

Mining Proteomics Data

PKA-regulated proteome of *Cryptococcus neoformans*

Learning objectives

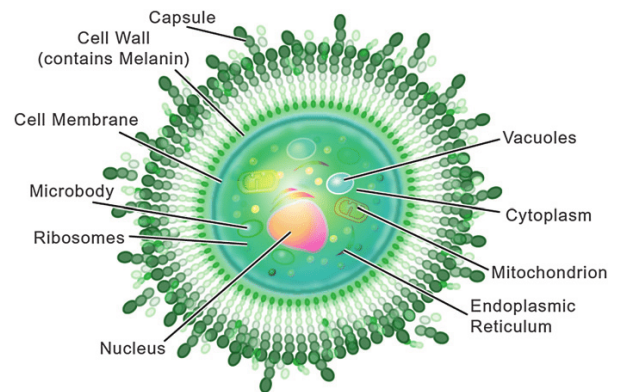
- Explore proteomics datasets on VEuPathDB
- Identify upregulated genes using the Quantitative Mass Spec. Evidence search

Introduction

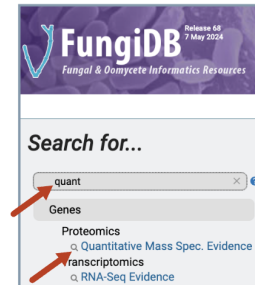
The opportunistic fungal pathogen *Cryptococcus neoformans* causes life-threatening meningitis in immunocompromised individuals. In *Cryptococcus*, the expression of virulence factors, including capsule and melanin, is in part regulated by the cyclic-AMP/protein kinase A (cAMP/PKA) signal transduction pathway.

Investigating the influence of PKA on the composition of the intracellular proteome can contribute to a comprehensive understanding of the regulation that underpins virulence. *C. neoformans* PGAL7::PKA1 strain can be used to induce the PKA pathway in galactose media and repress the pathway in glucose media.

In this exercise, the goal is to identify *Cryptococcus neoformans* genes that are upregulated in a protein kinase A dependent (PKA) manner and not in a non-PKA dependent manner.



1. Navigate to [FungiDB.org](https://fungi.fungi.org)
2. From the sidebar or header, search for “quantitative” and click on **Quantitative Mass Spec. Evidence**



3. Find genes upregulated in galactose media
 - a. Find the experiment called “Analysis of the protein kinase A-regulated proteome of *Cryptococcus neoformans* (Geddes et al.)”
 - b. Choose the Direct Comparison (DC) search.
 - c. Configure the direct comparison search to identify genes that are upregulated by 3-fold in galactose media. Run the search by clicking on “Get answer”.

Identify Genes based on Quantitative Mass Spec. Evidence

Legend: ☒ DC Direct Comparison ☐ FC Fold Change

Filter Data Sets: 11 rows

Organism	Data Set	Choose a Search
<i>Aspergillus clavatus</i> NRRL 1	Proteomics changes in response to human serum (Wiedner et al. 2013)	<input type="button" value="FC"/>
<i>Aspergillus fischeri</i> NRRL 181	Proteomics changes in response to human serum (Wiedner et al. 2013)	<input type="button" value="FC"/>
<i>Aspergillus fumigatus</i> Af293	Proteomics changes in response to human serum (Wiedner et al. 2013)	<input type="button" value="FC"/>
<i>Aspergillus fumigatus</i> Af293	<i>Aspergillus fumigatus</i> response to hypoxia (Barker et al. 2012)	<input type="button" value="FC"/>
<i>Aspergillus fumigatus</i> Af293	Development stage specific proteome (Suh et al.)	<input type="button" value="FC"/>
<i>Aspergillus fumigatus</i> Af293	Adaptive mechanisms of <i>Aspergillus fumigatus</i> /conidia to nutrient restriction Quant (Andjo et al.)	<input type="button" value="FC"/>
<i>Candida albicans</i> SC5314	Extracellular vesicle and whole cell lysate proteomes for DAY226 yeast/biofilm, ATCC90028 and ATCC10231 strains. (Dawson et al. 2020)	<input type="button" value="FC"/>
<i>Candida auris</i> strain B8441	Whole cell proteomics of <i>Candida auris</i> MMC1 and MMC2 strains (Zamith-Miranda et al. 2019)	<input type="button" value="FC"/>
<i>Cryptococcus neoformans</i> var. <i>grubii</i> H99	Secretome profiling of Pka-1 regulated proteins in <i>Cryptococcus neoformans</i> (16, 48, 72, and 120 hr post inoculation) (Geddes et al.)	<input type="button" value="FC"/>
<i>Cryptococcus neoformans</i> var. <i>grubii</i> H99	Analysis of the protein kinase A-regulated proteome of <i>Cryptococcus neoformans</i> (Geddes et al.)	<input checked="" type="button" value="DC"/>
<i>Neurospora crassa</i> OR74A	Circadian time course data from wild type and delta <i>csp-1</i> (Hurley et al.)	<input type="button" value="FC"/>

