

## Investigation of secondary metabolic processes in fungal species.

Secondary metabolite production is a hallmark of filamentous fungi. Secondary metabolites are important food toxins and they can also be a source of medically important compounds, from antibiotics and putative anti-cancer drugs.

**Exercise:** In this exercise we will (1) identify genes important for secondary metabolite processes in *Aspergillus nidulans*, (2) find orthologs of these genes in *Aspergillus fumigatus*, and (3) visualize gene clusters and metabolic pathways.

### 1. Start a search for secondary metabolites on FungiDB website.

Use *text search* to identify genes with “secondary metabolite” annotations in *A. nidulans*.

Identify Genes based on Text (product name, notes, etc.)

Organism

- Oomycetes
- Fungi
  - Agaricomycetes
  - Basidiomycetes
  - Chytridiomycetes
  - Eurotiomycetes
    - Ascomycetes
      - Aspergillus
        - Aspergillus aculeatus
        - Aspergillus carbonarius
        - Aspergillus clavatus
        - Aspergillus flavus
        - Aspergillus fumigatus
        - Aspergillus nidulans
        - Aspergillus niger
        - Aspergillus oryzae
        - Aspergillus terreus
      - Neurospora
      - Nectria
      - Talaromyces
    - Leotiomycetes
    - Pneumocystidomycetes
    - Pucciniomycetes
    - Saccharomycetes
    - Sclerosporomycetes
    - Sordariomycetes
    - Tremellomycetes
    - Ustilaginomycetes
    - Zygomycetes

Text term (use \* as wildcard)

Fields

- Alias
- EC descriptions
- Gene ID
- Gene notes
- Gene product
- GO terms and definitions
- Metabolic pathway names and descriptions
- Phenotype
- Protein domain names and descriptions
- PubMed
- Similar proteins (BLAST hits v. NRDB/POB)
- User comments

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FungiDB  
Fungal and Comparative Genomics Resources

Home New Search My Strategies My Basket (0) Tools Data Summary

Search for Genes   
Search for Isolates   
Search for Genomic Sequences   
Search for Genomic Segments (DNA Motif)   
Search for SNPs   
Search for ESTs   
Search for ORFs   
Search for Metabolic Pathways   
Search for Compounds   
View all available searches

Genomes, Organisms   
Genetic Maps   
Protein Attributes   
Protein Features   
Similarity/Pattern   
Transcript Expression   
Cellular Location   
Putative Function   
Evolution   
Population Biology

Click *Get answer*.

Your search should return 275 *A. nidulans* genes that have term “secondary metabolite” in EC description, Gene ID, Gene notes, GO terms, etc...

Text  
275 Genes  
Step 1

## 2. Find orthologs of the *A. nidulans* genes in other fungal species.

### Add Step 2 : Transform by Orthology

Organism select all | clear all | expand all | collapse all | reset to default

- Oomycetes
- Fungi
  - Agaricomycetes
  - Blastocladiomycetes
  - Chytridiomycetes
  - Eurotiomycetes
  - Leotiomycetes
  - Pneumocystidomycetes
  - Pucciniomycetes
  - Saccharomycetes
  - Schizosaccharomycetes
  - Sordariomycetes
  - Tremellomycetes
  - Ustilaginomycetes
  - Zygomycetes

select all | clear all | expand all | collapse all | reset to default

Syntenic Orthologs Only?  no

Run Step

Select Add Step > Transform by Orthology > Run Step



Total of 8845 genes were returned as secondary metabolite gene orthologs in fungal species.

Inspect the horizontal table: each number tells you how many genes are present in certain species.

Scroll horizontally and click on *A. fumigatus* results to view orthologs.

You will notice that the page was refreshed and now it displays only 190 genes in *A. fumigatus*.



Scroll down to view gene results. The table provides information about each gene, its genomic location, product prediction, and an ortholog name in *A. nidulans*.

Gene ID	Organism	Genomic Location	Product Description	Input Ortholog(s)	Ortholog Group	Paralog count
Afu8g00220	<i>A. fumigatus</i> Af293	AfumAf293_Chr8: 35,215 - 36,795 (-)	Cytochrome P450 with a predicted role in fumitremorgin B synthesis (fmE)	AN3394	OG5_126582	1
Afu8g00510	<i>A. fumigatus</i> Af293	AfumAf293_Chr8: 110,932 - 112,596 (-)	Predicted oxidoreductase; encoded in the <i>fma</i> (fumagillin) secondary metabolite gene cluster	AN3394	OG5_126582	1

Genes involved in secondary metabolic processes often are localized in gene clusters.

In the next section we will look for gene clusters and determine the directionality of genes within various chromosomes using *Genome View* tab. We will also explore other powerful tools in the *Analyze Results-BETA* tab.

### 3. Investigate gene clusters using Genome View tab.

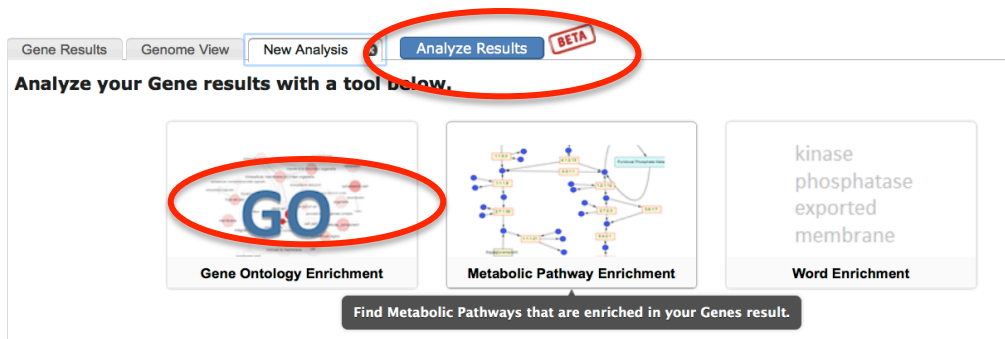


When you hover over purple or red bars you will be able view genes in forward and reverse orientation, respectively.

