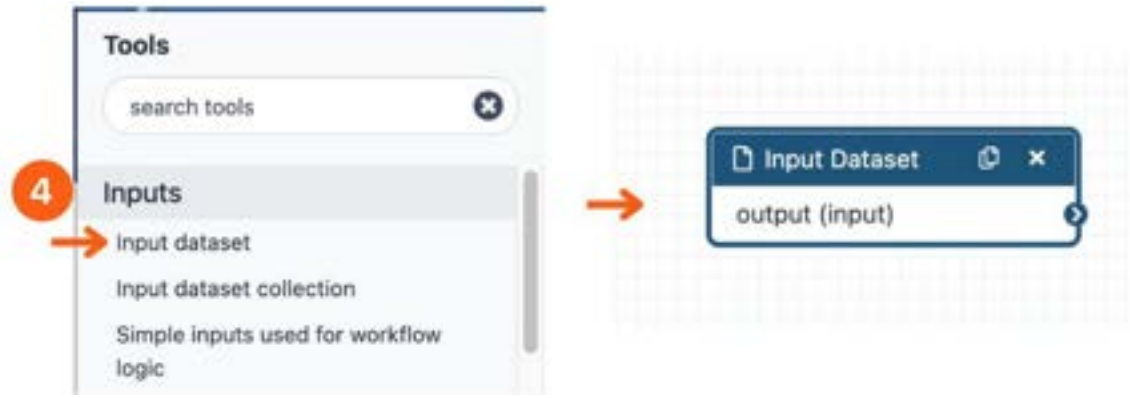
Viewing the VCF file results in the JBrowse genome browser.

- **Create a workflow to generate compressed VCF and index files to view your data in JBrowse.**

To view a VCF file in JBrowse, it must first be indexed and compressed. This is done using two tools: bgzip and tabix, respectively. You can run these tools sequentially, or you can set up a mini workflow and then run the workflow to generate the output files as follows:

1. Click on the “Workflow” menu.
2. Click on the “Create” button to start a new workflow.
3. Name the workflow (e.g., Compress and index VCF) and click the save button. This will open a workflow canvas.

4. All workflows must start with an input file, so add the “Input Dataset” step to the workflow using the menu on the left (you must click on the tool for it to appear in the workflow editor canvas).



5. Using the menu on the left, search for and add the “bgzip” tool.

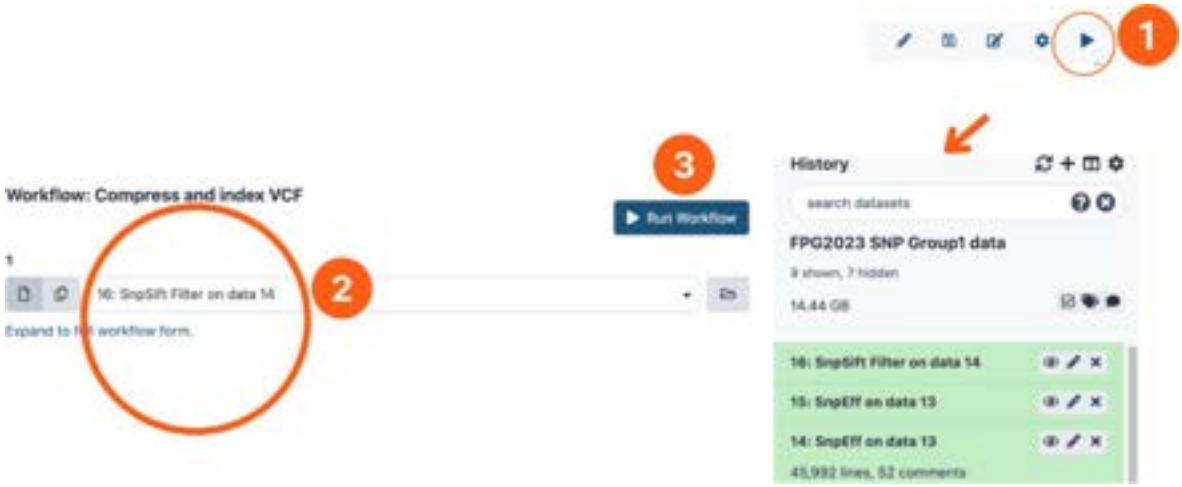
6. Using the menu on the left, search for and add the “tabix” tool. Left-click on the “tabix” icon and select “vcf” under “input selection” on the right (tool option section)

7. Connect each step/tool into a workflow and save it (the button is at the top of the screen)

- **Run the newly created workflow to generate a compressed vcf and index files.**
    1. Click on the “Play” button to start your workflow.
    2. Select the VCF file you want to process.

Note that the workflow produced several vcf files - SnpSift, SnpEff.. In the screenshot below, we will use a vcf filtered for high and moderate-impact SNPs, but if you are interested in all mutations, you may want to choose another file.

3. Click on the “Run Workflow” button.



After the workflow completes running, you should have 2 new files (tabix and bgzip) in the history on the right.

- **Download compressed vcf (vcf_bgzip) and index (tabix) files and view them in JBrowse.**

1. Download both files by clicking on the download icon. You will need both files.

2. After the files are downloaded, rename them as follows:
    a. The **vcf_bgzip** file to “**group#.vcf.gz”** (i.e. **group1.vcf.gz**)
    b. The **tabix** file to “**group#.vcf.gz.tbi**” (i.e. **group1.vcf.gz.tbi**)

3. Navigate to JBrowse in FungiDB and select the correct genome from the Genome drop-down menu.

4. Click on the Track menu and select "Open track file or URL".

5. Drag and drop your files into the window that appears. The file formats are autodetected. Then click on the “Open” button at the bottom of the pop- up.

You should now be able to view the SNPs in JBrowse.

SGD Variant Viewer

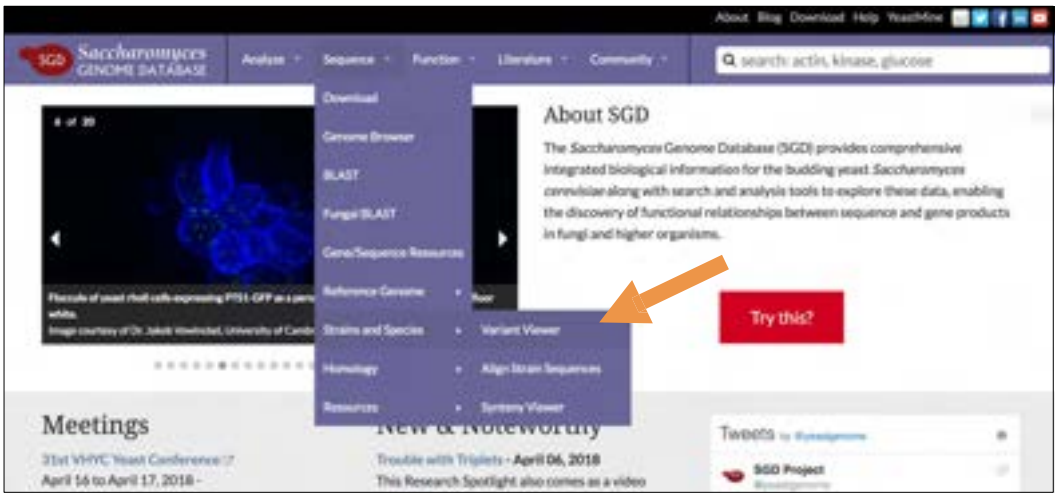
SGD's Variant Viewer (**https://yeastgenome.org/variant-viewer**) is an open-source web application that compares nucleotide and amino acid sequence differences between 12 common *S. cerevisiae* laboratory strains. For a given open reading frame, Variant Viewer breaks down the position and nature of any strain-specific sequence differences relative to the reference strain S288C. When used at a multi-gene level, it also provides a matrix of alignment scores that enables quick identification of genes with higher or lower variation.

Variant Viewer can be used to probe the genetic differences between *S. cerevisiae* strains that give rise to their unique phenotypes. For example, while haploid S288C cells exhibit an axial budding pattern, diploid cells exhibit a bipolar budding pattern. On the other hand, strain W303 shows bipolar bud site selection in both haploid and diploid cells.

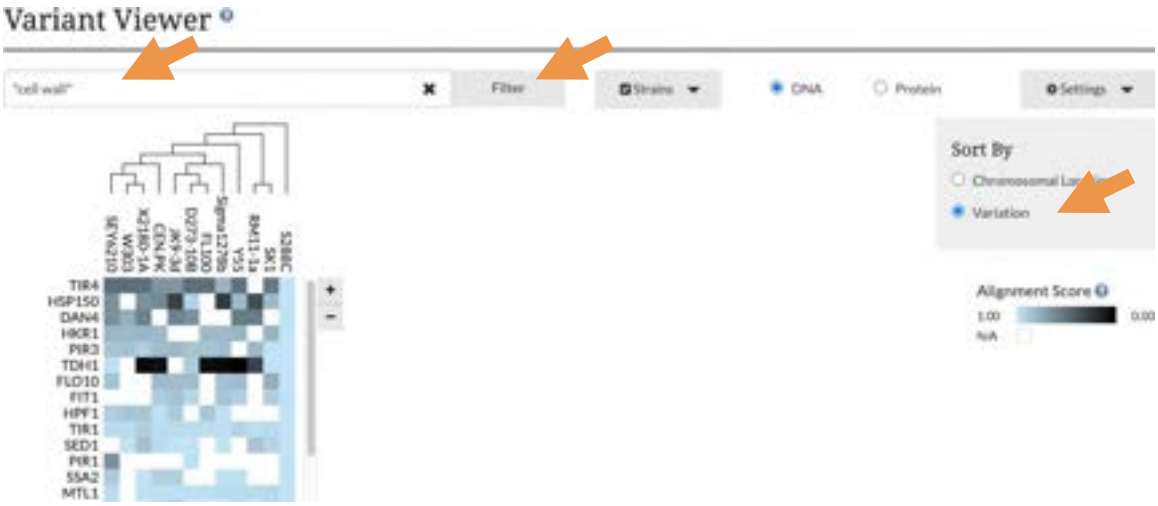
In this exercise, we will use Variant Viewer to find out what genetic differences between Sigma1278b and S288C explain why they differ in their ability to form pseudohyphae.

S288C vs. Sigma1278b: Cell Wall

- Open the SGD home page (www.yeastgenome.org), open the Sequence tab on top of the page, then select Strains and Species followed by Variant Viewer from the pull- down menus. Or just type in the URL: yeastgenome.org/variant-viewer

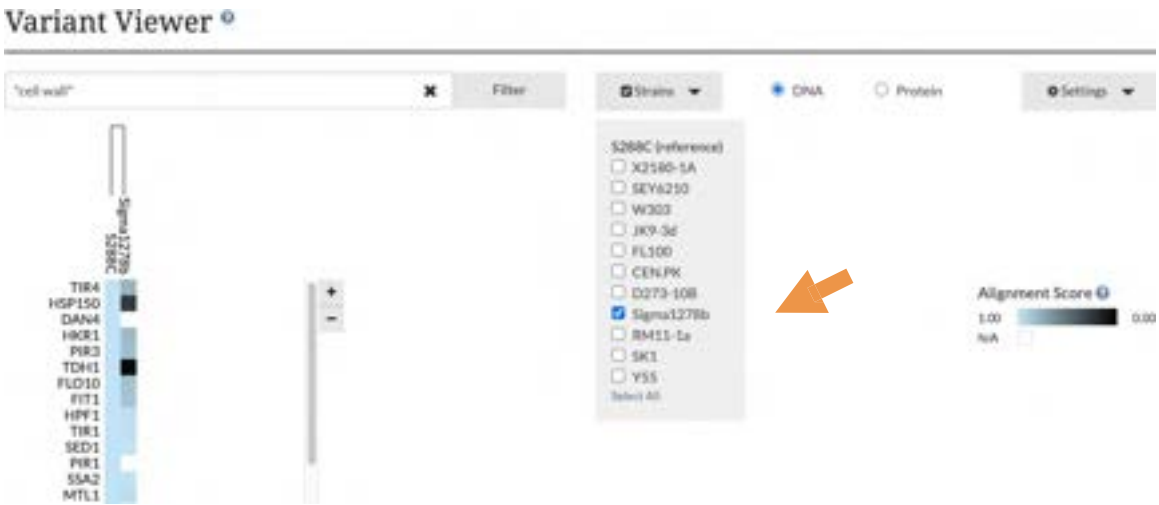


- The **Filter** box accepts one or more genes, as well as Gene Ontology (GO) terms. Because we are interested in genes involved in cell wall development, search for the GO term "**cell wall,**" sort by variation in the settings pull-down, and then click Filter.



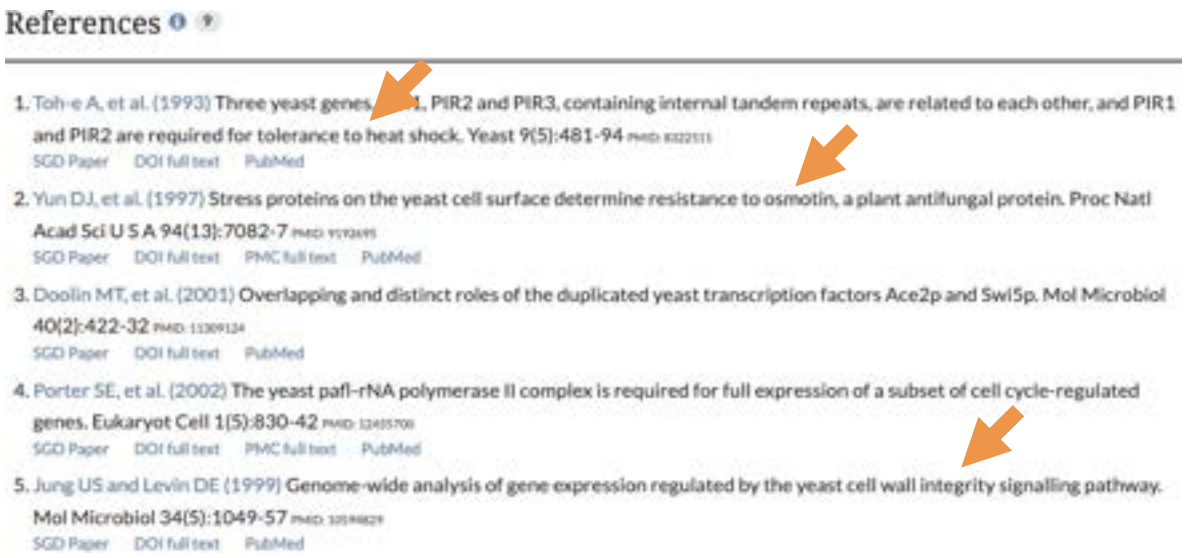
- The **matrix**, shown on the left, will have changed to only include the genes that localize to cell walls.
    - This matrix enables you to visualize high-level differences in multiple genes relative to strain S288C. Each square in the matrix corresponds to one of the twelve strains in Variant Viewer, shown at the top, and to an open reading frame, shown on the left.
    - The color of each square indicates how similar the sequence is relative to strain S288C. As indicated on the Alignment Score figure on the right, lighter shades of blue indicate high sequence similarity whereas darker shades indicate more dissimilarity. Note that if the square is white, it means a comparison could not be made.

- Next, we will want to make the matrix display only info for the strains we are interested in (S288C and Sigma1287b). Open the **Strains** pull-down menu, press Deselect All, then re-select Sigma1287b.



- Click on **PIR3** (O-glycosylated covalently bound cell wall protein) and in the sequence window select **Protein**. Scroll with your mouse along the green bar of sequence to see what the changes between strains are due to. Find the deletion beginning at Chr X1144715 and compare the protein sequences below.

- Now that we have identified that a deleted section of protein in a cell wall protein of Sigma1278b, we have a clue as to why this strain behaves differently from S288C. To examine PIR3 more closely, click the name in the upper left of the page to go to the locus summary page. From the PIR3 Locus Summary page, you can see in the Description that this protein is known to vary between strains.
- In the list of references below, you'll find papers referring to the role of this cell wall protein (and its relations) in heat shock, response to toxins, and cell wall integrity. The differences in this protein between strains might contribute to variations in behavior, such as differences in pseudohyphal growth for Sigma1278b relative to S288C



Variant Viewer: Sequence Tab

- Variant Viewer is also embedded in the Sequence tab of every gene page, with the data for the gene already pre-loaded from the results of the Variant Viewer search. This allows you to look at the variant information for a gene without starting from the tool's entry page.


BUD4 / YGR092W
Sequence Overview
Reference Strain: S288C
Alternative Reference Strains
Variants
Other Strains
History
Resources
Variants
Genomic DNA
Protein
Upstream IGR
Downstream IGR
Location: Chromosome X 598735..603078
Download
Legend

FungiDB: SNPs and Population Genetics

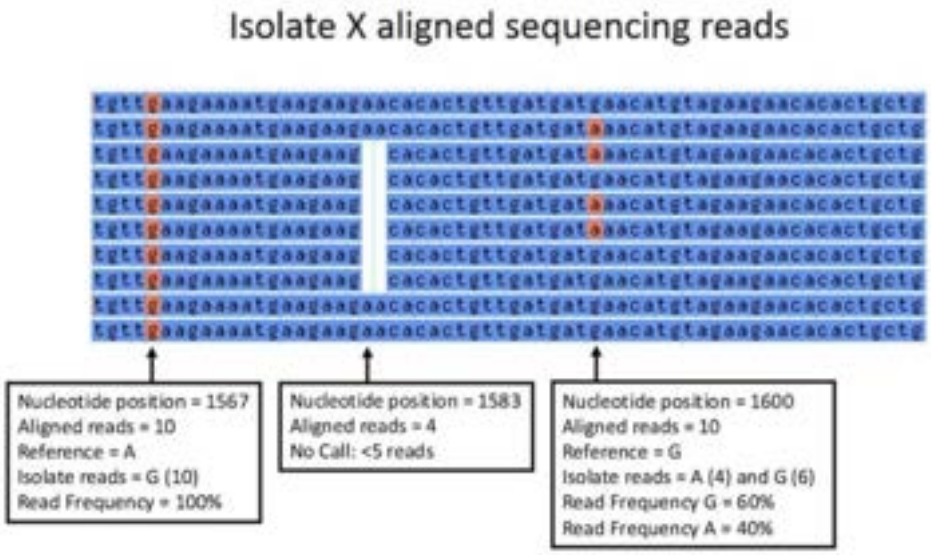
Learning Objective:

- Investigate SNP datasets using the following searches:
  - SNP characteristics,
  - SNPs between groups of isolates,
- Identify aneuploidy with the copy n umber variations earch.

Single nucleotide polymorphisms (SNPs) are genetic variations that may or may not have an impact on the function of a gene. Most SNPs do not affect gene function. However, some SNPs that lead to a change in the amino acid or a premature stop codon (nonsense) can directly affect protein function. SNPs that do not occur within genes are non-coding, but they may still influence processes such as splicing, mRNA stability and transcription. SNPs are useful for identifying similarities and differences between isolates or groups of isolates. They can also be used to identify genes that are under evolutionary pressure, either to remain unchanged (purifying selection) or to change (diversifying or balancing selection).

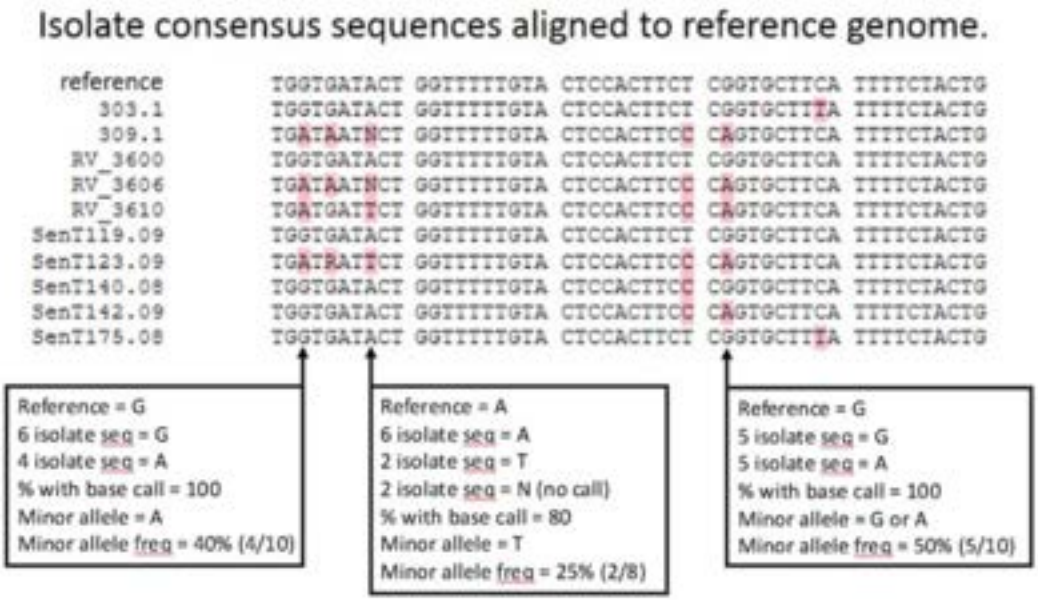
Read Frequency Threshold:

The parameter lets you control the quality of the sequencing data and the confidence of the SNP calls. Read Frequency Threshold is a particularly important parameter when dealing with diploid (or aneuploid) organisms since a read frequency of ~50% is expected for heterozygous SNPs.
Each isolate's sequencing reads are aligned to a reference genome and then each nucleotide position with 5 or more aligned reads is examined. A base call is made if the aligned reads meet your Read Frequency Threshold. For example, *Isolate X* has 10 aligned reads at nucleotide position 1600. If 6 reads are G and 4 reads are A, the read frequency is 60% for the G call and 40% for A. Running this search with the Read Frequency Threshold set to 80% will prevent a base call and consequently exclude *Isolate X* when returning SNPs for nucleotide position 1600. Running the search with the Read Frequency Threshold set to 60% will bring back a G for this isolate and a 40% threshold will return two calls (both G and A) at this position.



