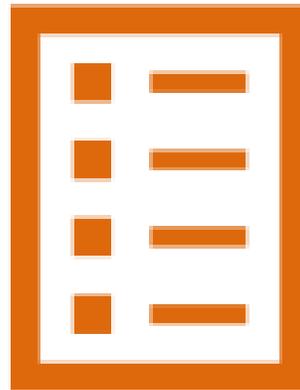


# ExpressAnalyst - Tutorial

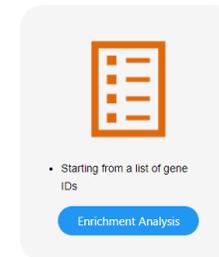
## Starting from a list

-- Comprehensive platform for gene expression and meta-analysis

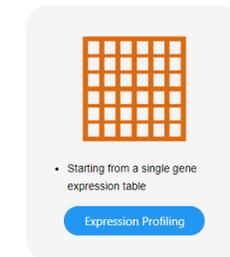


# Intro to ExpressAnalyst

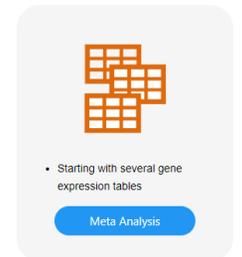
- Web platform for the analysis of gene expression data and meta-analysis
  - Previously part of NetworkAnalyst
- Designed for bench researchers rather than specialized bioinformaticians
- Integrates data processing, statistical analysis and data visualization to support:
  - Data comparisons
  - Biological interpretation
  - Hypothesis generation



Gene list



Single matrix



Meta-analysis

# Computer and browser requirements

- A modern web browser with JavaScript enabled
- Supported browsers include Chrome, Safari, Firefox, and Internet Explorer 9+
- For best performance and visualization, use:
  - Latest version of Google Chrome
  - A computer with at least 4GB of physical RAM
  - A 15-inch screen or bigger (larger is better)
- Browser must be WebGL enabled for 3D scatter visualization
- 50MB limit for data upload
  - ~300 samples for gene expression data with 20 000 genes

# Goals for this tutorial

- Differential Expression (DE) analysis is the cornerstone of transcriptomics data analysis.
- Functional interpretation of the resulting DE genes is a key challenge.
  - There is also a lack of tools supporting the analysis of multiple gene lists (gene list meta-analysis)
- ExpressAnalyst introduces several visual analytics techniques to facilitate functional interpretation of DE genes and the analysis of multiple gene lists.

# Data upload

Home > Upload > Quality Check > Meta-Analysis > Sig. Genes > Analysis Overview > GSEA Heatmap > Download

▼ Navigate to:

## Genes/protein list(s) upload

Please upload one or multiple lists of genes/proteins for network-based analysis and other data visualization, with built-in support for various functional enrichment analysis. For first time users, you are advised to explore the features using one of our example data sets.

The screenshot shows the 'Genes/protein list(s) upload' interface. It includes a 'Specify organism' dropdown menu (currently set to '----Not specified----'), a 'Set ID type' dropdown menu (currently set to '--- Not Specified ---'), and a large text area for pasting gene lists. Below the text area is an 'Upload' button. To the right of the interface, three blue callout boxes provide instructions: '1. Browse our list to select an organism', '2. Select an ID type', and '3. Copy-and-paste your list of genes'. A larger blue callout box at the bottom right explains the format: 'When entering your gene list, make sure to enter one gene per row. If you have multiple gene lists that you want to analyze, insert “//” in a new line to indicate the start of another list'. A blue callout box on the left side of the interface says: 'You can also try our examples to checkout the features and have an idea of the data format'. A 'Try Examples' link is visible in the interface.

<< Previous

>> Proceed

### Genes/protein list(s) upload

Please upload one or multiple lists of genes/proteins for network-based analysis and other data

...s, you are advised to explore the features using one of our example data sets.

Specify organism: ----Not specified----

Set ID type: --- Not Specified ---

For multiple gene lists, add a "/" line to separate different gene lists.

Copy-and-paste one or multiple gene lists (Insert a "/" line to indicate the start of a new gene list, or [click here](#) to upload multiple gene list files)

[Try Examples](#)

Upload

Select "Gene list 1"

#### Example Gene Lists

Name	Parameter	Description
<input checked="" type="radio"/> Gene list 1	<b>Organism:</b> Human <b>ID Type:</b> Entrez Gene ID	Endotoxin tolerance gene signature (99 genes)
<input type="radio"/> Gene list 2	<b>Organism:</b> Mouse <b>ID Type:</b> Entrez Gene ID	Trem-1 activation gene signature (96 genes)
<input type="radio"/> Gene list 3	<b>Organism:</b> Fruit fly <b>ID Type:</b> Uniprot ID	Example gene list for testing purpose (41 genes)
<input type="radio"/> Gene list 4	<b>Organism:</b> C. elegans <b>ID Type:</b> Uniprot ID	Example gene list for testing purpose (74 genes)
<input type="radio"/> Gene list 5	<b>Organism:</b> S. cerevisiae <b>ID Type:</b> Uniprot ID	Example gene list for testing purpose (47 genes)
<input type="radio"/> Gene list 6	<b>ID Type:</b> Seq2Fun IDs	Example gene list for testing purpose (50 genes)
<input type="radio"/> Multiple Lists	<b>Organism:</b> Mouse <b>ID Type:</b> Symbol	Three lists of genes for testing purposes

Submit Cancel

### Genes/protein list(s) upload

Please upload one or multiple lists of genes/proteins for network-based analysis and other data visualization, with built-in support for various functional enrichment analysis. For first time users, you are advised to explore the features using one of our example datasets.

