

DAY 2

Complex Search Strategies

Learning objectives:

- Create a complex search strategy using different types of data

Navigate to your account and open the RNA-Seq strategy created previously - <https://fungidb.org/fungidb/app/workspace/strategies/import/3574a8adf546ee33>

In this strategy, we have looked for genes that are upregulated in *Fusarium* during saprotrophic growth conditions.

a) Identify genes from Step 2 that have non-synonymous mutations based on field isolates data for *Fusarium*

- Modify the existing strategy to look for 4-fold upregulated genes
- Click on Add Step button
- Locate the SNP Characteristics search
- Set parameters as shown below
- Click on the Run Step button

Set of Samples

7 Set of Samples Total
expand all | collapse all
Find a variable

Sample type

- Average mapping coverage
- Proportion mapped reads
- Fungal organism
- Host organism
- Data Set**
- Fungal strain
- Geographic location

6 of 7 Set of Samples selected [Data Set](#)

Data Set

☐ Keep checked values at top

7 (100%) of 7 Set of Samples have data for this variable

| | Remaining Set of Samples | Set of Samples | Distribution | % |
|--|--------------------------|----------------|--------------|--------|
| <input type="checkbox"/> Fusarium graminearum PH-1 Genome Sequence and Annotation | 1 (14%) | 1 (14%) | | (100%) |
| <input checked="" type="checkbox"/> SNP calls on WGS of six F. graminearum strains collected from wheat fields in France | 6 (86%) | 6 (86%) | | (100%) |

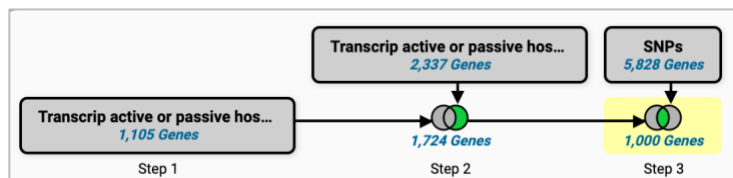
SNP Class

Non-Synonymous

Number of SNPs of above class >=

1

How many genes were returned by the search?



How would you identify which gene products are enriched in the Step 3?

b) Perform GO term enrichment (Molecular function)

- Click on the Analyze Results tab, which is located above the results table
- Select Gene Ontology Enrichment
- Set GO enrichment analysis parameters as shown below

1,000 Genes (817 ortholog groups)

Gene Results Genome View **Analyze Results**

1,000 Genes (817 ortholog groups)

Gene Results Genome View New Analysis

Analyze your Gene results with a tool below.

Gene Ontology Enrichment

Metabolic Pathway Enrichment

Word Enrichment

kinase
phosphatase
exported
membrane

Organism *Fusarium graminearum* PH-1

Ontology

☒ Molecular Function
☐ Biological Process
☐ Cellular Component

Evidence

☒ Computed
☒ Curated
[select all](#) | [clear all](#)

Limit to GO Slim terms

☒ No
☐ Yes

P-Value cutoff 0.05 (0 - 1)

Submit

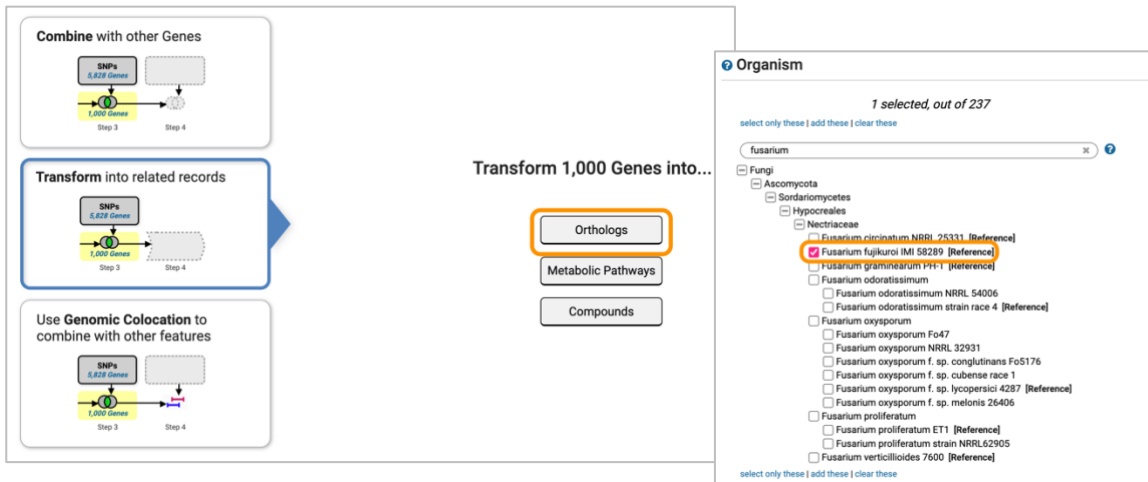
Do the results make sense? Would you expect hydrolases or secondary metabolism pathways being activated during saprophytic growth?