Minor allele frequency:

The minor allele frequency refers to the least common base call for a single nucleotide position across all isolates. The default setting for this parameter is 0% and returns all SNPs - instances where at least one isolate has a base call that differs from reference. Increase the Minor allele frequency to ensure that SNPs returned by the search are shared by a larger percentage of isolates in your group.



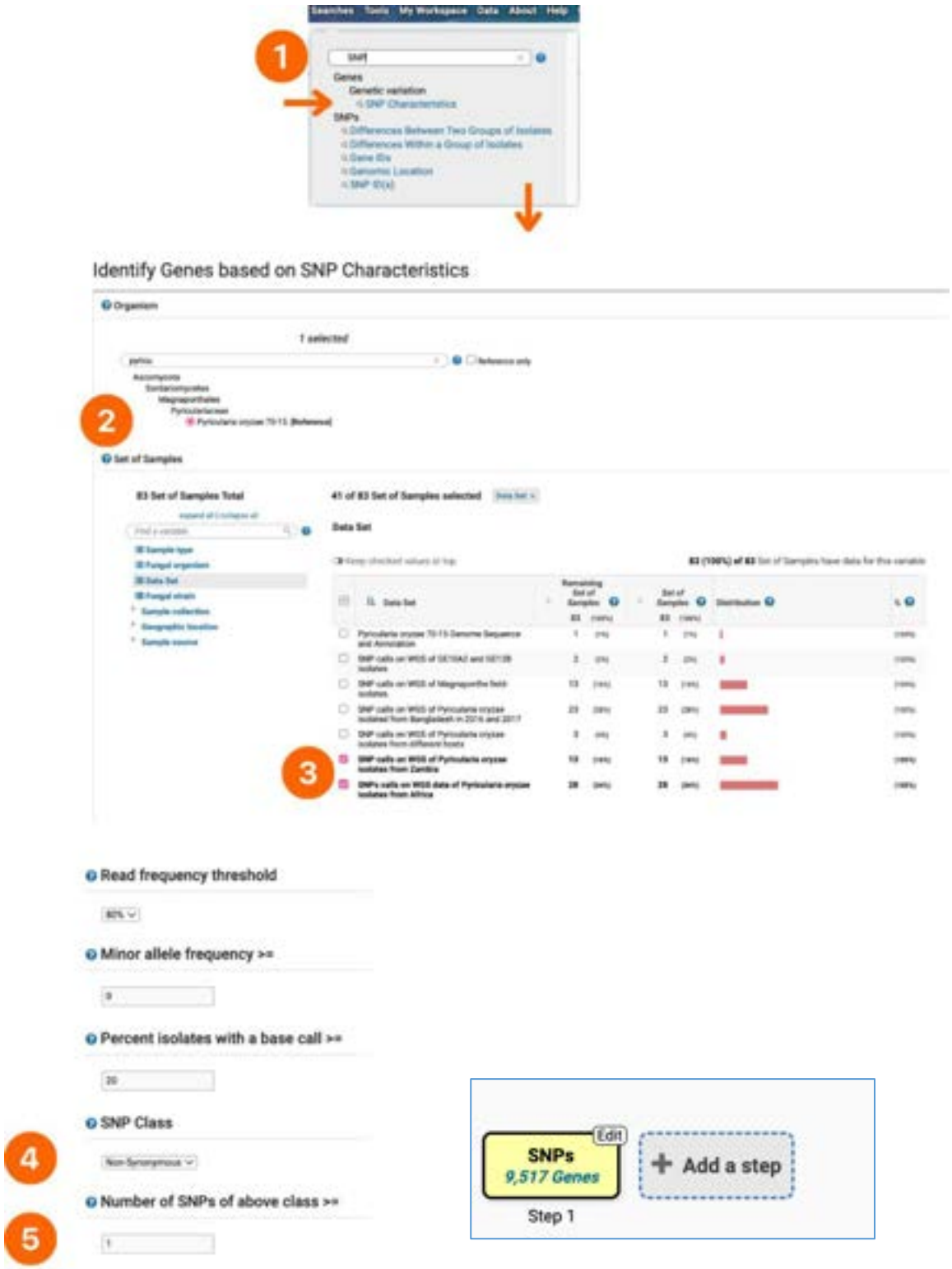
Percent isolates with a base call:

Sometimes an isolate does not have a base call at a certain nucleotide position because the Read Frequency Threshold was not met or because there were less than 5 aligned sequencing reads for that nucleotide position. In this case, an SNP can be returned by the search based on a subset of your isolate group. The 'Percent isolates with a base call' parameter defines the fraction of isolates that must have a base call before an SNP is returned for that nucleotide position. The default setting for this parameter is 80%, or 8 out of 10 isolates in your group must have a base call for an SNP to be returned by the search. The higher this parameter, the more likely the SNP is to be high quality as regions difficult to align or difficult to sequence will tend to have a lower percentage of calls since the coverage and/or quality will be lower in that region.

A. Identify Genes with the 'SNP Characteristics' search. Identify putative nuclear effectors with at least 1 non-synonymous SNP in *Pyricularia oryzae*.

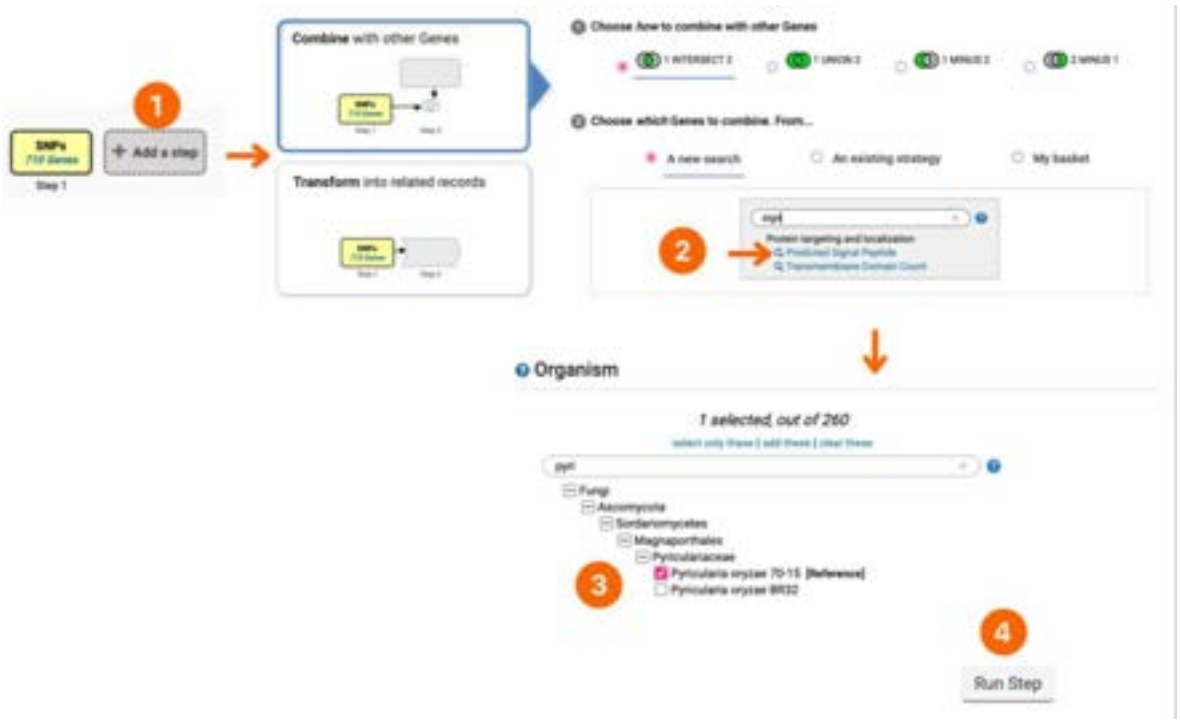
Pyricularia oryzae (also known as *Magnaporthe oryzae*) is a pathogen that affects rice crops, causing a severe disease known as rice blast. During the infection process,
P. oryzae and other plant pathogens make use of various types of effectors to manipulate the plant's immune system. Nuclear effectors are a type of effector that contain both a secretion signal and a DNA-binding domain. In the upcoming exercise, we will be analyzing a collection of *P. oryzae* isolates obtained from rice plants in different parts of Africa. Our goal is to identify genes with at least one non-synonymous SNP, which also exhibit characteristics of nuclear effectors.

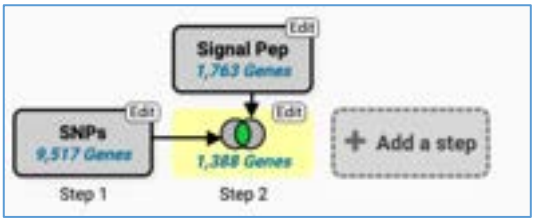
- **Identify genes with at least 1 non-synonymous SNP.**
    1. Deploy the “SNP characteristics’ search.
    2. Select *Pyricularia oryzae* 70-50 from the organism tree.
    3. In the Data Set section, select the datasets where isolates were collected in Zambia and other African fields.
    4. Set the “SNP Class” parameter to “Non-Synonymous”.
    5. Choose to identify genes with at least 1 non-synonymous SNPs and click on the “Get Answer” button.



- **dentify putative nuclear effectors based on the presence of both a secretion signal and the DNA-binding domains IPR007219 or IPR009071.**

    1. Click on the “Add a Step” button.
    2. Use the “Combine with Other Genes” option to deploy the “Predicted Signal Peptide” search.
    3. Set the genome to *Pyricularia oryzae* 70-50.
    4. Click on the “Run Step” button.

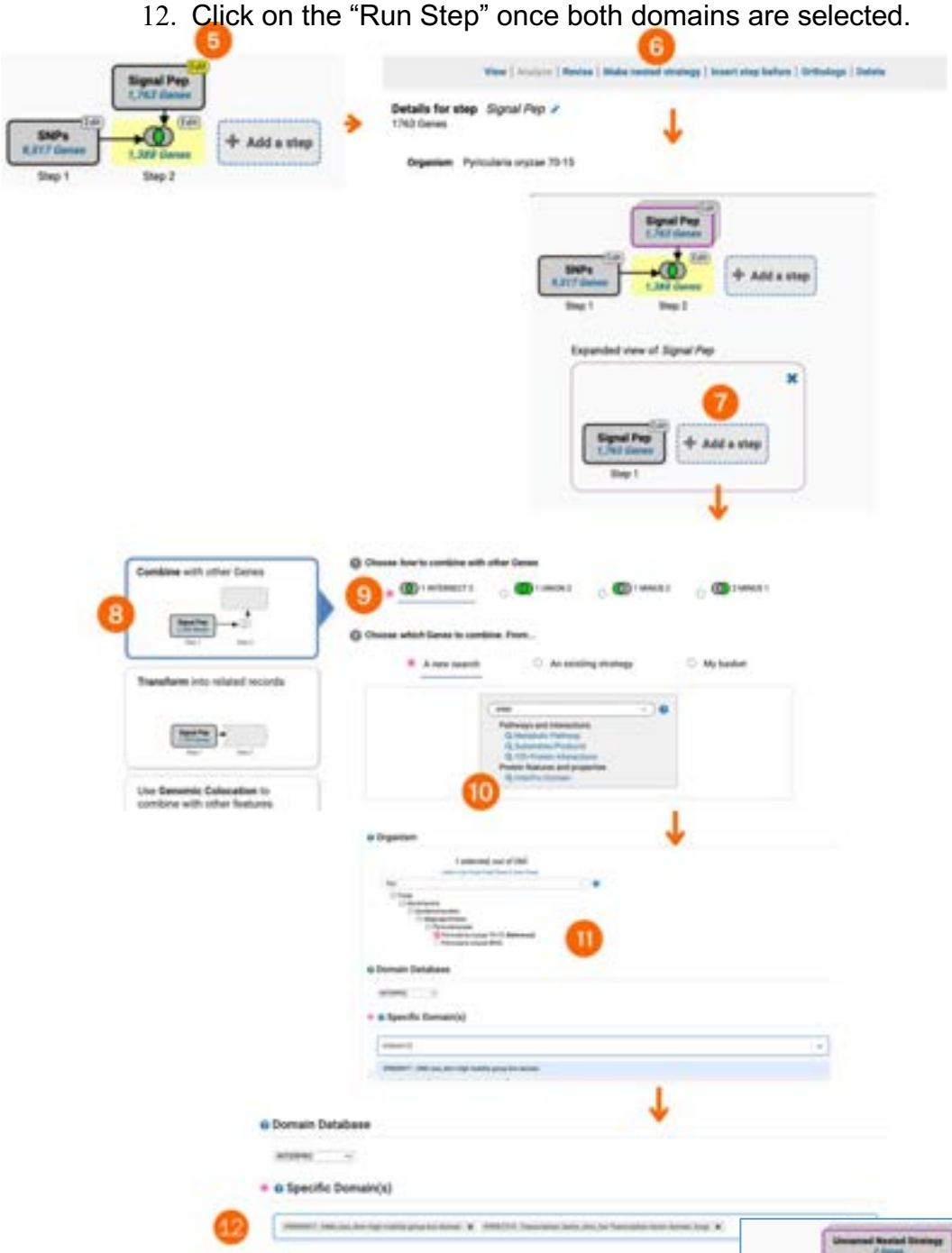




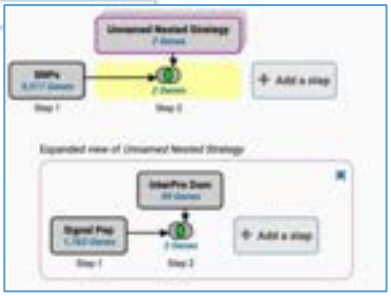
Note that currently, our strategy returns genes with at least 1 SNP and a predicted signal peptide domain. How can we identify genes with at least 1 SNP and a predicted signal peptide domain AND a DNA-binding domain? (Hint: you can do this with a nested strategy as described below).

5. Hover over the “Signal Pep” search box and click on the “Edit” option.
6. Select the “Make nested strategy” option at the top.
7. Click on the “Add a Step” button within the “Expanded view of *Signal Pep”* (nested) strategy.
8. Select the “Combine with other Genes” search.
9. Set the Boolean operator to “1 intersect 2”.
10. Deploy the “InterPro Domain” search.

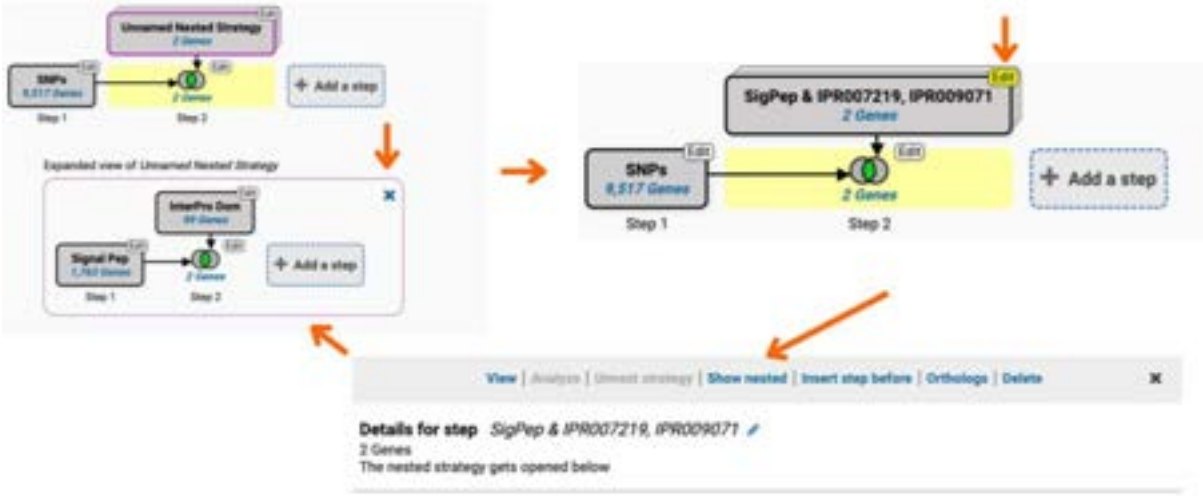
11. Set the genome to *Pyricularia oryzae* 70-50 and set the "Domain database" to InterPro and enter and select the following DNA binding domains from the dropdown menu: IPR007219, IPR009071.
12. Click on the "Run Step" once both domains are selected.



How many genes were identified to be putative nuclear effectors that have non-synonymous SNPs in the isolates collected in Africa?