Specify organism: H. sapiens (human) [v]

Set ID type: Entrez ID [v]

Try Examples

#Entrez	logFC
4495	61.12
4496	51.06
4499	23.79
6354	21.04
6369	19.76
4494	16.24
4501	14.76
11026	14.04
199675	12.65
4316	12.04
771	8.19
6346	7.07
6367	6.97
5473	6.76
2357	5.71
5265	5.65
1462	5.27
2358	4.92
22918	4.58
67700	4.50

Upload

Note: words after “#” sign are ignored by the program

1. Click on “Upload”

1. Click on “Proceed”

**OK**  
All IDs are unique. You can click the **Proceed** button to proceed.

**Welcome**  
Please upload your data, or try our example(s).

# Visual analytics

For gene list input, only enrichment network, ridgeline chart (if fold change value is provided) and ORA heatmap can be used. If multiple gene lists are uploaded, upset diagram is usable as well.

🏠 > Upload > Analysis Overview > Ridgeline > Download



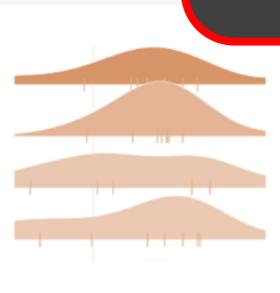
- Interactive volcano plot to display the DE genes.

Volcano Plot



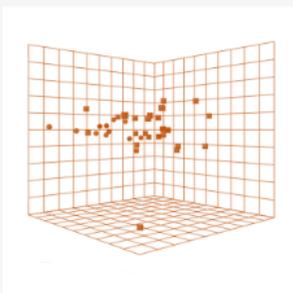
- Visualize functional categories that are enriched in a network.

Enrichment Network



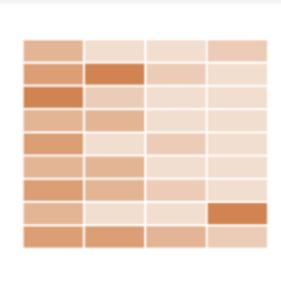
- Visualize fold-change distribution of enriched pathways

Ridgeline Chart



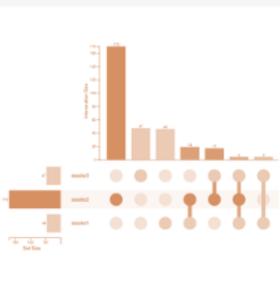
- Explore overall samples and genes in 3D space

