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*.





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...



Get Answer

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



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 Results Genome View Analyze Results										
Advanced Paging										
		Gene ↓ ID	韋 Organism 🍳	Genomic Location	Product Description	 ▲ Input ♥ Ortholog(s) 	Cortholog 🕹 🔒	≑ Paralog 🚳 📊		
		Afu8g00220	A. fumigatus Af293	AfumAt293_Chr8: 35,215 - 36,795 (-)	Cytochrome P450 with a predicted role in fumitremorgin B synthesis (ftmE)	AN3394	OG5_126582	1		
		Afu8g00510	A. fumigatus Af293	AfumAf293_Chr8: 110,932 - 112,596 (-)	Predicted oxidoreductase; encoded in the fma (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.

Gene Result Genome View Analyze Results										
Genes on re	ward strand; versed strand;									
Showing 1 to 8 of 8 entries Previous 1 Next Show 25 c entries Search:										
Sequence	Organism	Chromosome		Characteristic Length	Gene Locations					
AfumAf293_Chr3	Aspergillus fumigatus Af293	3	36	4079167						
AfumAf293_Chr6	Aspergillus fumigatus Af293	6	32	3778736						
AfumAf293_Chr2	Aspergillus fumigatus Af293	2	27	4844372						
AfumAf293_Chr4	Aspergillus fumigatus Af293	4	23	3927224						
AfumAf293_Chr1	Aspergillus fumigatus Af293	1	21	4918979						
AfumAf293_Chr5	Aspergillus fumigatus Af293	5	18	3948441						
AfumAf293_Chr8	Aspergillus fumigatus Af293	8	18	183312						
AfumAf293_Chr7	Aspergillus fumigatus Af293	7	15	2058334						
Sequence	Organism	Chromosome	#Genes	Length	Gene Locations					
Showing 1 to 8 of 8 entries Previous 1 Next										

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 Results Genome View Gene Ontology Enrichment	Gene Ontology Enrichment	(2) Analyze	Results BETA					
	[Rename This Analysis Copy These Parameter Values							
Find Once Onteland terms that are entitled in terms and the Dead Mark	Gene On	ology Enrici	intent (2)					
3 Gene Ontology terms that are enriched in your gene result. Read More Parameters								
> Parameters								
Analysis Results:				This s	upolycic roc	ult may be	Download	Analysis Resu
Got a total of 310 results Filter :				1118 6	indiysis rea	To save thi	s analysis resul	t, please downloa
\$ GO ID \$ GO Term	Genes in the \$\$ bkgd with this term	Genes in your cresult with this term	Percent of bkgd			P- value	Senjamini	Sonferroni
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 conduced. 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.