Note: Nested strategy can be collapsed and expanded later as needed:



Strategy URL:
https://fungidb.org/fungidb/app/workspace/strategies/import/bd657f5629cac5df

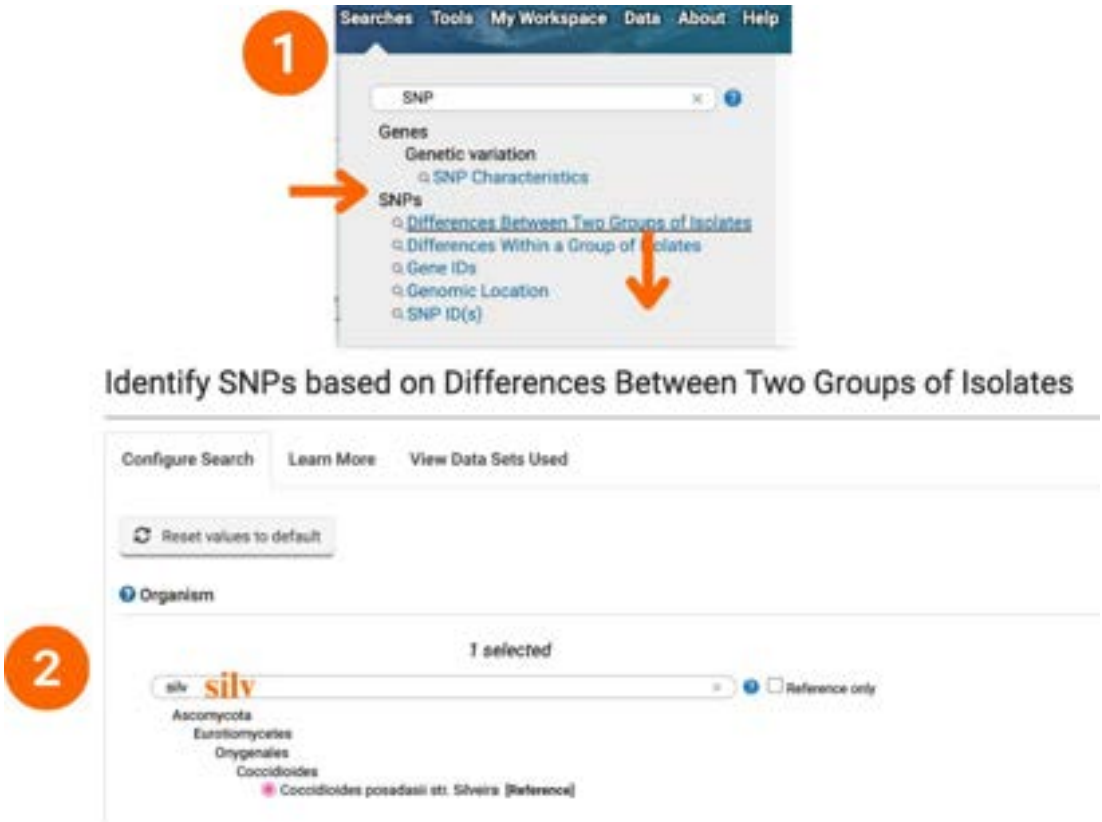
References: https://www.nature.com/articles/s41467-020-19624-w

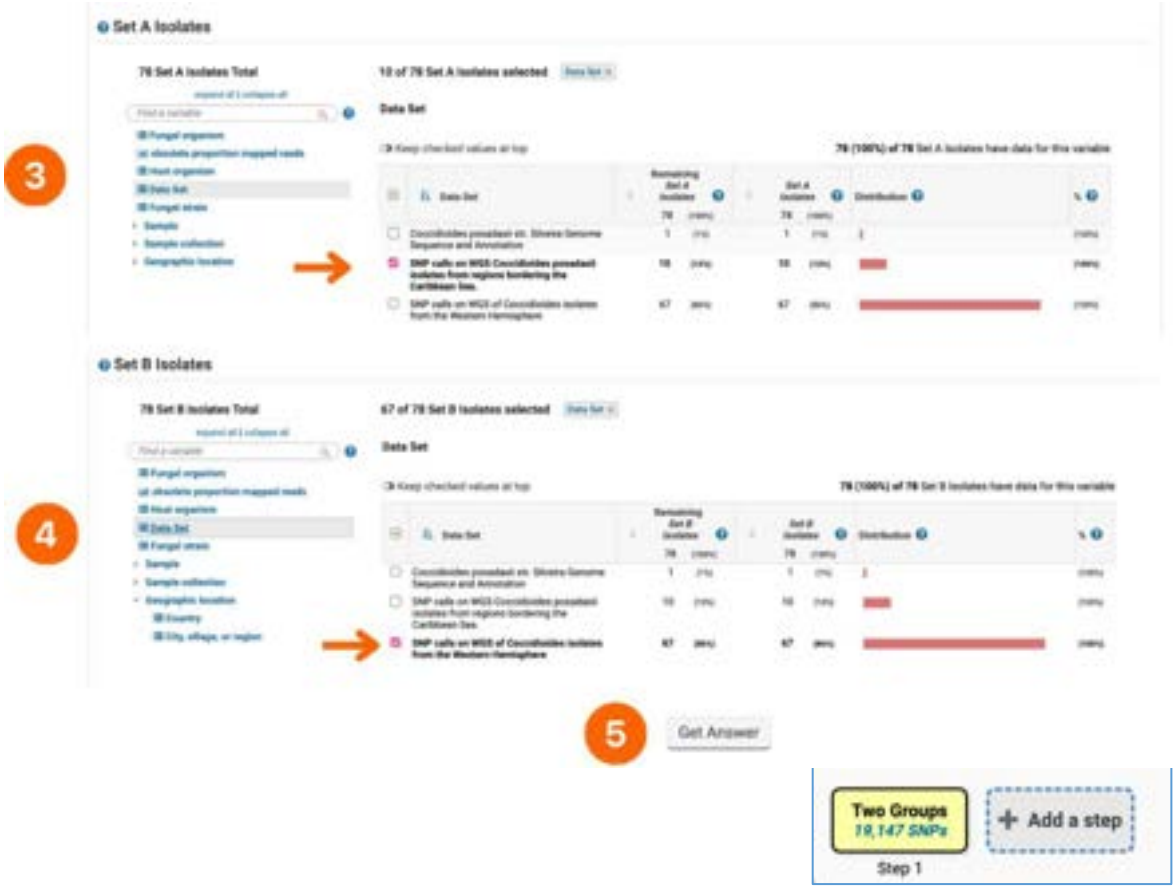
B. Identify SNPs based on Differences Between Two Groups of Isolates

Coccidioidomycosis, also known as Valley fever, is a disease caused by two closely related species of fungi – *Coccidioides immitis* (*C. immitis*) and *Coccidioides posadasii* (*C. posadasii*). The disease is associated with high morbidity and mortality rates that affect tens of thousands of people every year. These two fungal species are found in several regions in the Western Hemisphere, but recent studies suggest that their geographic range is expanding. The following example describes the identification of SNPs (single nucleotide polymorphisms) in *C. posadasii* str. Silveira isolates that were collected from different geographic locations.

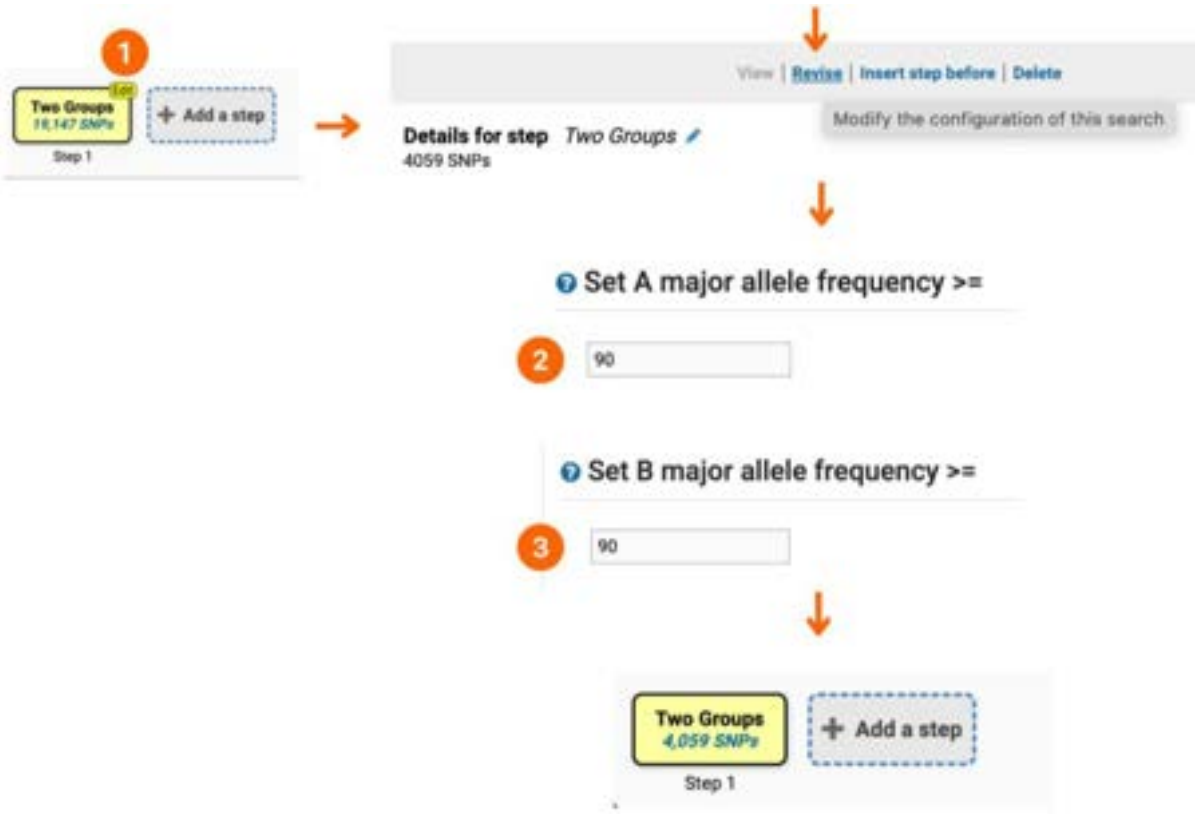
- **Identify SNPs between two groups of *C. posadasii* str. Silveira isolates**

1. Deploy the “Difference Between Two Groups of Isolates” search.
2. Set the genome to *Coccidioides posadasii* strain Silveira.
3. Select Set A isolates from the Data Set menu: Caribbean dataset.
4. Select Set B isolates from the Data Set menu: Western hemisphere dataset.
5. Click on the “Get Answer” button to get the results.





- **Change the stringency of your search to major allele frequency >= 90%**



The search strategy returns SNPs rather than genes, which are classified by genomic location within the results table. When individual SNPs fall within a gene, its corresponding Gene ID is listed next to the SNP record (Gene ID column).

- Each SNP is linked to its own record page. Click on the NGS_SNP.GL636536.6075.

SNP location, allele summary, associated GeneID, major and minor allele records can be found at the top of the page, followed by DNA polymorphism summary and SNP records table that is searchable by isolate IDs.

A summary of all SNPs detected in this gene across all datasets integrated into FungiDB is displayed in the SNP Genomic Context section:

SNPs are denoted by diamonds that are coloured based on the coding potential:

- noncoding (yellow diamonds)
- non-synonymous (dark blue)
- synonymous (light blue)
- nonsense (red)

In **the SNP alignment section**, you can choose to align a group of selected isolates based on the metadata filters:

The **Country Summary** section provides a global overview of the major and minor alleles per country:

Country Summary

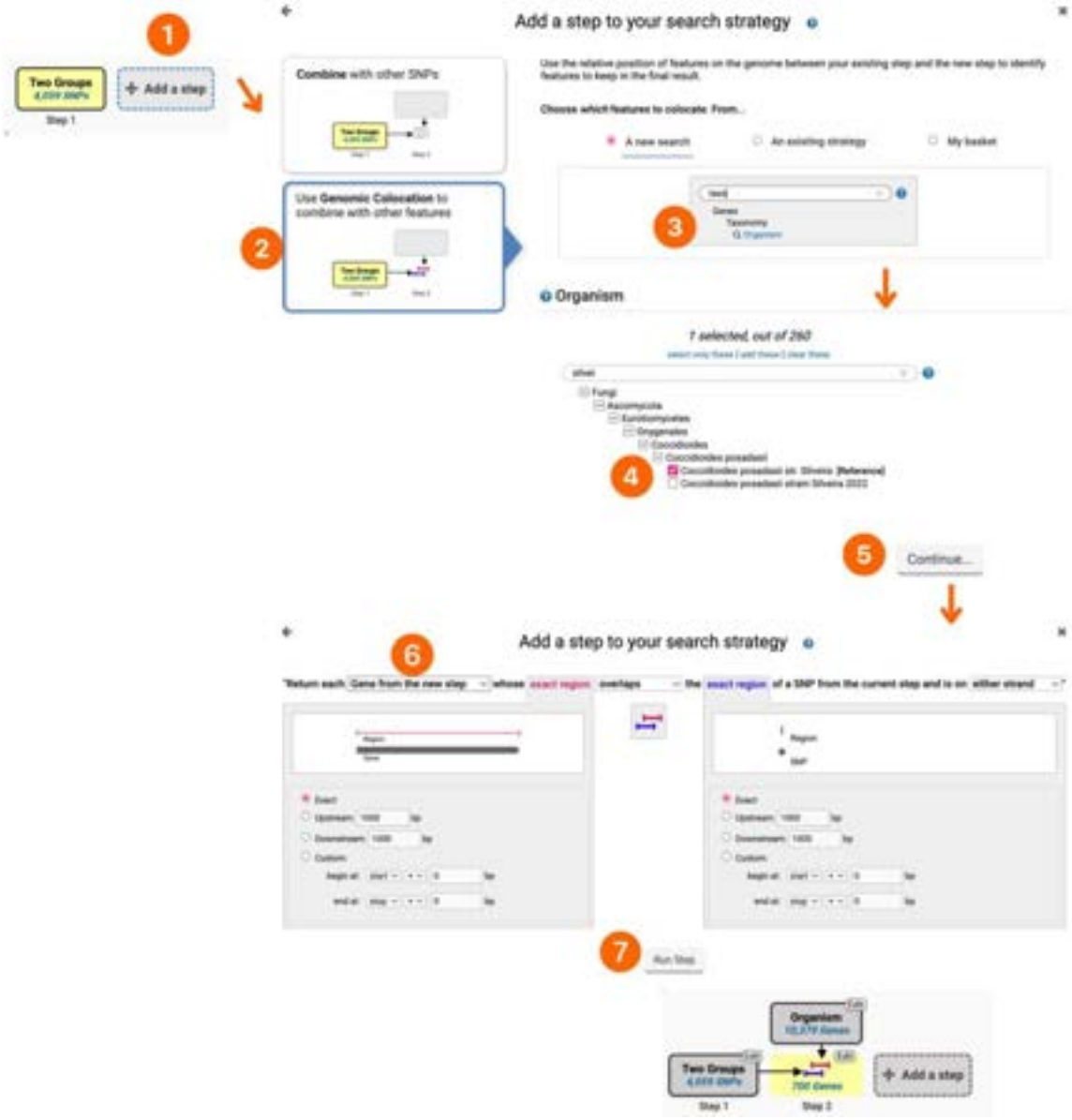
Geographic Location	#Alleles	Major Allele	Minor Allele	Other Allele
United States of America	65	C (.62)	T (.38)	N/A
Mexico	15	C (.53)	T (.47)	N/A
Venezuela	10	T (.7)	C (.3)	N/A
Guatemala	6	C (.83)	T (.17)	N/A
Argentina	2	C (.5)	T (.5)	N/A
Brazil	2	C (.5)	T (.5)	N/A
Paraguay	2	C (.5)	T (.5)	N/A
unknown	1	C (1)	N/A	N/A

DNA-seq reads can be viewed by clicking on the *view DNA-seq reads* link from within the table.

Venezuela	JTORRES	EUSMPL0102-1-7	C	G	C	75	100	view DNA-seq reads

Clicking on the “view DNA-seq reads” link will re-direct you to a JBrowse highlighting SNPs detected. You can select more tracks to examine by clicking on the Select Tracks tab on the left.

- **Map SNPs from Step 1 to genes in *C. posadasii* str. Silveira.**
    1. Click on the “Add a step” button.
    2. Select the “Use Genomic Colocation to combine with other features” tool.
    3. Filter searches on “taxonomy” to identify the “Organism” search.
    4. Select *C. posadasii* strain Silveira genome.
    5. Click on the “Continue…” button to specify colocation search parameters.
    6. Select to return ‘Gene from the new step’ whose exact region overlaps the SNP.
    7. Click on the “Run Step” button for results.



In this strategy we compared SNPs in C. posadasii collected in different geographical regions and identified 700 genes that overlap with these SNPs. For those genes that are not well characterized (e.g., conserved hypothetical proteins) you can use other searches and tools to understand their function.

Strategy URL:
https://fungidb.org/fungidb/app/workspace/strategies/import/d9d0fff2dbda229d

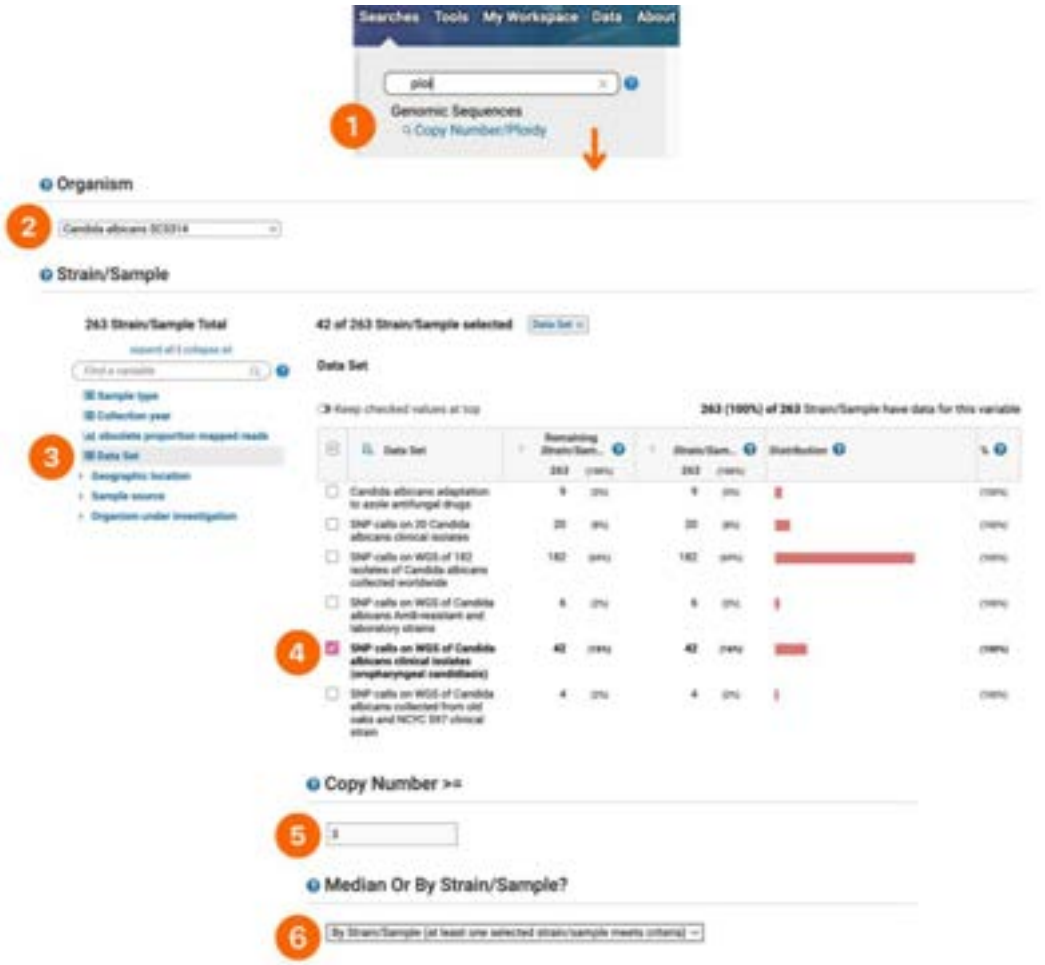
C. Copy number variation & ploidy searches.

Gene copy number variation can be caused by deletions or duplications. In addition to being useful for variant calling, high-throughput sequencing data can be used to determine regions with copy number variation (CNV). All reads in FungiDB are mapped to the same reference strain as SNP datasets, and, as a result, we can estimate a gene's copy number in each of the aligned strains.

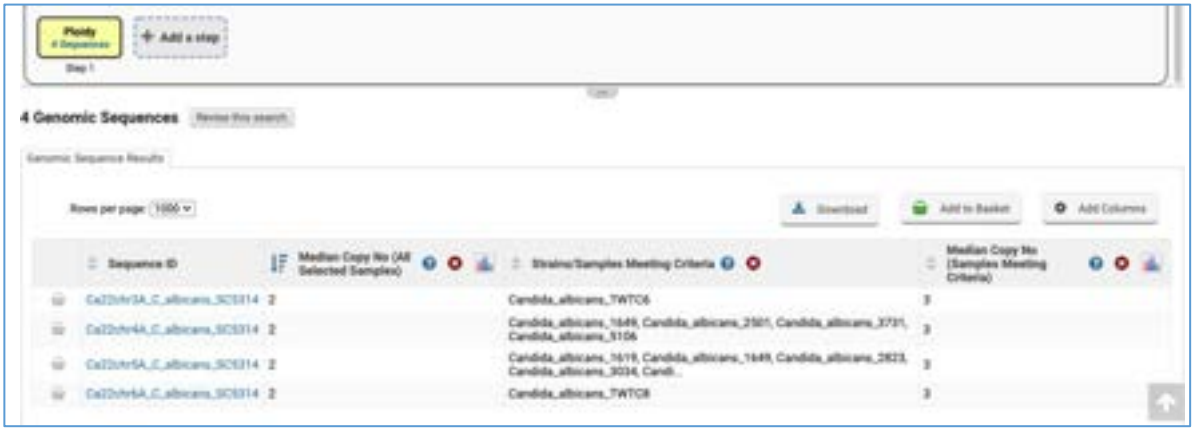
C.1. Copy Number/Ploidy search (Genomic Sequences)

Find genomic sequences or chromosomes based on their estimated copy number in resequenced strains. Genomic sequences returned by the search will either have a median estimated copy number greater than or equal to the value you entered for the Copy Number across the selected strains/samples **or** will have an estimated copy number greater than or equal to the value you entered for the Copy Number in at least one of the selected strains/samples.

- **Identify trisomic chromosomes in clinical isolates of *Candida albicans*.**
    1. Deploy the "Copy Number/Ploidy" search.
    2. Set the genome to Candida albicans SC5314.
    3. Navigate to the Data Set section.
    4. Select the dataset called "SNP calls on WGS of Candida albicans clinical isolates (oropharyngeal candidiasis)".
    5. Set the Copy Number to "3".
    6. Select to identify ploidy "By strain/sample" and click on the "Get Answer" button.



The search by strain/sample (i.e., at one or more of the selected strains must match the criteria rather than the median of the selected strains matching) is intended to find chromosomes where the whole chromosome is duplicated. It may find chromosomes where partial aneuploidy involves most of the chromosome but is unlikely to find chromosomes where partial aneuploidy only covers a small region. Also, because this search currently relies on coverage alone, it will not find instances of global genome duplication (e.g., all chromosomes became triploid).