Notice that some bars are wider and have slightly different appearance. Notice that chromosomes 4 and 8 contain clusters of 3 and more genes. Hover over or click on the selected areas to view more details.

### 4. Explore advanced analysis tools in Analyze Results tab.

FungiDB offers several advanced options for genome investigation. *Analyze Results-BETA* tool allows you to enrich for different classes of gene function (GO enrichment), and also visualize gene framework and a sequence of chemical reactions (Metabolic Pathway enrichment).



Let's explore **Gene Ontology Enrichment** function. It offers three different ways to view your results. You can choose to display your results based on **molecular function** (molecular activities of gene products), or **cellular component** (where gene products are active), or **biological process** (pathways and larger processes that are made up of the activities of multiple gene products). For this exercise choose *biological process*.

### Gene Ontology Enrichment (2)

Find Gene Ontology terms that are enriched in your gene result. [Read More](#)

**Parameters**

**Analysis Results:** [Download Analysis Results](#)  
This analysis result may be lost if you change your gene result.  
To save this analysis result, please download.

Got a total of 310 results Filter:

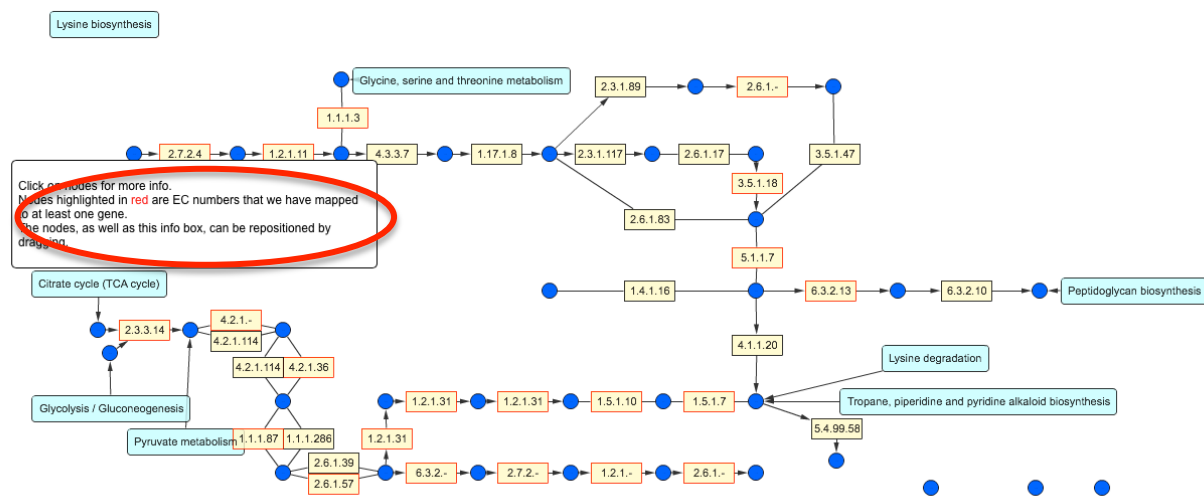
GO ID	GO Term	Genes in the bkgd with this term	Genes in your result with this term	Percent of bkgd Genes in your result	Fold enrichment	Odds ratio	P-value	Benjamini	Bonferroni
GO:0019748	secondary metabolic process	186	72	38.7	19.93	31.48	4.27e-67	1.32e-64	1.32e-64
GO:0044550	secondary metabolite biosynthetic process	153	60	39.2	20.19	29.05	1.72e-55	2.66e-53	5.32e-53
GO:0044711	single-organism biosynthetic process	418	67	16.0	8.25	12.2	1.26e-40	1.30e-38	3.91e-38
GO:0044710	single-organism metabolic process	1796	111	6.2	3.18	6.25	1.08e-33	8.36e-32	3.35e-31
GO:0042181	ketone biosynthetic process	58	32	55.2	28.41	33.96	5.83e-33	3.61e-31	1.81e-30
GO:0042180	cellular ketone metabolic process	66	32	48.5	24.96	29.82	1.41e-31	7.28e-30	4.37e-29
GO:1901617	organic hydroxy compound biosynthetic process	108	35	32.4	16.69	20.23	1.27e-29	5.60e-28	3.92e-27

The table above depicts the summary of GO term enrichment within the search you conducted. It also provides other information including p-value, a statistical confidence factor, for classifications shown. If you click on individual GO IDs, specific pathways included in each category will be displayed.

### 5. Analyze Results tab, continued: Metabolic Pathway Enrichment

Click on Metabolic Enrichment icon. This particular function of the website allows you to visualize enzymatic and chemical flows within biosynthetic pathways.

Explore *Lysine Biosynthesis Pathway ec0300*.



This pathway has well-defined components, such as genes, substrates, and enzymes, and you can gather more information about particular steps by examining nodes and boxes within the map shown above.