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*.

   ![FungiDB search interface](image)

   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...
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*.

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.
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.