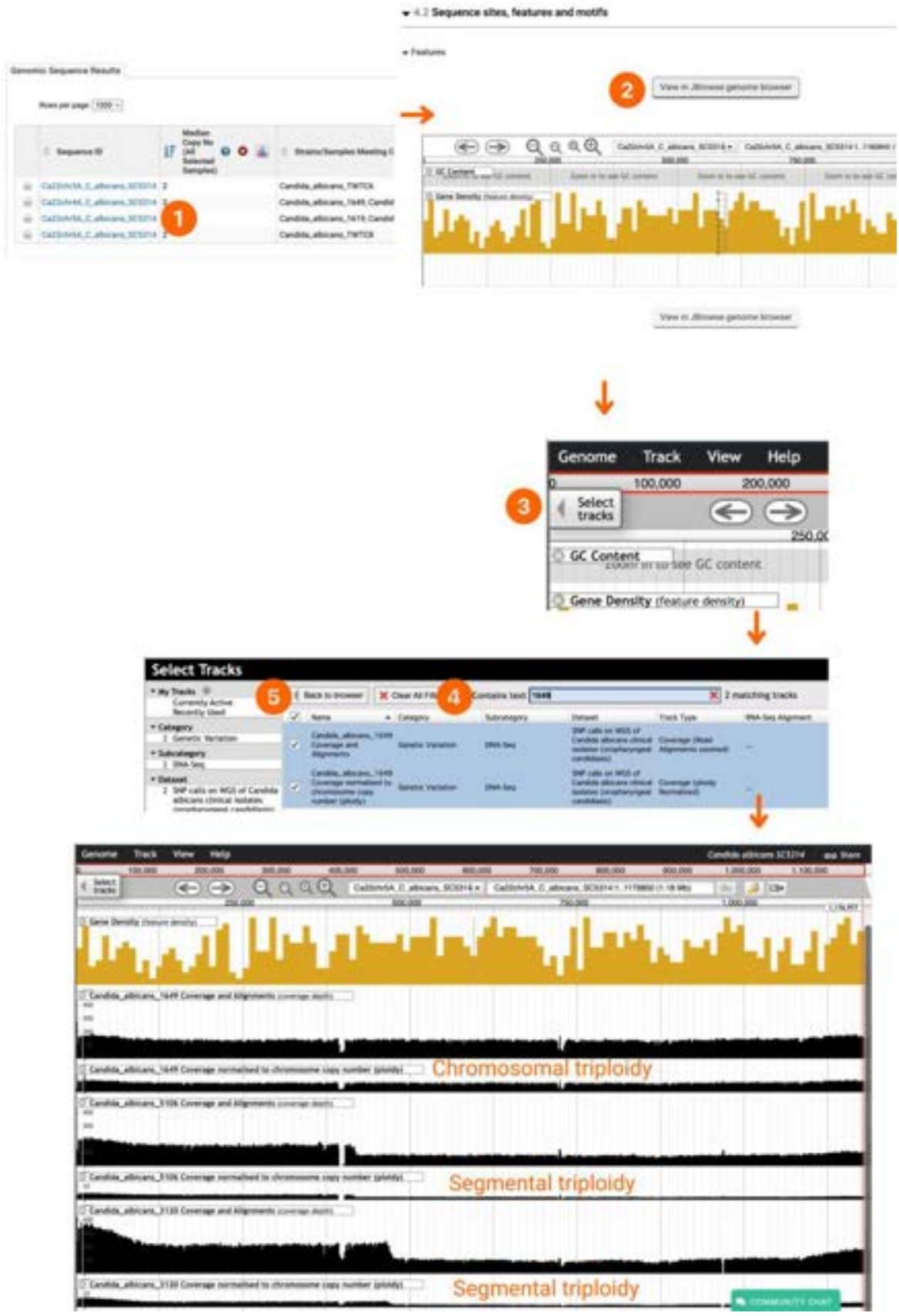


- **Explore segmental aneuploidy in JBrowse.**

JBrowse has two coverage tracks:

- Raw coverage from the alignment (available for every isolate where we have whole genome sequencing, whether we ran the copy number pipeline or not)
- Normalized coverage in bins (only available for isolates where we have run the copy number pipeline)

1. Click on one of the Sequence ID Ca22chr5A_C_albicans_SC5314 (in blue).
2. Navigate to JBrowse by clicking on the “View in JBrowse genome browser” button.
3. When in JBrowse, click on the Select tracks tab to customize your view.
4. Use the “Contains text” filter to identify and select tracks for the following isolates: 1649, 5106, and 3120.
5. Click on the “Back to browse” tab to return to JBrowse view with selected tracks.



Notice examples of chromosomal (1649) and segmental triploidy (5106 and 3120). The whole chromosome is shown in both screenshots, and both tracks are shown for each sample. Note that VEuPathDB is not currently normalizing for telomere proximity.

URL:
https://fungidb.org/fungidb/jbrowse/index.html?loc=Ca22chr5A_C_albicans_SC5314%3A1..1190845&data=%2Ffungidb%2Fservice%2Fjbrowse%2Ftracks%2FcalbSC5314&tracks=gcContent%2CgeneDensity%2CCandida_albicans_1649%20Coverage%20and%20Alignments%2CCandida_albicans_1649%20Coverage%20normalised%20to%20chromosome%20copy%20number%20(ploidy)%2CCandida_albicans_5106%20Coverage%20and%20Alignments%2CCandida_albicans_5106%20Coverage%20normalised%20to%20chromosome%20copy%20number%20(ploidy)%2CCandida_albicans_3120%20Coverage%20and%20Alignments%2CCandida_albicans_3120%20Coverage%20normalised%20to%20chromosome%20copy%20number%20(ploidy)&highlight=

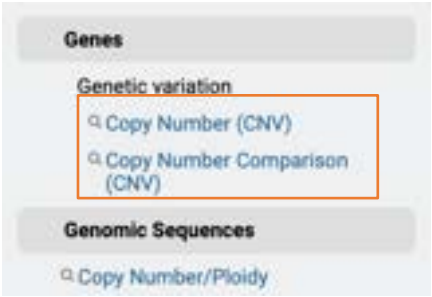
Strategy URL:
https://fungidb.org/fungidb/app/workspace/strategies/import/6dc86b214d14a5f3
References: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4383195/

C.2. Copy Number search

(Genes) Using Gene

Searches

One way to discover regions of potential segmental aneuploidy is to use the searches for genes by copy number. We have two searches: Gene searches taking advantage of sequence alignment data can be found under the "Genetic Variation" category. Two available searches that define regions of CNV are:



- **Copy number:** This search returns genes that are present at copy numbers (haploid number or gene dose) within a range that you specify.

- **Copy number comparison:** This search compares the estimated copy number of a gene in the re-sequenced strain with the copy number in the reference annotation. The copy number in the reference annotation is calculated as the number of genes that are in the same ortholog group as the gene of interest. We infer that these genes have arisen as a result of tandem duplication of a common ancestor.

Different metrics for defining copy number:

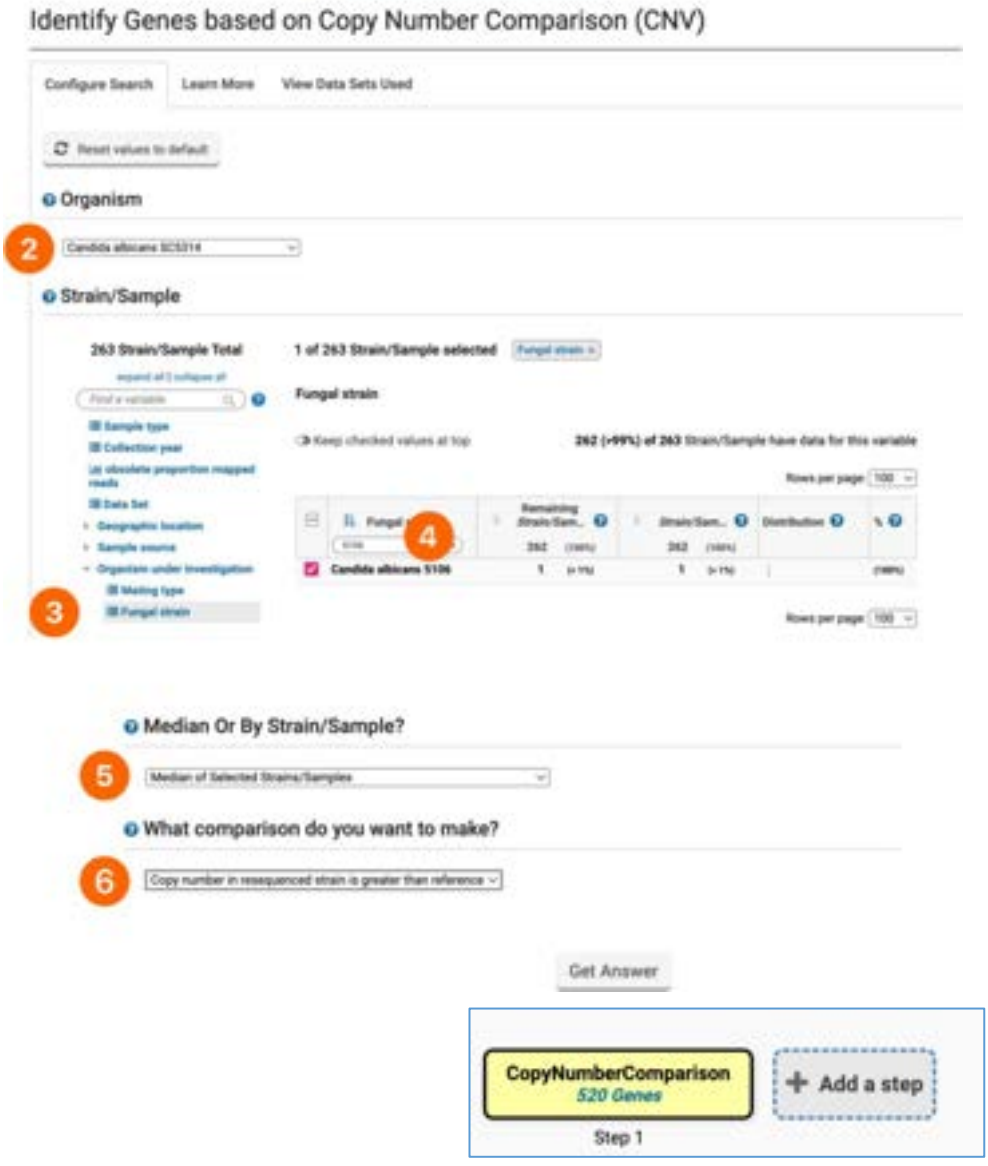
- **Haploid number** is the number of genes on an individual chromosome.
- **Gene dose** is the total number of genes in an organism, accounting for copy number of the chromosome.

For example, a single-copy gene in a diploid organism has a haploid number of 1 and a gene dose of 2. You can choose to search for genes where at least one of your selected isolates meets your cutoff criteria for the chosen metric (By Strain/Sample), or where the median of the chosen metric across all the selected isolates meets the cutoff (Median of Selected Strains/Samples)

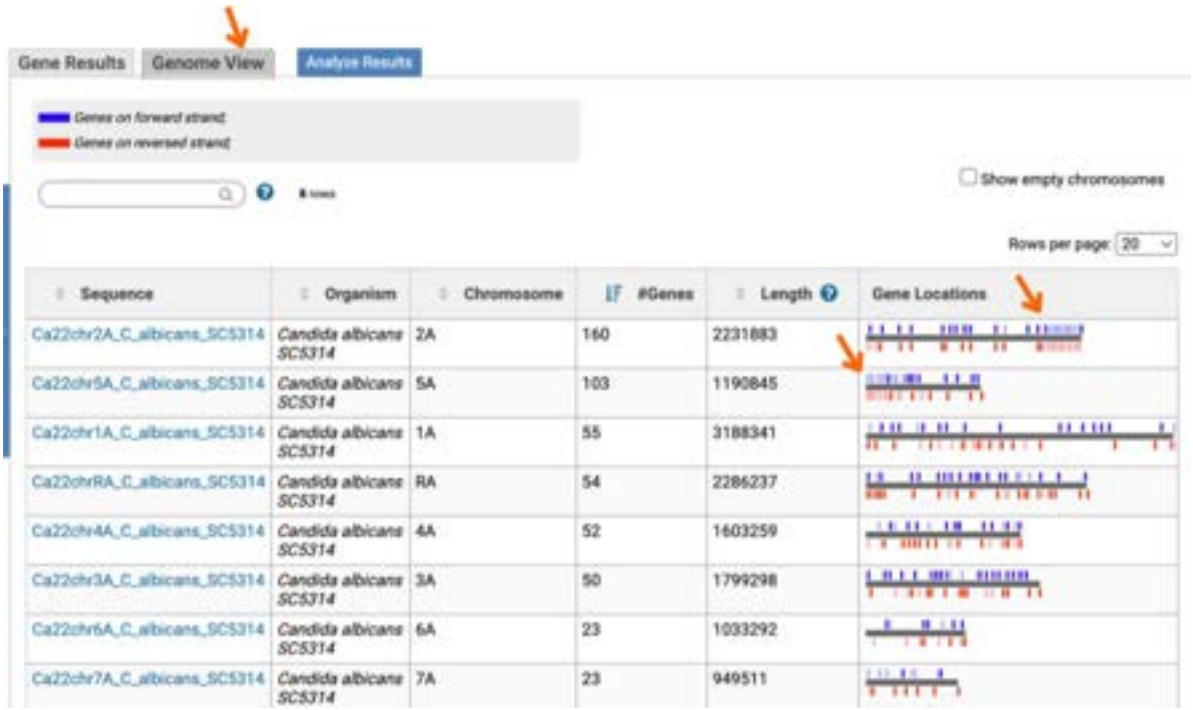
- **Discover regions of potential segmental aneuploidy in *Candida albicans* isolate 5106.**
    1. Deploy the “Copy Number Comparison (CNV)” search.
    2. Select the genome for “Candida albicans”.
    3. Navigate to the Fungal strain” metadata field.
    4. Filter isolates for “5106” and check the box to select this isolate.
    5. Leave the “Median or By Strain/Sample” parameter at default.

    Note: Choosing Median or By Strain/Sample will only make a difference if you have multiple strains.

    6. From the drop-down menu select the “Copy number in resequenced strain is greater than reference” option.



Examine the results using the Genome View option.



Sequence	Organism	Chromosome	#Genes	Length	Gene Locations
Ca22chr2A_C_albicans_SC5314	*Candida albicans SC5314*	2A	160	2231883	
Ca22chr5A_C_albicans_SC5314	*Candida albicans SC5314*	5A	103	1190845	
Ca22chr1A_C_albicans_SC5314	*Candida albicans SC5314*	1A	55	3188341	
Ca22chrRA_C_albicans_SC5314	*Candida albicans SC5314*	RA	54	2286237	
Ca22chr4A_C_albicans_SC5314	*Candida albicans SC5314*	4A	52	1603259	
Ca22chr3A_C_albicans_SC5314	*Candida albicans SC5314*	3A	50	1799298	
Ca22chr6A_C_albicans_SC5314	*Candida albicans SC5314*	6A	23	1033292	
Ca22chr7A_C_albicans_SC5314	*Candida albicans SC5314*	7A	23	949511	

As you can see in the highlighted regions, large numbers of genes predicted to have increased copy numbers are clustered at the right-hand end of chromosome 2 and the left- hand end of chromosome 5, corresponding to the segmental aneuploidies shown in the JBrowse session above.

Strategy URL:
https://fungidb.org/fungidb/app/workspace/strategies/import/07b439e0de5e9c6a

Exercise: Exploring variants in Ensembl Fungi

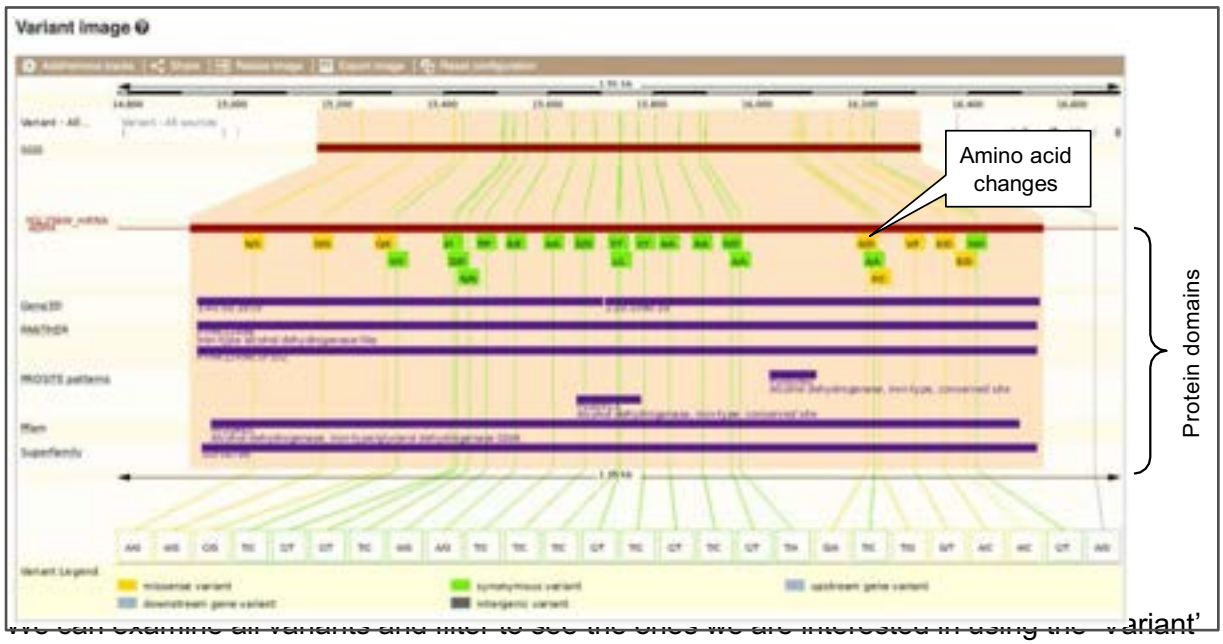
Links to be clicked shown in blue, text to be entered shown in red.

In any of the sequence views shown in the 'Gene' and 'Transcript' tabs, you can view variants on the sequence. You can do this by clicking on Configure this page 

from any of these views.

Let's take a look at the Gene sequence view for *ADH4* (gene stable ID: YGL256W). This gene is a ribonuclease protein in *Saccharomyces cerevisiae* (R64-1-1). Select *Saccharomyces cerevisiae* R64-1-1 under Favourite genomes on the Ensembl Fungi homepage. Search for YGL256W and go to the Variant image view.

This view shows variants mapped to the gene structure and protein domains.



We can examine all variants and filter to see the ones we are interested in using the 'Variant' table. Click on the Variant table link on the left-hand menu.

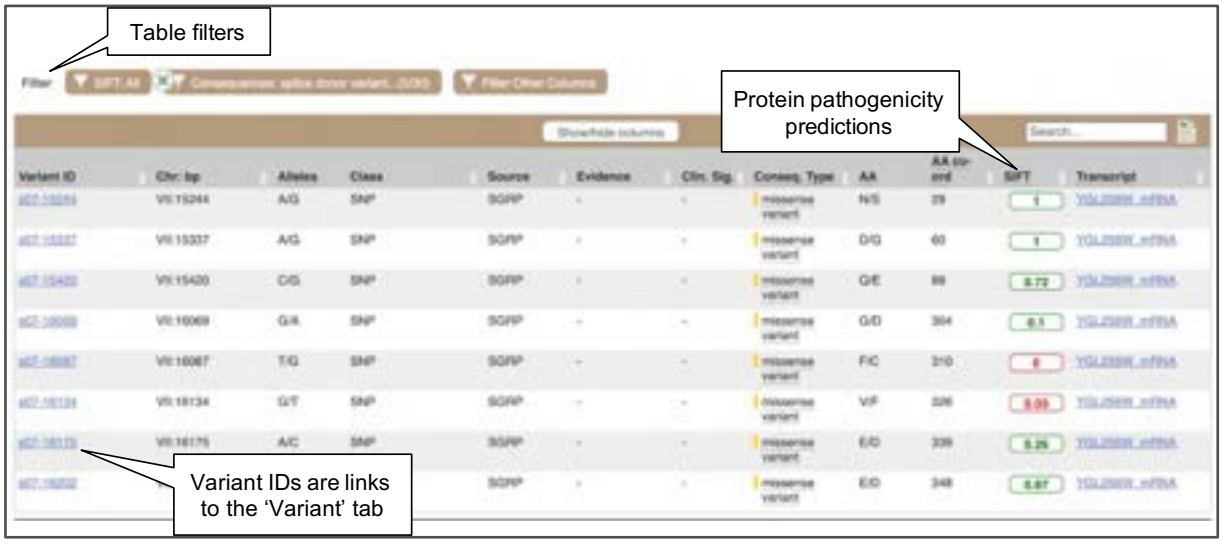
This table shows the variants in order of their occurrence throughout the genome, and they are reported on the forward strand. The gene *ADH4* is located on the forward strand, so we are first shown variants upstream of the gene (starting at the 5' upstream region).

