

# 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. Next, we will:

### 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
- Data Set**
- Fungal strain
- Geographic location

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

☐ Keep checked values at top

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

	Data Set	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%)

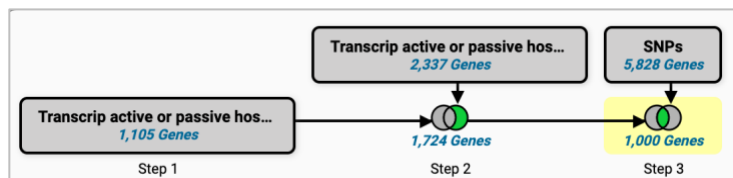
**SNP Class**

**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

Do the results make sense? It looks like hydrolases or secondary metabolism pathways are enriched within gene results which is consistent with 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

**Combine with other Genes**

SNPs 5,828 Genes  
1,000 Genes  
Step 3 Step 4

**Transform into related records**

SNPs 5,828 Genes  
1,000 Genes  
Step 3 Step 4

**Use Genomic Colocation to combine with other features**

SNPs 5,828 Genes  
1,000 Genes  
Step 3 Step 4

Transform 1,000 Genes into...

Orthologs

Metabolic Pathways

Compounds

**Organism**

1 selected, out of 237

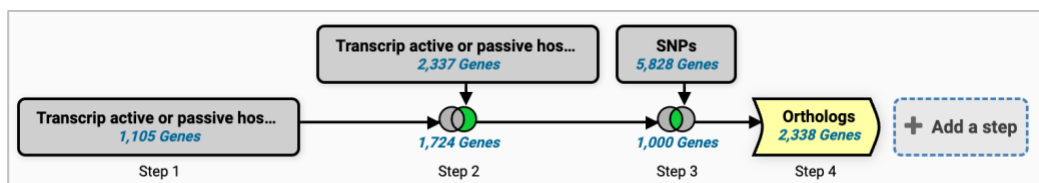
select only these | add these | clear these

fusarium

- ☐ Fungi
- ☐ Ascomycota
- ☐ Sordariomycetes
- ☐ Hypocreales
- ☐ Nectriaceae
- ☒ *Fusarium circinatum* NRRL 25331 [Reference]
- ☒ *Fusarium fujikuroi* IMI 58289 [Reference]
- ☒ *Fusarium graminearum* Y19-1 [Reference]
- ☐ *Fusarium odoratissimum*
- ☐ *Fusarium odoratissimum* NRRL 54006
- ☐ *Fusarium odoratissimum* strain race 4 [Reference]
- ☐ *Fusarium oxysporum*
- ☐ *Fusarium oxysporum* Fo47
- ☐ *Fusarium oxysporum* NRRL 32931
- ☐ *Fusarium oxysporum* f. sp. conglutinans Fo5176
- ☐ *Fusarium oxysporum* f. sp. cubense race 1
- ☐ *Fusarium oxysporum* f. sp. lycopersici 4287 [Reference]
- ☐ *Fusarium oxysporum* f. sp. melonis 26406
- ☐ *Fusarium proliferatum*
- ☐ *Fusarium proliferatum* ET1 [Reference]
- ☐ *Fusarium proliferatum* strain NRRL62905
- ☐ *Fusarium verticillioides* 7600 [Reference]

select only these | add these | clear these

How many orthologs were identified?



Modify the strategy 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

**Orthologs**  
2,338 Genes  
Step 4

**Edit**

View | Analyze | **Revise** | Insert step before | Orthologs | Delete

Modify the configuration of this search

**Details for step Orthologs**

2338 Genes

Organism *Fusarium fujikuroi* IMI 58289

Syntenic Orthologs Only? no

Give this search a weight

**Syntenic Orthologs Only?**

**yes**

```

graph LR
    S1[Transcript active or passive hos...  
1,105 Genes  
Step 1] --> S2[Transcript active or passive hos...  
1,724 Genes  
Step 2]
    S2 --> S3[SNPs  
5,828 Genes  
Step 3]
    S3 --> S4[Orthologs  
449 Genes  
Step 4]
  
```

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