Direct Comparison

Identify Genes based on *C. neoformans* var. *grubii* H99 Analysis of the protein kinase A-regulated proteome of *Cryptococcus neoformans* Proteomics (direct comparison)

Configure Search Learn More View Data Sets Used

Experiment

Analysis of the protein kinase A-regulated proteome of *Cryptococcus neoformans*

Direction

up-regulated

Comparison

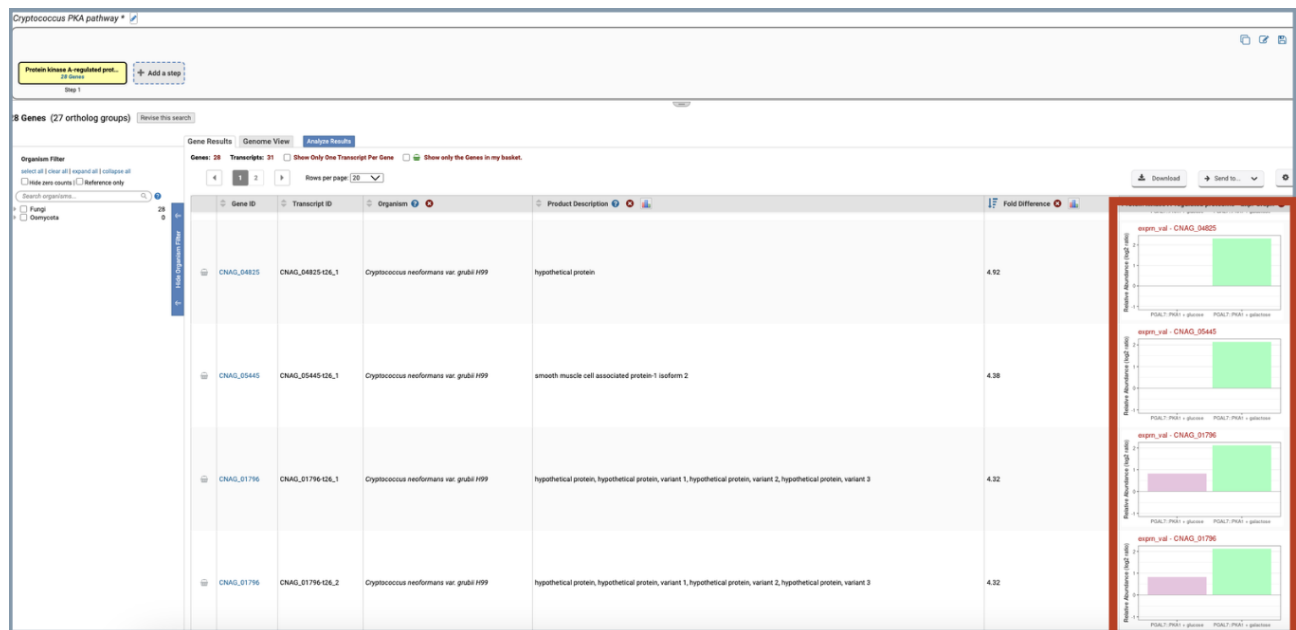
☐ PGAL7:PKA1 + glucose

☒ PGAL7:PKA1 + galactose

Fold difference >=

3

4. Explore your results.



Do the expression graphs in the table of gene results meet the criteria you selected?

5. Add a step and remove from this list any gene that is upregulated by 1.5-fold in glucose media. How many genes did you identify?

The workflow consists of the following steps:

- Step 1:** Cryptococcus PKA pathway (28 Genes).
- Step 2:** Add a step to your search strategy. Choose how to combine with other Genes: 1 MINUS 2. Choose which Genes to combine. From: A new search. Search for Genes by Quantitative Mass Spec. Evidence. The results will be 1 subtracted from the results of Step 1.
- Search Results:** A list of 11 rows of data sets. The results are filtered to show 1 row: Analysis of the protein kinase A-regulated proteome of Cryptococcus neoformans (Geddes et al.).
- Direct Comparison:** Configure Search. Experiment: Analysis of the protein kinase A-regulated proteome of Cryptococcus neoformans. Direction: up-regulated. Comparison: PGAL7-PKA1 + glucose. Fold difference >= 1.5. Run Step.
- Final Results:** Cryptococcus PKA pathway (28 Genes) minus Protein kinase A-regulated prot... (25 Genes) equals 3 Genes.

6. Can you reconfigure the above searches to identify genes that are downregulated as opposed to upregulated? Did your results change?