(a) How many variants in this gene are predicted to be missense?

You can filter the table to view variants that alter the protein sequence. Click on the Consequences: All button above the table. Click the option 'PTV and Missense' in the pop-up, then Apply. You can also filter by other columns such as variant Class.

(b) Are there any known variants in this gene predicted to be deleterious?

The SIFT scores (https://doi.org/10.1093/nar/gkg509) predict the consequence of the variant on the function of the protein taking into account chemical changes and conservation of amino acids. Scores <0.05 and coloured red are 'deleterious' while scores >0.05 and coloured green are 'tolerated'.



Let's have a look at a specific variant. Click on the top result in the filtered table, or

search for s07-15244. This will open the 'Variant' tab.

The icons show you what information is available for this variant.

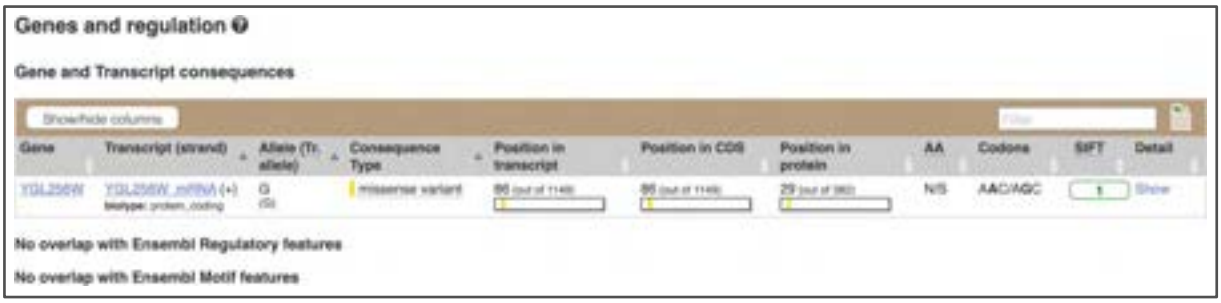
(c) What are the genomic coordinates of this variant?

(d) What is the reference allele? (*Hint: Ensembl always reports alleles on the forward strand. The reference allele is given first.*)

You can find some background information on variants, alleles and haplotypes here: https://www.ebi.ac.uk/training/online/courses/human-genetic-variation-introduction/what-is-genetic-variation/what-are-variants-alleles-and-haplotypes/. The reference allele for s07-15244 is A.

(e) How many genes are affected by this variant? Does it have the same consequence across different transcripts of different genes?

Click on the Genes and regulation icon, or follow the link in the left-hand panel.



This variant overlaps one gene. It causes a change in the protein sequence (missense variant) in the YGL256W gene we were looking at (note that only missense variants have SIFT scores).

(f) Which allele is major in the *Saccharomyces* Genome Resequencing Project (SGRP) study?

Click on Genotype frequency in the left-hand menu. Note that the reference allele A is more frequent than the alternative allele G in this case.

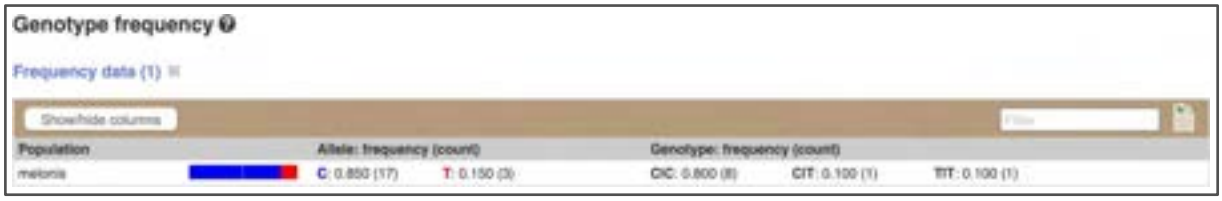
Additional Exercise – Variation data in *Fusarium oxysporum*

(a) Select the *Fusarium oxysporum* FO2 genome and search for FOXG_13574T0 gene. One of its upstream variants is SNP tmp_10_6610. What are the possible alleles for this polymorphic position? Which one is on the reference genome?
(b) What is the most frequent allele at this position? How many heterozygous individuals were observed in the melonis population?
(c) Which individuals have got genotypes C|T and T|T?

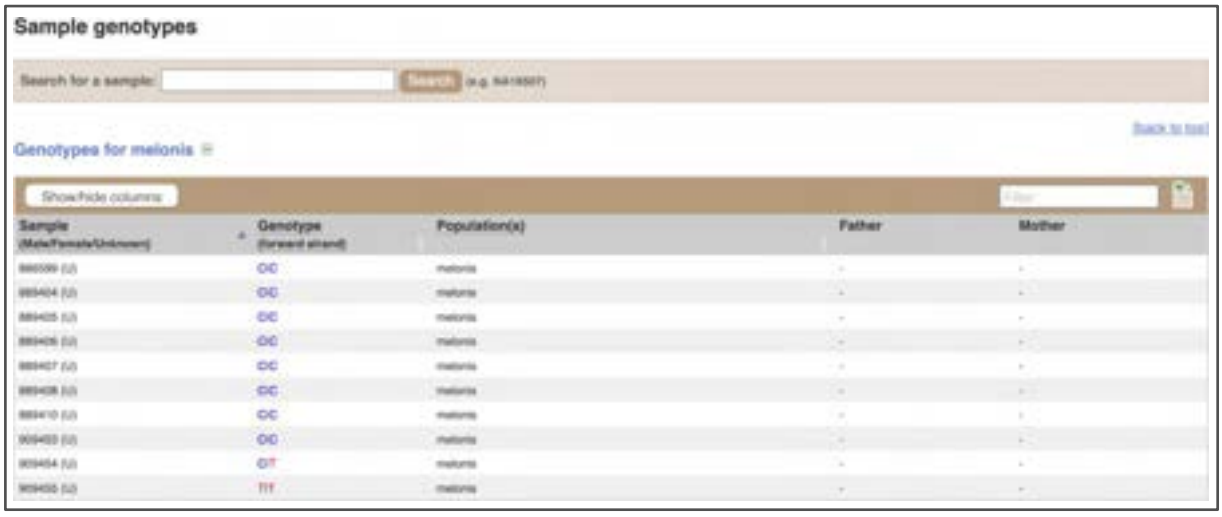
Answers

(a) You can find the alleles in the summary information at the top of the 'Variant' tab. The reference allele for tmp_10_6610 is C and the alternative allele is T.

(b) Click on Genotype frequency in the left-hand panel. The most frequent allele is C. There is one heterozygous individual in the melonis population.



(c) Click on Sample genotypes in the left-hand panel. Individual 909454 is heterozygous (C|T genotype) and individual 909455 is homozygous for the minor allele (T|T genotype).


Sample genotypes
Search for a sample:
Genotypes for melonis
Show/hide columns
Sample
Genotype
Population(s)
Father
Mother

Exercise: The Ensembl Fungi Variant Effect Predictor (VEP)

We have identified four variants in *Verticillium dahliae* JR2: chromosome 5, C->G at 698711, G->T at 698935, G->A at 700313 and C->A at 701484. Use the Ensembl VEP to determine:

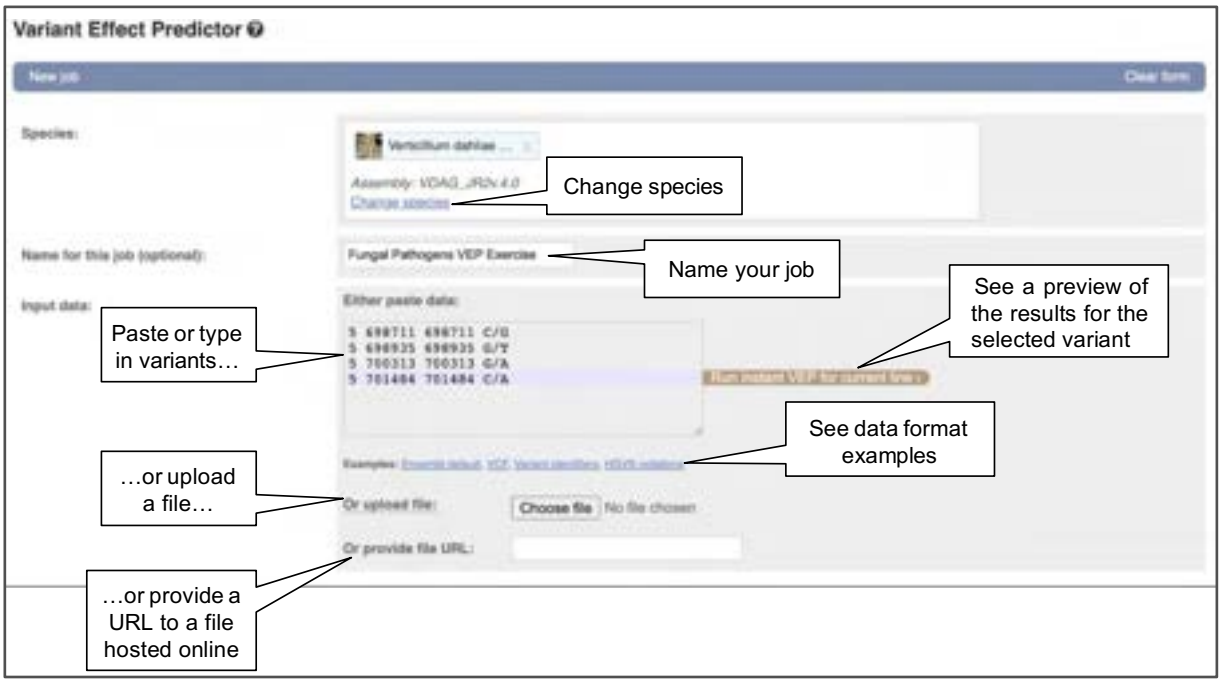
(a) Are your variants novel or have they already been annotated in Ensembl?
(b) What genes are affected by your variants?
(c) Do any of your variants affect gene regulation?

Click on Tools in the top brown bar from any Ensembl Fungi page, then Variant Effect Predictor to open the input form. You will need to change the species to *Verticillium dahliae* JR2 and paste your input data in the provided text box.

The VEP recognises a number of input formats including the Ensembl default format, variant call format (VCF), variant identifiers and HGVS notations. The HGVS nomenclature is a globally recognised standard for describing variants. You can read more about this here: https://hgvs-nomenclature.org/stable/.

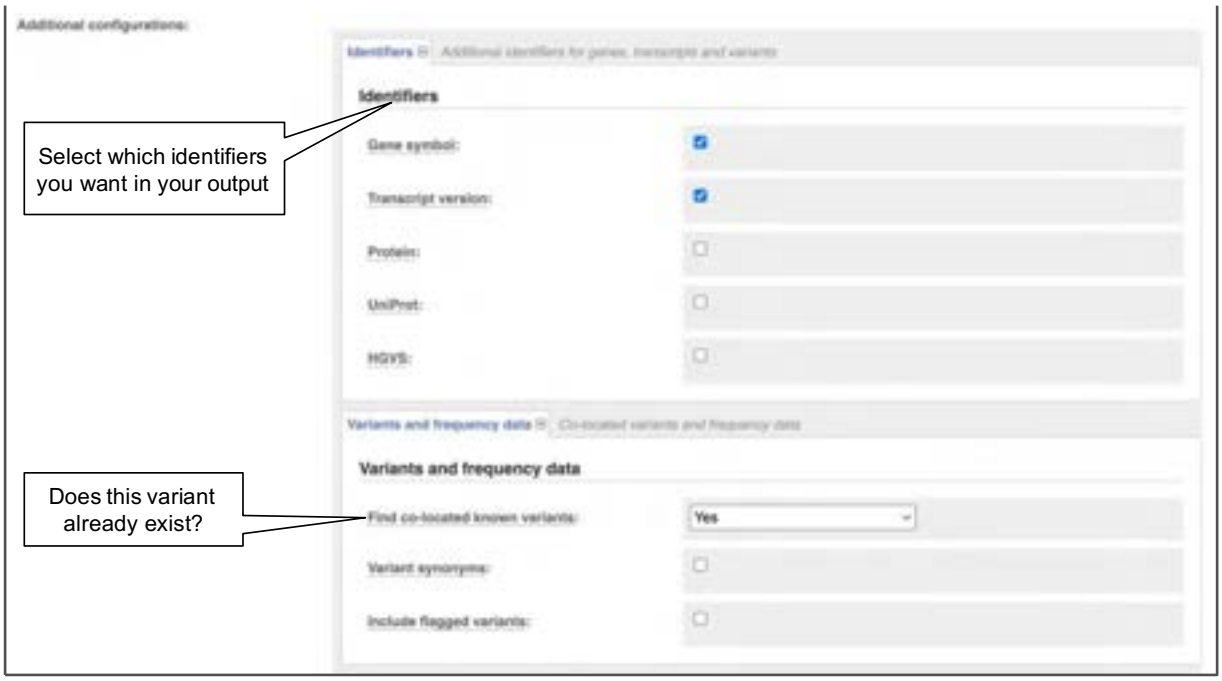
The Ensembl default format is composed of four compulsory columns and additional 'strand' column: Chromosome, Start Position, End Position, Alleles (reference/alternate), Strand (1 for forward; -1 for reverse), with one line per variant. Your variants in this format would look like this:

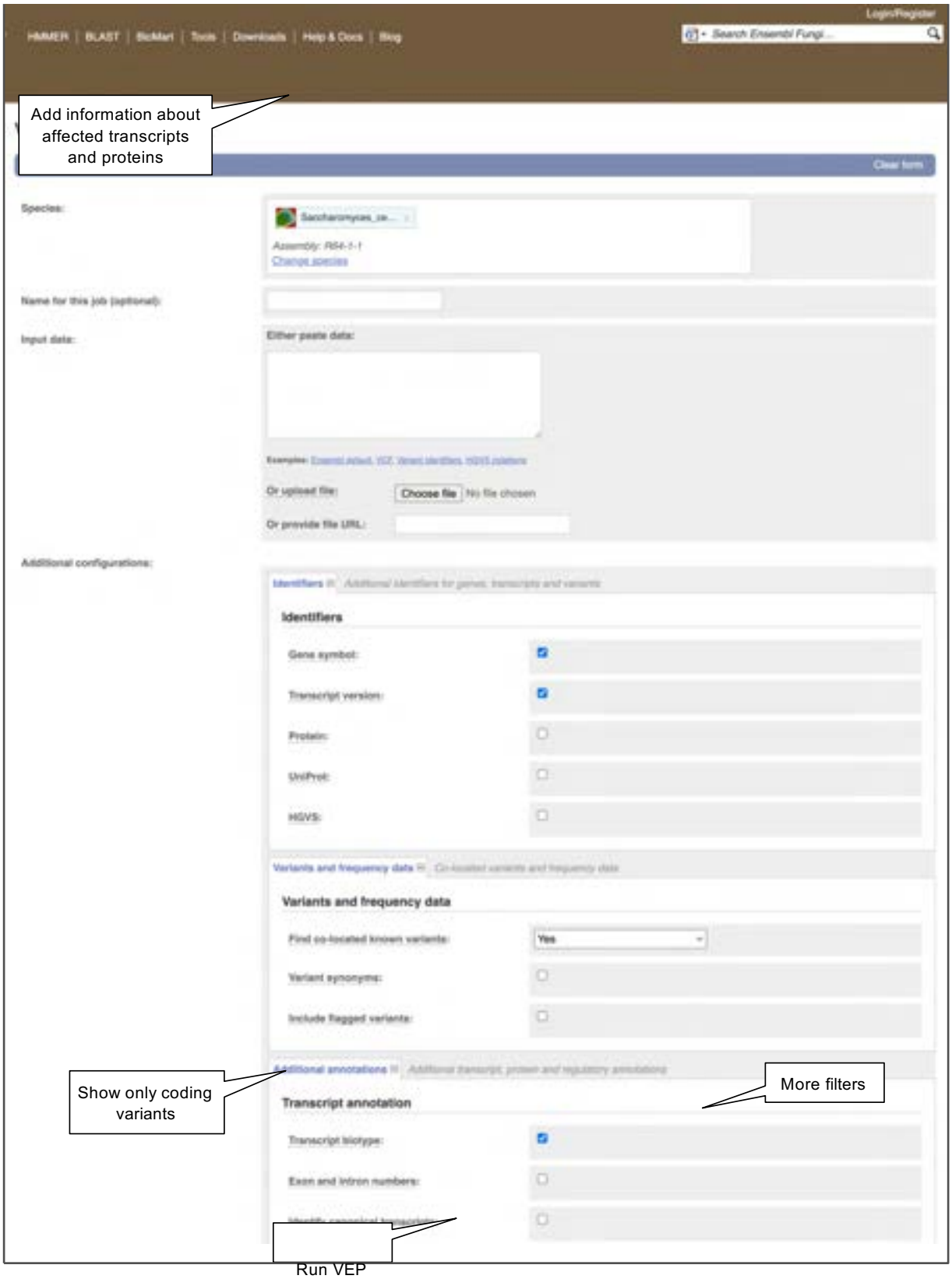
5 698711 698711 C/G
5 698935 698935 G/T
5 700313 700313 G/A
5 701484 701484 C/A



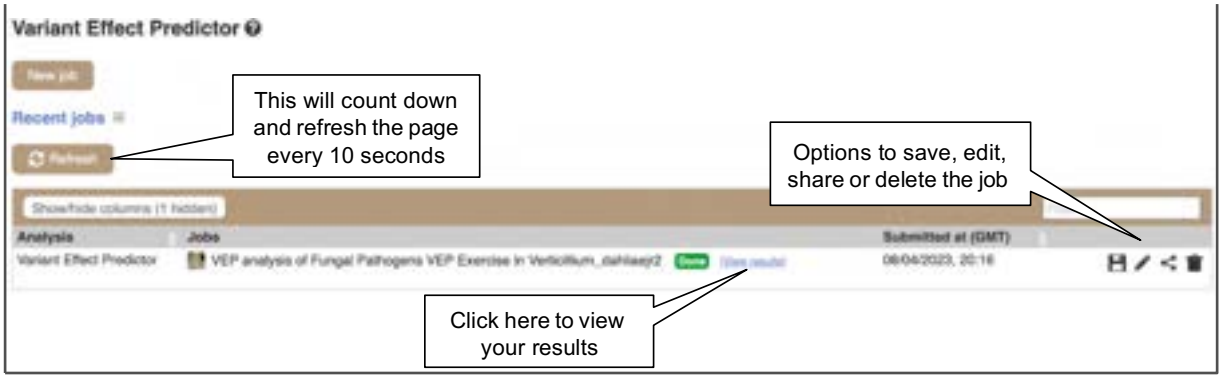
The VEP will automatically detect that the data is in Ensembl default format. Clicking on the Run instant VEP for current line option will generate a pop-up with summarised results for that individual variant.

There are further options that you can choose for your output. These are categorised as Identifiers, Variants and frequency data, Additional annotations, Predictions, Filtering options and Advanced options. Let's open all the menus and take a look.





Hover over the options to see definitions. When you've selected everything you need, scroll to the bottom of the page and click Run.

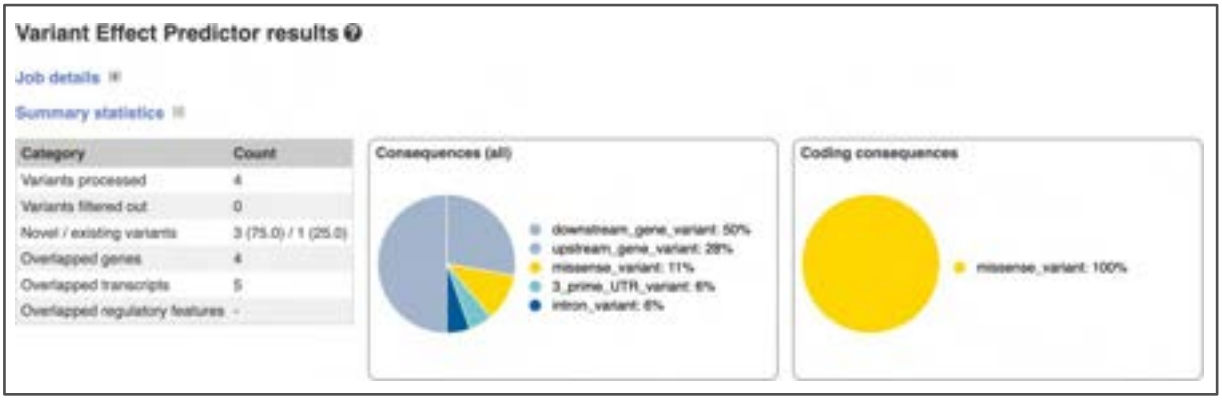


A table display will show you the status of your job. It will say Queued, then automatically switch to Done when the job is done, you do not need to refresh the page. You can edit or discard your job at this time. If you have submitted multiple jobs, they will all appear here.