| GO ID | GO Term | Genes in the bkgd with this term | Genes in your result with this term | Percent of bkgd genes in your result | Fold enrichment | Odds ratio | P-value | Benjamini |
|------------|--|----------------------------------|-------------------------------------|--------------------------------------|-----------------|------------|---------|-----------|
| GO:0005200 | structural constituent of cytoskeleton | 21 | 10 | 47.6 | 7.21 | 13.07 | 2.64e-7 | 9.14e-5 |
| GO:0004553 | hydrolase activity, hydrolyzing O-glycosyl compounds | 205 | 33 | 16.1 | 2.44 | 2.82 | 1.40e-6 | 2.43e-4 |
| GO:0016798 | hydrolase activity, acting on glycosyl bonds | 219 | 33 | 15.1 | 2.28 | 2.60 | 6.18e-6 | 7.13e-4 |

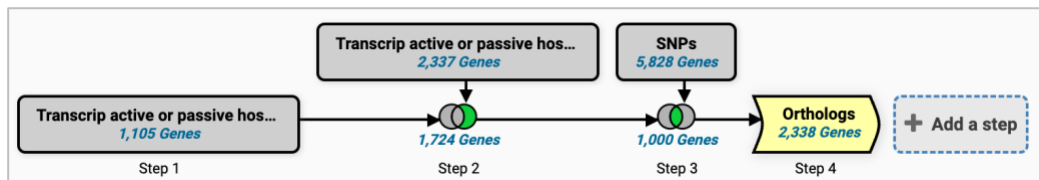
How would you go about finding orthologs of *Fg*PH-1 genes in *Fusarium fujikuroi*?

c) Find orthologs of *Fg* genes from the Step3 in *Fusarium fujikuroi*

- Click on the Add Step button
- Select the “Transform into Related Records” search
- Choose the “Orthologs” option
- Identify the correct genome and deploy the search

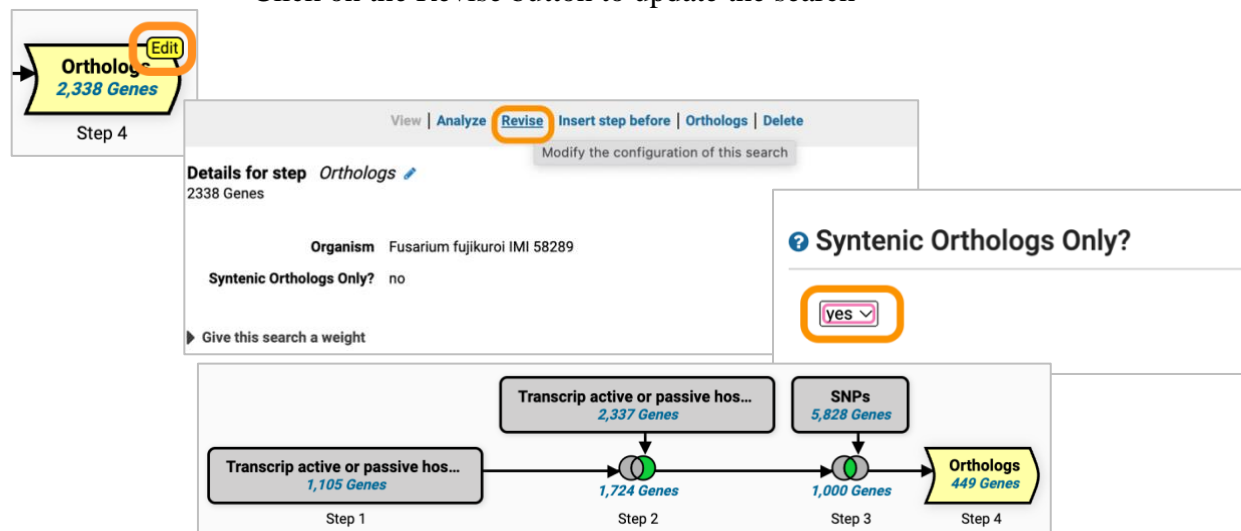


How many orthologs were identified?



What would happen if the strategy modified to look for syntenic orthologs only?

- Hover over the last step
- Click on the Edit button
- Select Revise
- Change the “Syntenic Orthologs Only?” parameter to “Yes”
- Click on the Revise button to update the search



<https://fungidb.org/fungidb/app/workspace/strategies/import/c1fe02e2de87c46c>