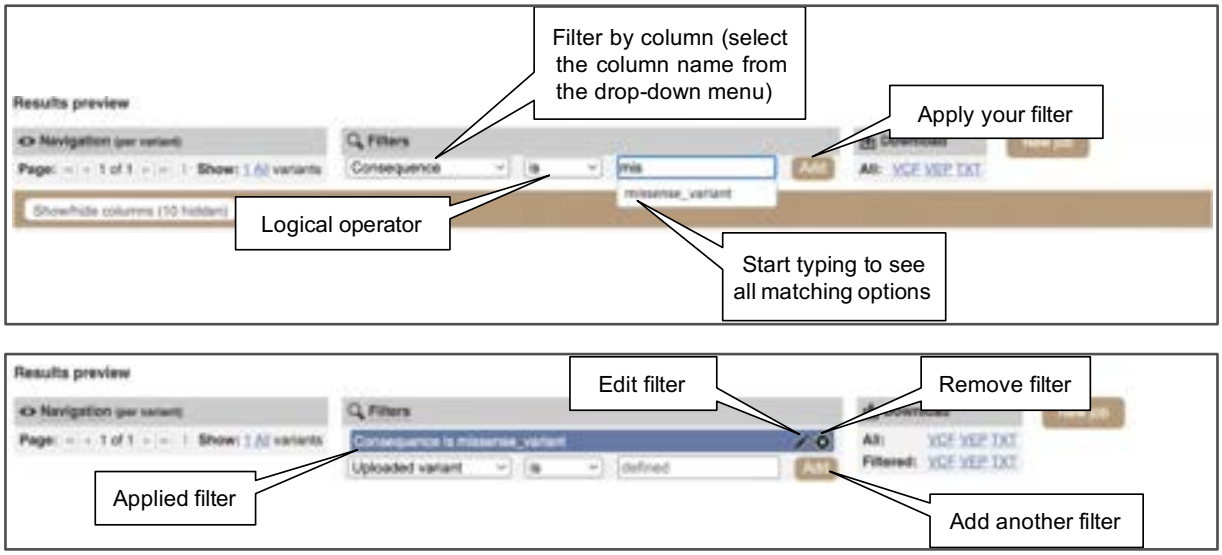
Click View results once your job is done. In your results you will see a graphical summary of your data, as well as a table of your results.

Let's come back to our questions:

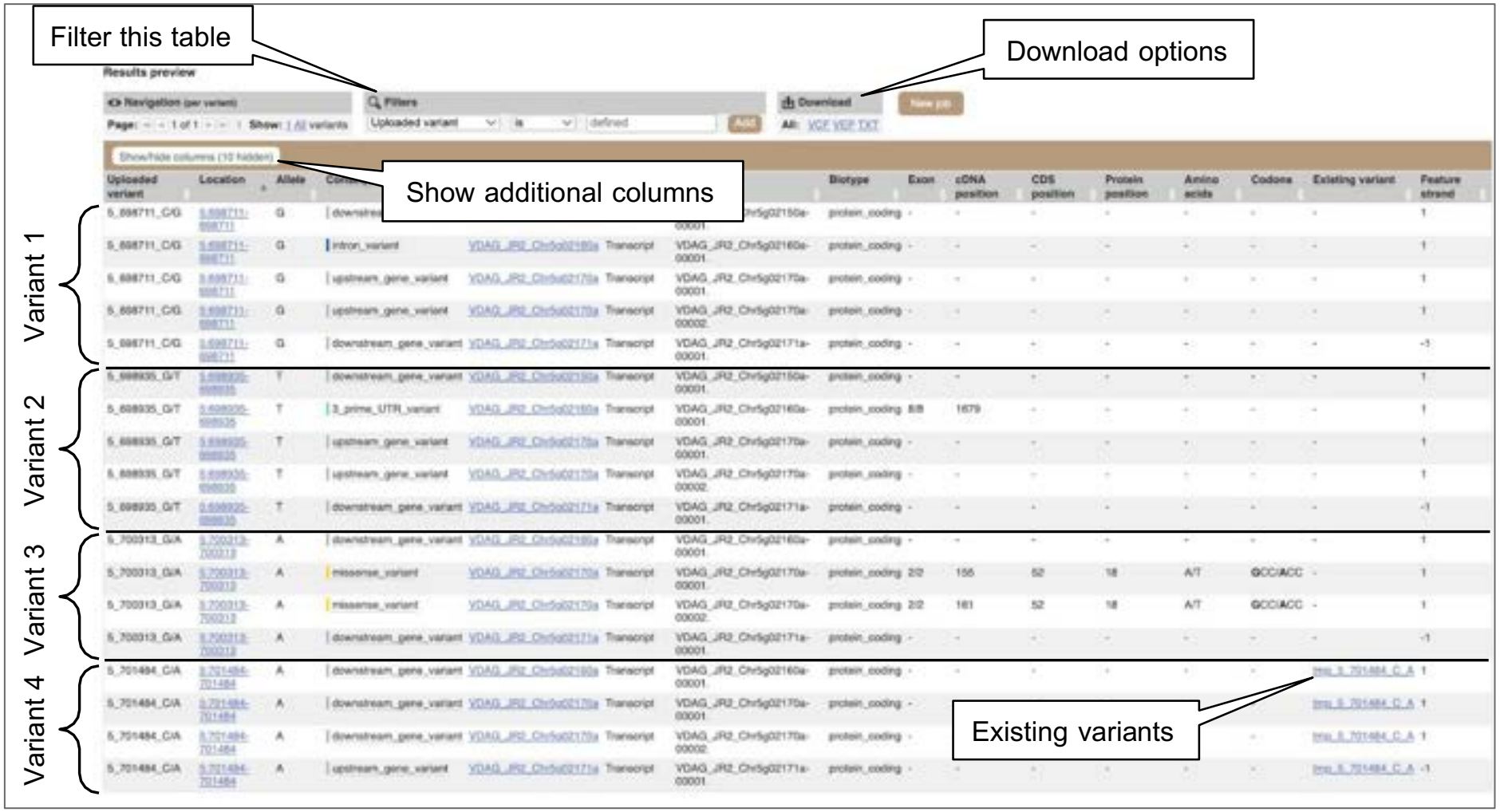
(a) Are your variants novel or have they already been annotated in Ensembl?
(b) What genes are affected by your variants?
(c) Do any of your variants affect gene regulation?



The output table reports one variant consequence per row. If your variants have multiple alternate alleles, hit multiple genes or transcripts, you'll find few lines per variant. If the output table is large, you might want to use the filter option to narrow it down. Once you've added a filter, it will appear in the filter box, allowing you to add other filters.



Filter text box is by default set to ‘defined’, which can be used to filter out empty values, e.g. ‘Existing variant’ ‘is’ ‘defined’ will filter out variants with empty values in the ‘Existing variant’ column, leaving you with known variants only. Note that you should not type ‘defined’ in the search box, just leave it as it is.


Filter this table
Download options
Show additional columns
Existing variants
Variant 1
Variant 2
Variant 3
Variant 4

Additional Exercise: The Ensembl Fungi Variant Effect Predictor (VEP)

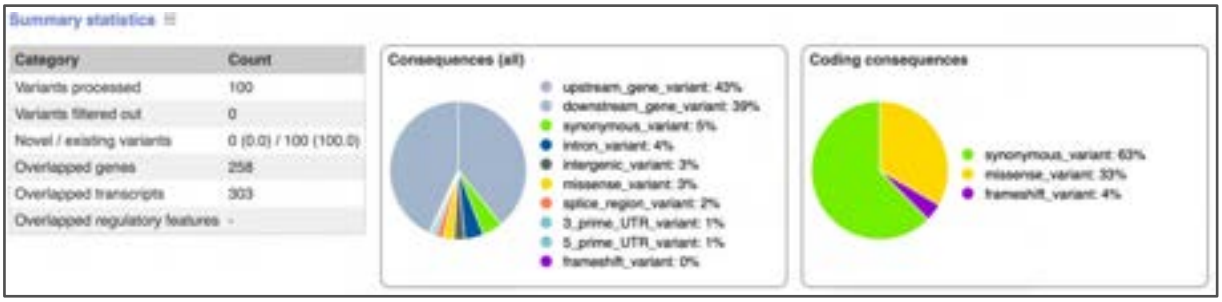
On the course file page, you will find a VCF file labelled VEP_exercise.vcf. This is a small subset of the outcome of *Puccinia graminis* (Ug99) whole genome sequencing and variant calling experiment. This file can also be found on our FTP site under the following link: http://ftp.ebi.ac.uk/pub/databases/ensembl/training/2021/FungalPathogens/VEP_exercise.vcf

Run the file through the VEP by downloading and uploading it from your computer, or by attaching it as a remote file hosted online (you will need to provide the FTP file URL).

(a) How many variants have been processed?
(b) How many genes and transcripts are overlapped by variants in this file?
(c) Do any of the variants change the amino acid sequences of any proteins? What genes? What is the amino acid change? (*Hint: use the filters above the table to filter by consequences*.)
(d) What are the HGVSp notations of missense variants falling in known protein domains?
(e) How many variants are frameshift? Which gene(s) do they fall in and which exons? Can you find a UniParc ID of protein(s) affected by this variant?

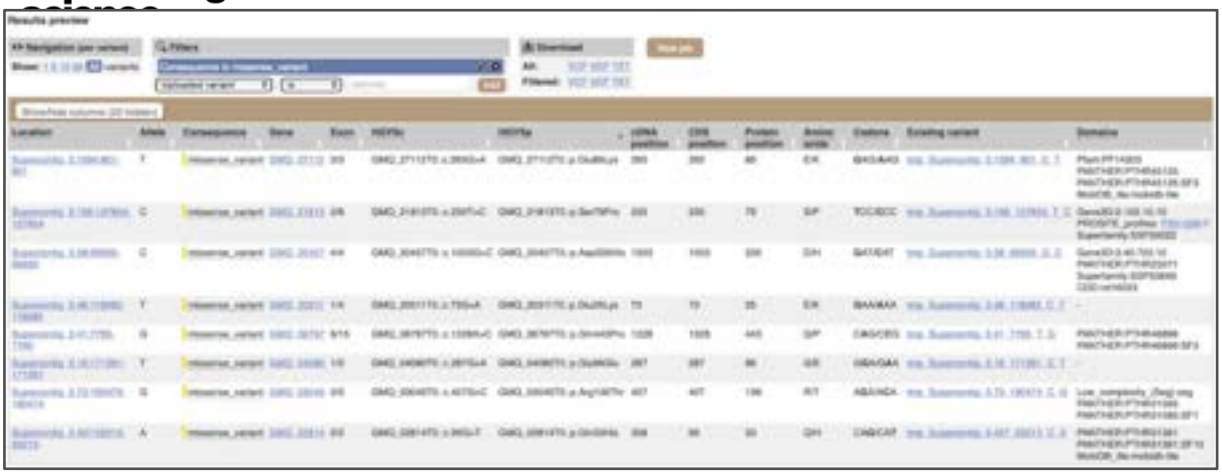
Answer

(a) 100 variants have been processed.
(b) The variants overlap 258 genes and 303 transcripts.



(c) Apply the Consequence is missense_variant under 'Filters'. Under 'Navigation' (to the left of the filter options, click on All. 8 variants change the amino acid sequence in the encoding protein. The affected genes are:

GMQ_21813
GMQ_27112
GMQ_04080
GMQ_06767
GMQ_02814
GMQ_20311
GMQ_20457
GMQ_03045



(d) Ensure you selected the following additional configurations in the VEP input form:

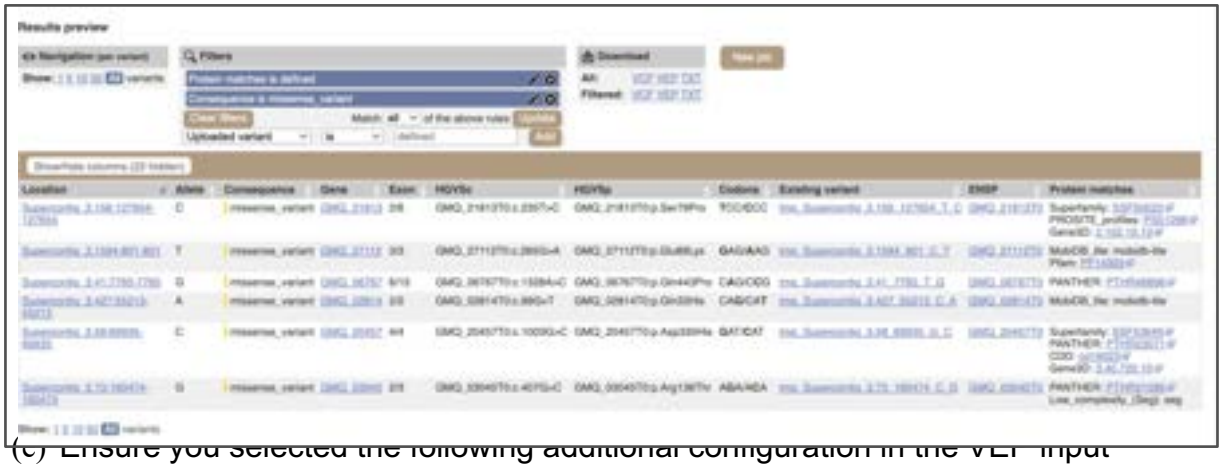
Identifiers: Protein (to include protein position information), HGVS (to include the HGVS notations)
Additional annotations: Protein matches (to include any overlapping protein domains)

In the VEP results table, apply the following filters:

Consequence is missense_variant
Protein matches is [leave text box empty]

Under 'Navigation' (to the left of the filter options, click on All. Ensure the columns HGVSp and Protein matches are visible under the Show/hide columns option above the table. The HGVSp notations of missense variants falling in known protein domains (see 'Protein matches' column) are as follows:

GMQ_21813T0:p.Ser79Pro
GMQ_27112T0:p.Glu89Lys
GMQ_06767T0:p.Gln443Pro
GMQ_02814T0:p.Gln33His
GMQ_20457T0:p.Asp335His
GMQ_03045T0:p.Arg136Thr



(c) Ensure you selected the following additional configuration in the VEP input form:

Identifiers: UniProt (to display any associated UniProt accession IDs, including UniProtKB/Swiss-Prot and UniParc)

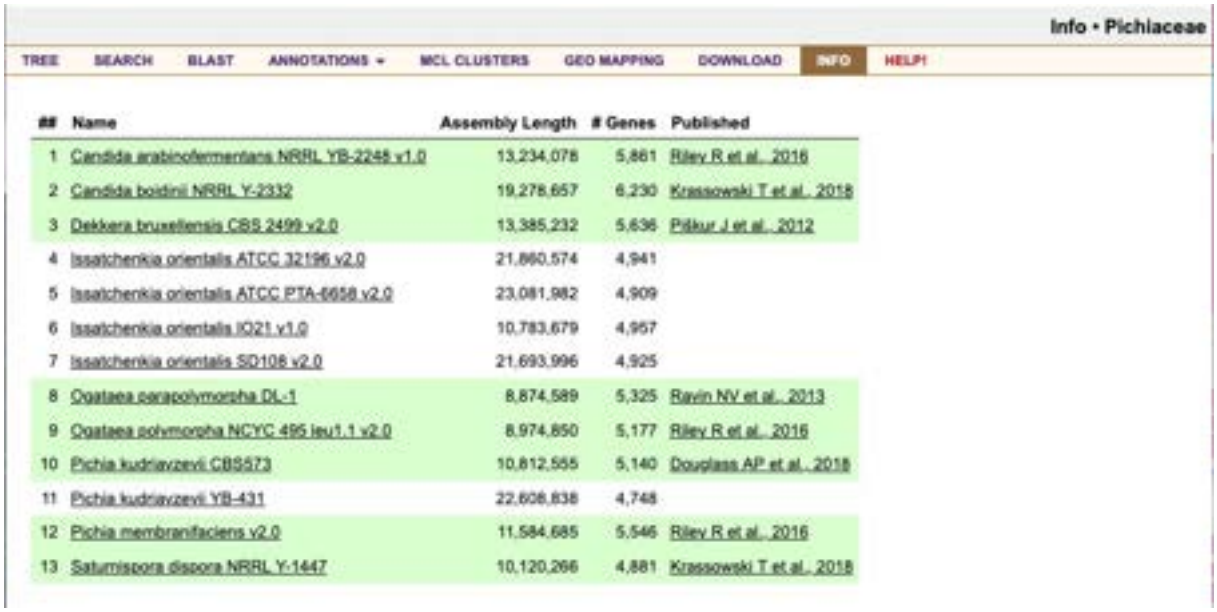
Apply the Consequence is frameshift_variant under ‘Filters’. There is 1 frameshift variant which falls in the GMQ_27001 gene on exon 1 (out of 3). The UniParc ID is UPI0004E9C5AE.

MycoCosm: KEGG Browser

KEGG (Kyoto Encyclopedia of Genes and Genomes; http://www.genome.jp/kegg/), is a resource which maintains a curated set of annotated enzymes and their associated metabolic pathways. Each portal's KEGG Browser facilitates display and discovery of MycoCosm's KEGG-annotated genes. Using the KEGG browser, one can search or browse through KEGG metabolic and regulatory pathways to retrieve information about the enzymes, pathways, and proteins associated with the KEGG annotations.

Scenario: You have plated a variety of yeasts on a variety of carbon sources, and discovered that some members of the Pichiaceae grow on galactose (e.g., *Dekkera bruxellensis*) and some do not (e.g., *Pichia membranifaciens*). Use MycoCosm to find genes that could explain this metabolic difference.

1) Go to the MycoCosm Pichiaceae PhyloGroup at mycocosm.jgi.doe.gov/Pichiaceae:



2) To verify that *Dekkera* (which grows on galactose) and *Pichia* (which does not) are sibling taxa, click on "**TREE**":

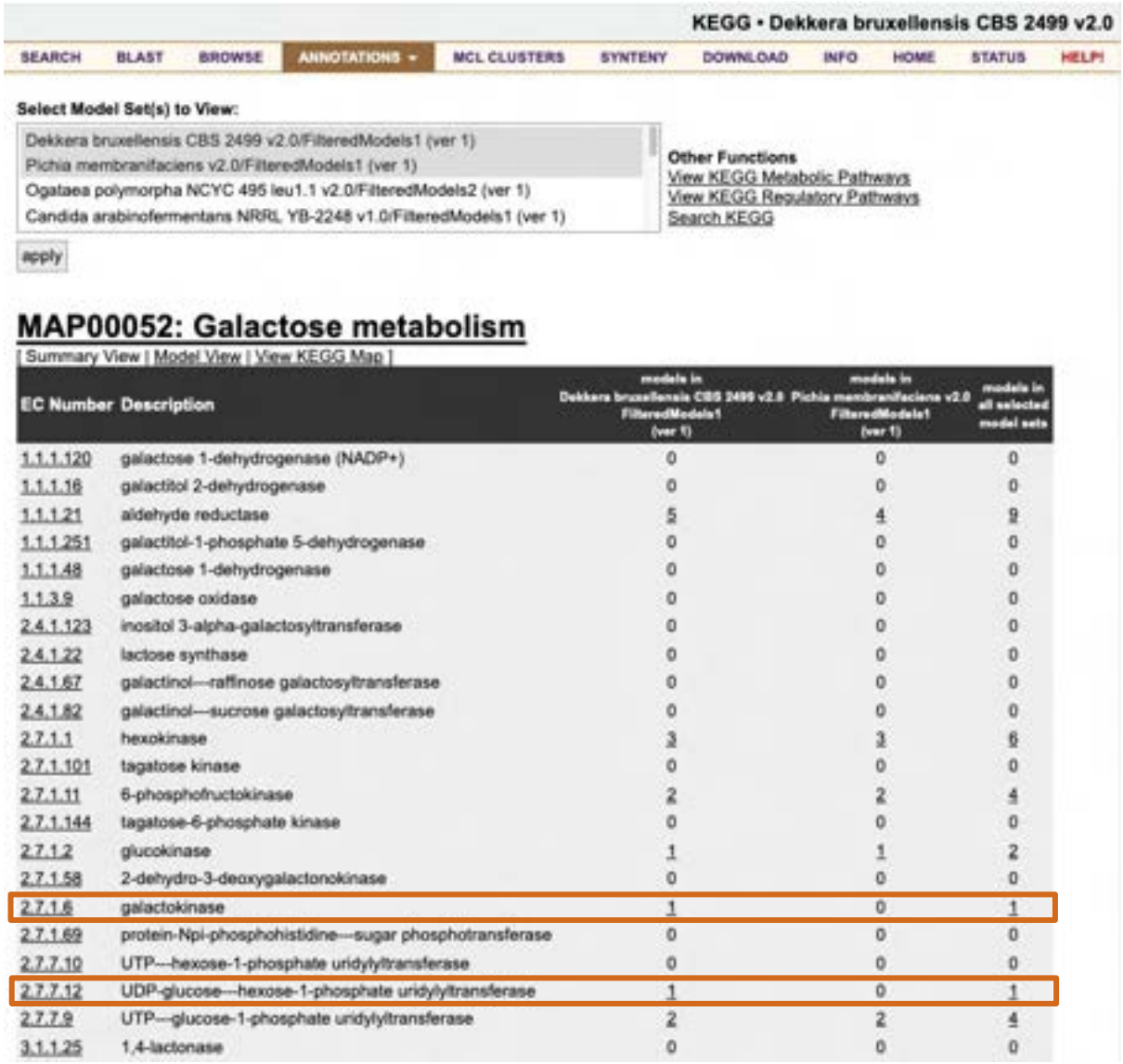
3) Click on '**Dekkera bruxellensis CBS 2499 v2.0**' to go to its genome portal:

4) Click on "**ANNOTATIONS** => **KEGG**" to go to the portal's KEGG browser:

5) Scroll down to the '**Carbohydrate Metabolism**' section, and find the subsection '**Galactose metabolism**'. *Dekkera* has 24 genes annotated to this metabolic pathway:

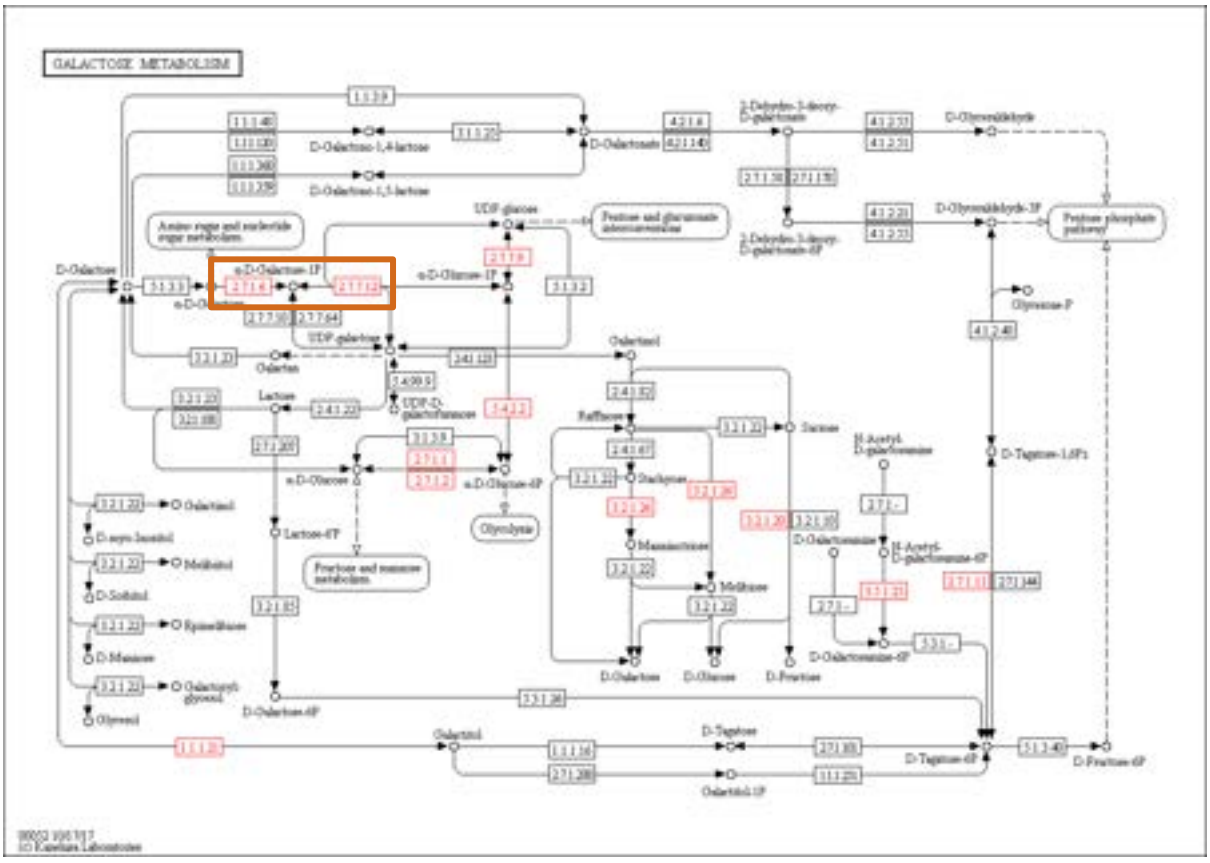
6) Click on '**Galactose metabolism**' to drill down into the KEGG hierarchy and list the EC numbers associated with that pathway.

7) Go to the '**Select Model Set(s) to View**' list box, select *Dekkera bruxellensis* and *Pichia membranifaciens,* and click the '**apply**' button. The *Dekkera* and *Pichia* galactose metabolism gene counts are side-by-side and may be directly compared. Galactokinase (EC = 2.7.1.6) and UDP-glucose--hexose-1-phosphate uridylyltransferase (2.7.7.12) are each present in *Dekkera* but not in *Pichia*:



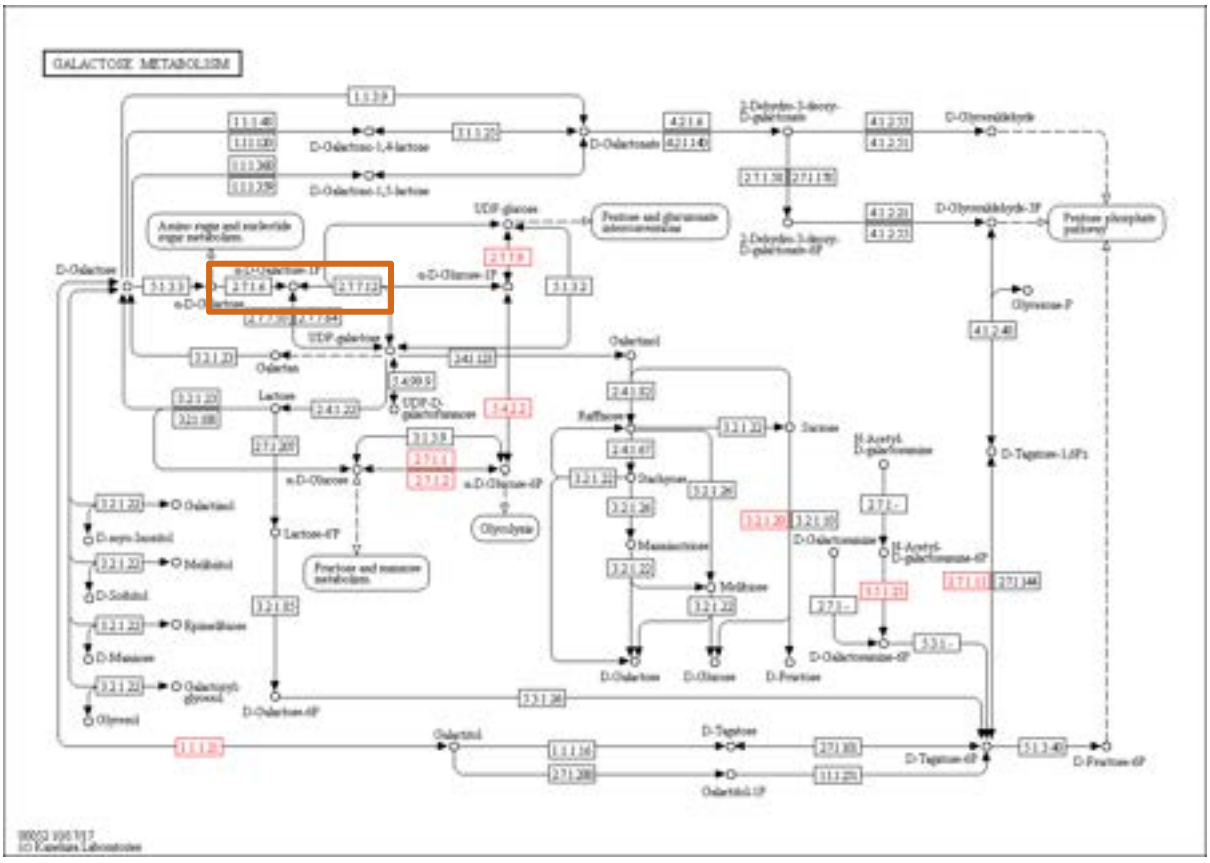
8) Scroll back up to the '**Select Model Set(s) to View**' list box and select *Dekkera bruxellensis* only. Click '**apply**' to show the *Dekkera* counts only.

9) Click '**View KEGG Map**' to see a graphical display of the pathway. Here, the red boxes indicate enzymes present in in *Dekkera*. These include both 2.7.1.6 (Galactokinase) and 2.7.7.12 (UDP-glucose--hexose-1-phosphate uridylyltransferase):



10) Use the web browser back button return to the *Dekkera* galactose metabolism page and select *Pichia* only. Click '**apply**' to show the *Pichia* counts only.

11) Click '**View KEGG Map**' again. This time, note that neither 2.7.1.6 nor 2.7.7.12 are colored red. No wonder *Pichia* cannot grow on galactose – it is missing the genes coding for key enzymes in the galactose utilization pathway.



Exercise:

Based on the KEGG annotations, can you predict whether *Ogataea polymorpha*, *Saccharomyces cerevisiae*, and *Nadsonia fulvescens* can grow on galactose?

Reference:

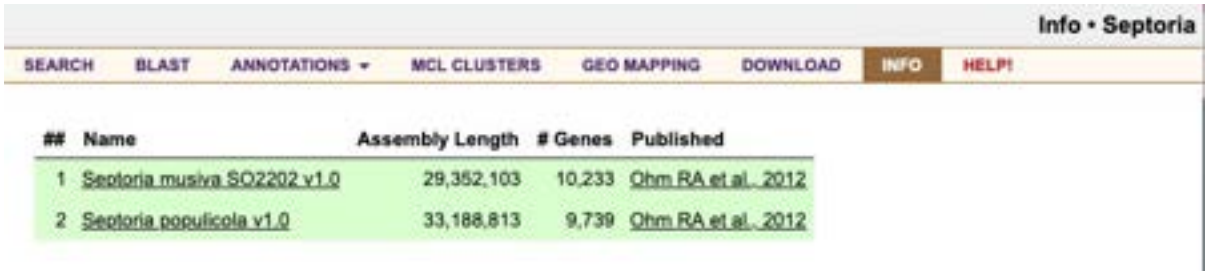
- Riley R, Haridas S, Wolfe KH, Lopes MR, Hittinger CT, Göker M, Salamov AA, Wisecaver JH, Long TM, Calvey CH, Aerts AL, Barry KW, Choi C, Clum A, Coughlan AY, Deshpande S, Douglass AP, Hanson SJ, Klenk HP, LaButti KM, Lapidus A, Lindquist EA, Lipzen AM, Meier-Kolthoff JP, Ohm RA, Otillar RP, Pangilinan JL, Peng Y, Rokas A, Rosa CA, Scheuner C, Sibirny AA, Slot JC, Stielow JB, Sun H, Kurtzman CP, Blackwell M, Grigoriev IV, Jeffries TW. Comparative genomics of biotechnologically important yeasts. Proc Natl Acad Sci U S A. 2016 Aug 30;113(35):9882-7. doi: 10.1073/pnas.1603941113. Epub 2016 Aug 17. PubMed PMID: 27535936; PubMed Central PMCID: PMC5024638.

MycoCosm: Secondary Metabolism Clusters Browser

In fungi, secondary metabolite (SM) genes are often organized in chromosomal clusters dedicated to that metabolite's biosynthetic pathway. Each portal's SM Clusters Browser facilitates display and discovery of MycoCosm's SM-annotated genes.

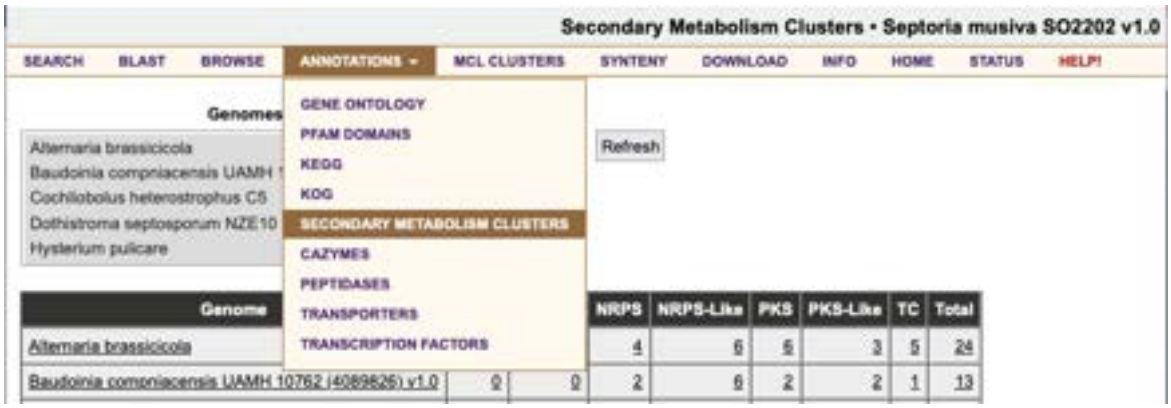
Scenario: You have identified a toxic SM produced by *Septoria musiva*, a pathogenic fungus that induces cankers in the poplar tree, but not produced by *Septoria populicola*, which infects a different species of poplar and does not induce cankers. The SM's structure suggests that its biosynthetic pathway may have as its core enzyme a hybrid PKS-NRPS (polyketide synthase-nonribosomal peptide synthetase). Use MycoCosm to find candidate gene clusters for this pathway.

1) Go to the MycoCosm *Septoria* PhyloGroup at mycocosm.jgi.doe.gov/Septoria. Both species are represented in the group:

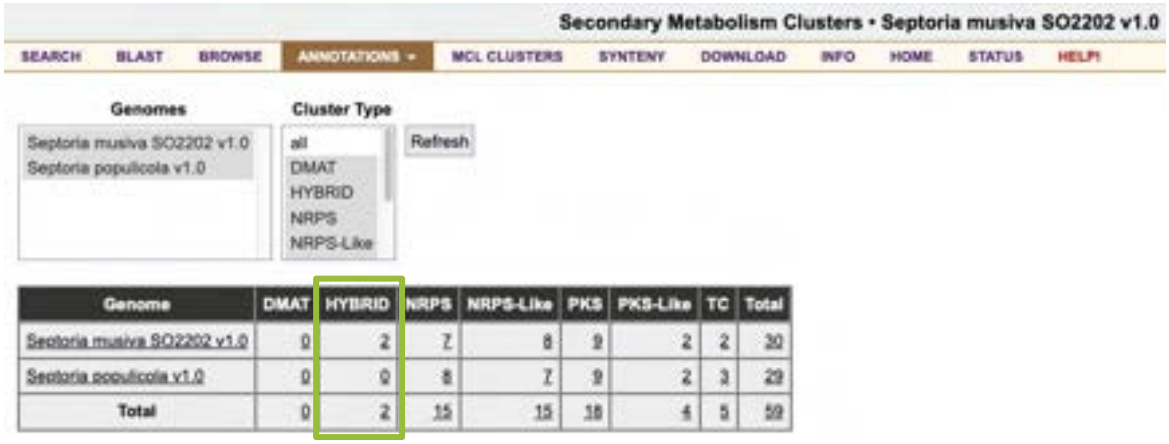


2) Click on '***Septoria musiva* SO2202 v1.0**' to go to its genome portal:

3) Click on "**ANNOTATIONS** => **SECONDARY METABOLISM CLUSTERS**" to go to the portal's SM clusters browser:

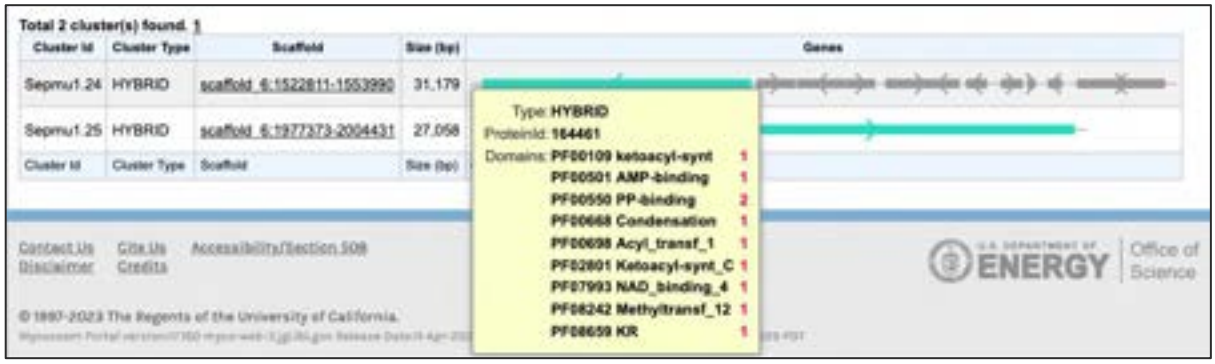


4) Scroll through the 'Genomes' list box and select both '*Septoria musiva*' and '*Septoria populica*', and only those 2 species. Click the '**Refresh**' button. Only the SM cluster core gene counts of the 2 *Septoria* spp. are shown, and may be directly compared. *S. musiva* has 2 hybrid core genes (PKS-NRPS genes) while *S. populicola* has none:

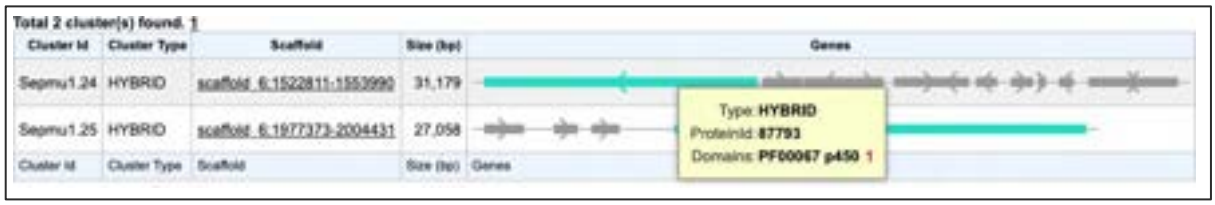


5) There are a total of 2 genes in the Hybrid column. Click on the number to show a graphical representation of the 2 gene clusters in *S. musiva*. The 'Size' column displays each cluster's length, and the 'Genes' column displays each cluster's core PKS-NRPS gene (in color) and its accessory, decorator, and other genes (in gray). A core hybrid gene is typically very large, but the total cluster size can be highly variable. To resize the 2 clusters to scale to each other, go to the 'Scale' pull-down menu, select 'Across All Clusters', and click on the 'Refresh' button:

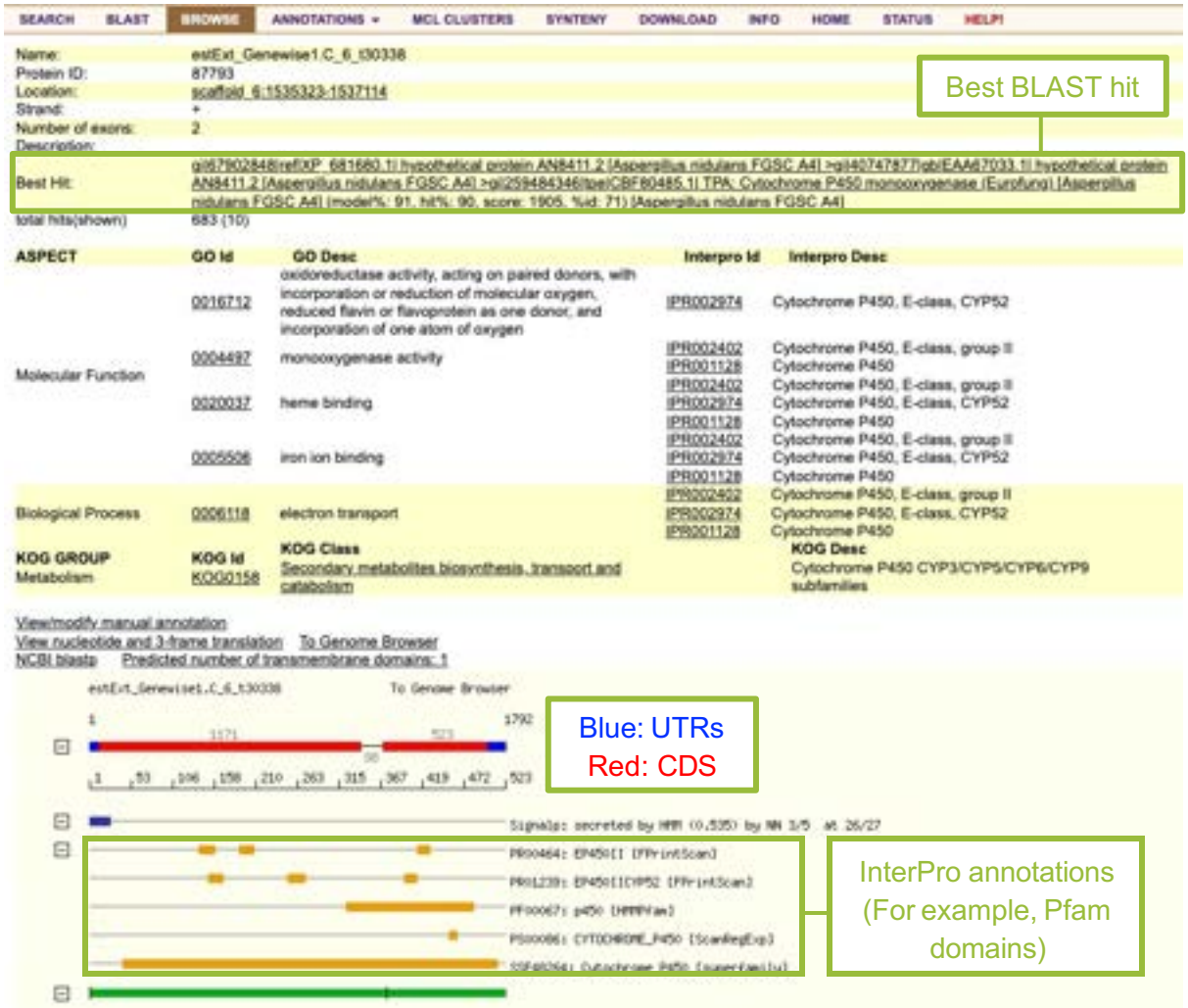
6) Each gene in the clusters is represented by an arrow with a single pair of fletching that indicates the gene's 5' to 3' direction. Mouse-over the top cluster's core gene to get more information about the PKS-NRPS hybrid. The listed domains are typical of a hybrid enzyme:



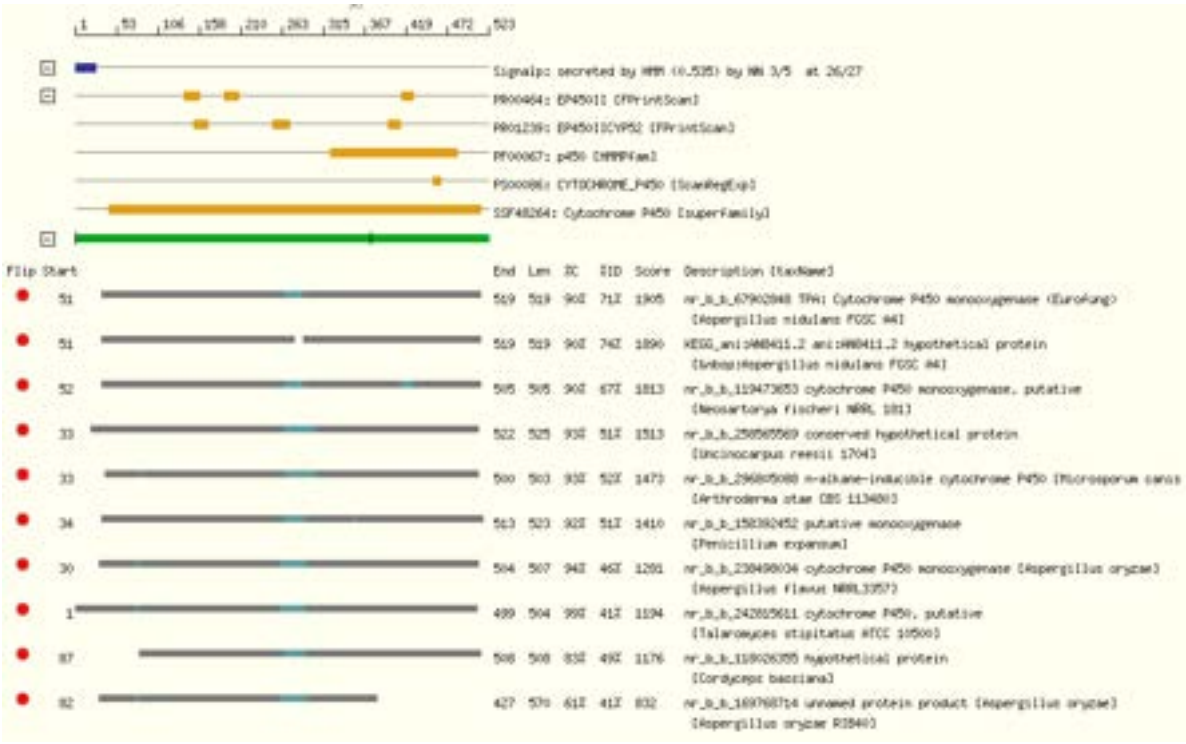
7) To get domain information about the other genes in the SM cluster, mouse-over them too. The next gene 3' to the core gene has a p450 domain:



8) To get more detailed information about a gene, click on it directly. Click on the gene with the p450 domain to see its 'protein page'. Examination of the protein page reveals that:
    a) The gene is expressed. The blue bars represent UTRs, which can be inferred only from transcriptomic data.
    b) The protein has p450 Pfam and other annotations indicative of a cytochrome p450 monooxygenase.
    c) The best Blast hit in nr is a cytochrome p450 monooxygenase from *Aspergillus nidulans*, which belongs to a different class of fungi (Eurotiomycetes) from *Septoria* (Dothideomycetes).



9) Based on the annotations and top hits, it seems that this gene is indeed a cytochrome p450 monooxygenase, a class of enzymes that often modify core structures of SM biosynthetic pathways. Similar perusal of the other genes of the cluster says that this cluster is an excellent candidate for synthesis of your SM.



10) One explanation for *S. musiva* having this cluster and the congeneric *S. populica* not is that the former acquired the cluster by horizontal gene transfer from a phylogenetically distant source. The 'best Blast hit' of the cytochrome p450 enzyme supports this hypothesis. To see if the core enzyme can shed some light, click the web browser back button to go back to the SM CLUSTERS graphic, and click on the same PKS-NRPS core gene we moused over earlier. The protein page is rich in details, including domains and the top 10 hits. All of the hits are high quality and are from Eurotiomycetes. This cluster is an excellent candidate for horizontal gene transfer from the Eurotiomycetes!

References:

- Dhillon B, Feau N, Aerts AL, Beauseigle S, Bernier L, Copeland A, Foster A, Gill N, Henrissat B, Herath P, LaButti KM, Levasseur A, Lindquist EA, Majoor E, Ohm RA, Pangilinan JL, Pribowo A, Saddler JN, Sakalidis ML, de Vries RP, Grigoriev IV, Goodwin SB, Tanguay P, Hamelin RC. Horizontal gene transfer and gene dosage drives adaptation to wood colonization in a tree pathogen. Proc Natl Acad Sci U S A. 2015 Mar 17;112(11):3451-6. doi: 10.1073/pnas.1424293112. Epub 2015 Mar 2. PubMed PMID: 25733908
- Schümann J, Hertweck C. Molecular basis of cytochalasan biosynthesis in fungi: gene cluster analysis and evidence for the involvement of a PKS-NRPS hybrid synthase by RNA silencing. J Am Chem Soc. 2007 Aug 8;129(31):9564-5. Epub 2007 Jul 18. PubMed PMID: 17636916.

FungiDB: Secondary Metabolites and clusters

Learning objectives:

- Explore InterPro search in FungiDB
- Cross-reference the results with MycoCosm data

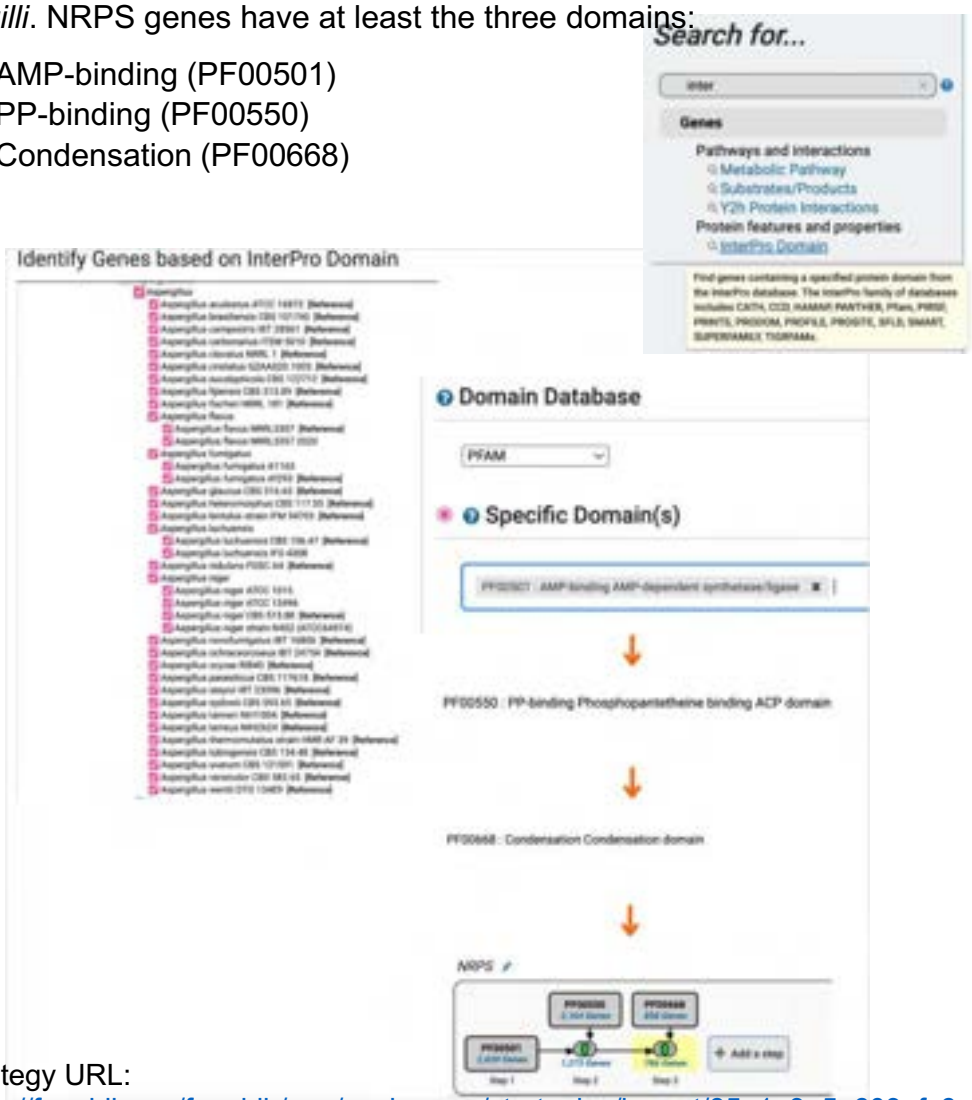
- **Finding secondary metabolites and gene clusters**

Fungi produce a plethora of secondary metabolites. The secondary metabolites can be segregated into groups based on the first step of their biosynthesis, more specifically, the "key enzymes" that are required: Non-ribosomal peptide synthetases (NRPSs), NRPS-like, Polyketide synthases (PKSs), PKS-like, Hybrid PKS – NRPS, Prenyltransferases (DMAT), Terpene cyclases/synthase (TC).

1. Use the InterPro search to identify NRPS genes in all

Aspergilli. NRPS genes have at least the three domains:

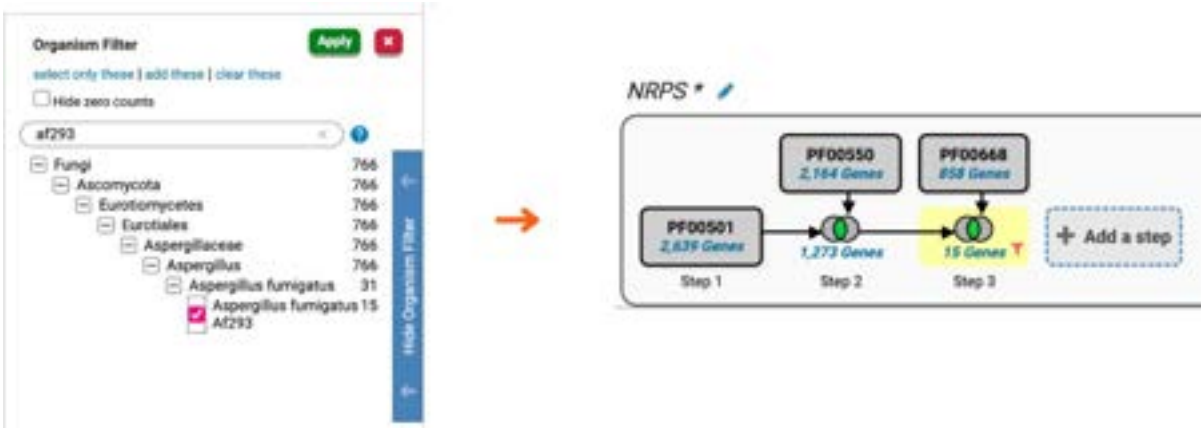
- AMP-binding (PF00501)
- PP-binding (PF00550)
- Condensation (PF00668)



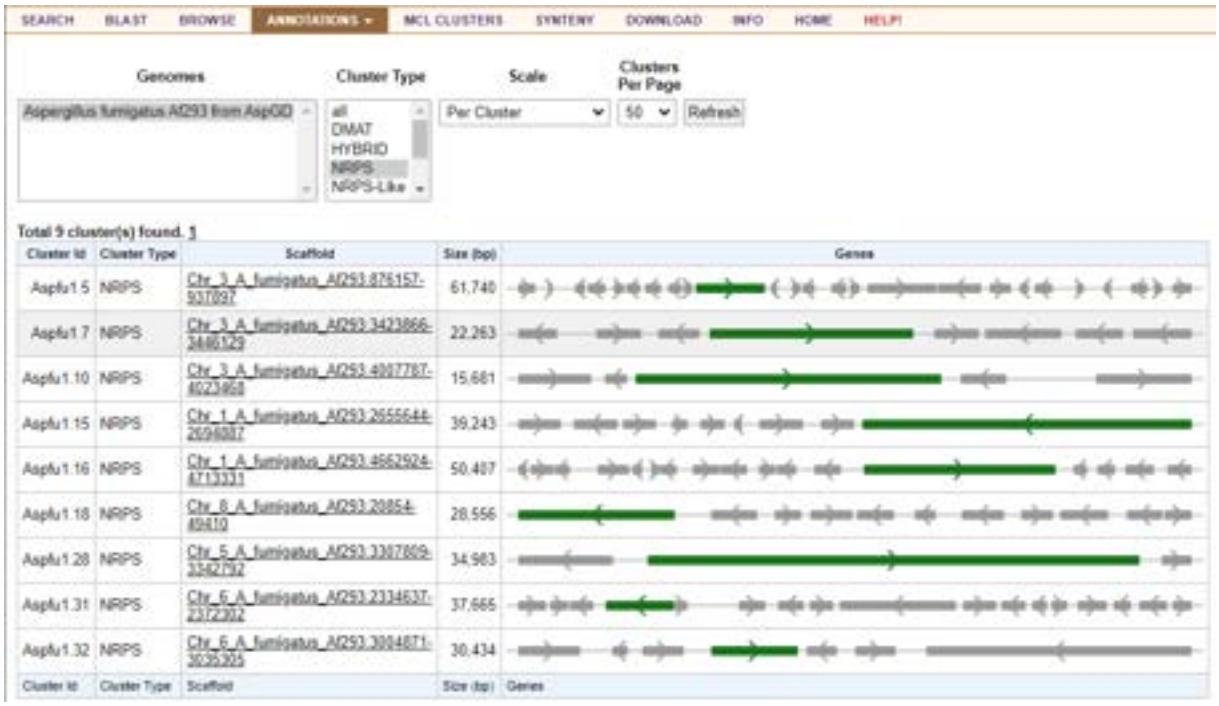
Strategy URL:
https://fungidb.org/fungidb/app/workspace/strategies/import/85a1e3a5a603efc6

- How many genes were identified in *Aspergillus fumigatus* Af293?

Hint: use the organism filter on the left to limit your search results to Af293 genes only.



- Create a search for NRPS genes in MycoCosm. Access the *A. fumigatus* Af293 portal (https://mycocosm.jgi.doe.gov/Aspfu1) and navigate to the Secondary Metabolism Clusters page (under the 'Annotations' tab). How many genes did you get?



- What do you think may be causing the difference in the predicted gene number?

- This view on MycoCosm allows you to analyze backbone and auxiliary proteins across the entire predicted secondary metabolism cluster. How conserved are these secondary metabolite clusters across related Aspergilli? Click on the scaffold coordinates for Aspfu1.5 and analyze the Vista curve tracks in the genome browser. How many related Aspergilli show some synteny with this region? Repeat this exercise for the next cluster, Aspfu1.7.
    - Answer: Synteny is observed across most Aspergilli for Aspfu1.5, raising the possibility that this SM cluster is widespread across the genus. However,

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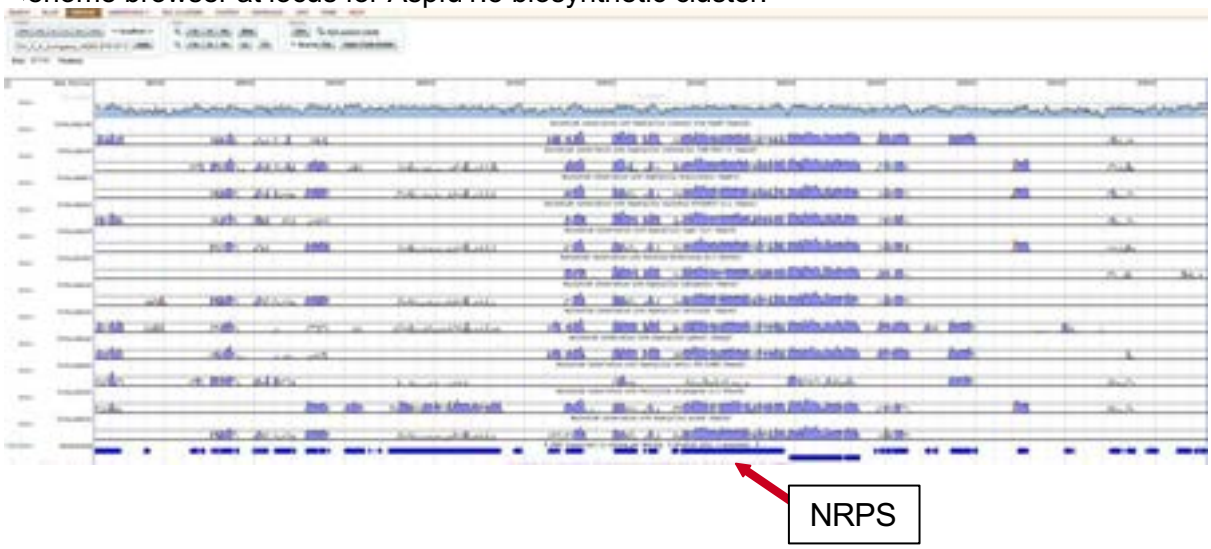
Aspfu1.7 shows no synteny except for at a couple auxiliary genes in *Aspergillus wentii*, suggesting that it is possibly lineage specific.

Genome browser at locus for Aspfu1.5 biosynthetic cluster:



Genome browser at locus for Aspfu1.7 biosynthetic