Dimension Reduction



- Interactive heatmap to explore gene expression pattern

ORA   GSEA



- Visualize intersections of multiple sets

Upset Diagram

Refer to tutorial 3 and 4 for info about Enrichment Network

⏪ Previous   Downloads

# Ridgeline diagram

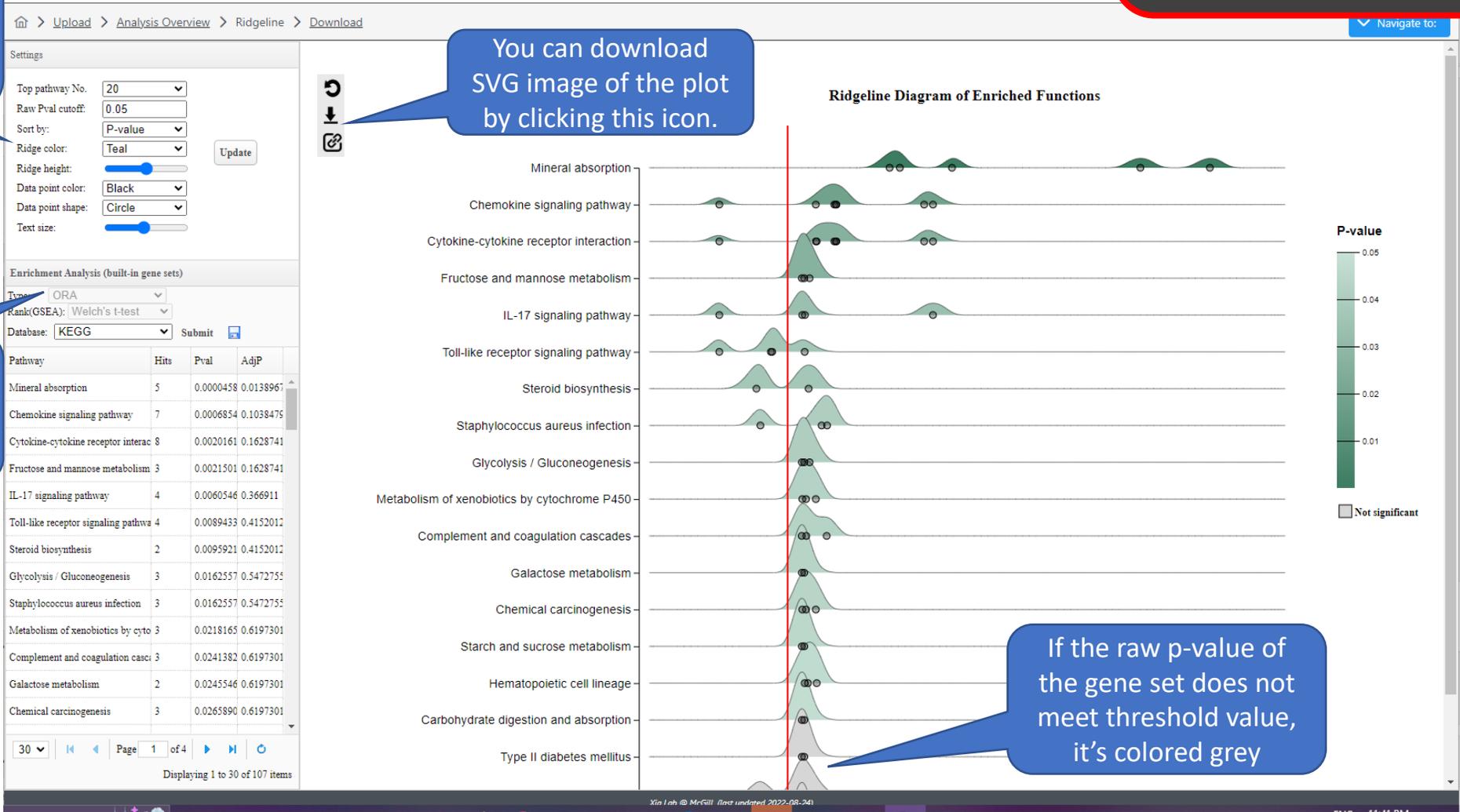
This analysis option is only available if fold change is provided in the gene list. It is useful for visualizing the overall fold-change distribution of the members of gene sets of interest.

Settings panel is used to adjust ridgeline plot style and number of pathways displayed

GSEA option is only available when whole matrix is provided

You can download SVG image of the plot by clicking this icon.

If the raw p-value of the gene set does not meet threshold value, it's colored grey



### Genes/protein list(s) upload

Please upload one or multiple lists of genes/proteins for network-based analysis and other data visualization, with built-in support for various functional enrichment analysis. For first time users, you are advised to explore the features using one of our example data sets.

Specify organism

H. sapiens (human)

Set ID type

Entrez ID

#Entrez logFC  
4495 61.12  
4496 51.06  
4499 23.79  
6354 21.04  
6369 19.76  
4494 16.24  
4501 14.76  
11026 14.04  
199675 12.65  
4316 12.04  
771 8.19  
6346 7.07  
6367 6.97  
5473 6.76  
2357 5.71  
5265 5.65  
1462 5.27  
2358 4.92  
22918 4.58  
66730 4.38

Copy-and-paste one or more gene lists (Insert a "/" line to indicate the start of a new gene list, or [click here](#) to upload multiple gene list files)

[Try Examples](#)

#### Example Gene Lists

Name	Parameter	Description
<input type="radio"/> Gene list 1	Organism: Human ID Type: Entrez Gene ID	Endotoxin tolerance gene signature (99 genes)
<input type="radio"/> Gene list 2	Organism: Mouse ID Type: Entrez Gene ID	Trem-1 activation gene signature (96 genes)
<input type="radio"/> Gene list 3	Organism: Fruit fly ID Type: Uniprot ID	Example gene list for testing purpose (41 genes)
<input type="radio"/> Gene list 4	Organism: C. elegans ID Type: Uniprot ID	Example gene list for testing purpose (74 genes)
<input type="radio"/> Gene list 5	Organism: S. cerevisiae ID Type: Uniprot ID	Example gene list for testing purpose (47 genes)
<input type="radio"/> Gene list 6	ID Type: Seq2Fun IDs	Example gene list for testing purpose (50 genes)
<input checked="" type="radio"/> Multiple Lists	Organism: Mouse ID Type: Symbol	Three lists of genes for testing purposes

Submit

Cancel

Go back to upload page and try "Multiple Lists" option

Multiple gene lists analysis is a type of meta-analysis where the DE genes of individual studies are integrated and compared.

Home > Upload > Analysis Overview > Ridgeline > Download

### Genes/protein list(s) upload

Please upload one or multiple lists of genes/proteins for network-based analysis and other data visualization, with built-in support for various functional enrichment analysis. For first time users, you are advised to explore the features using one of our example data sets.

Specify organism: M. musculus (mouse) ▼

Set ID type: Official Gene Symbol ▼

Copy-and-paste one or more gene lists (Insert a "//" line to indicate the start of a new gene list, or [click here](#) to upload multiple gene lists)

- Dram1
- C1qc
- Tlr2
- Casp1
- Laptn5
- Hist1h2ak
- Ifi272a
- Top2a
- Tmsb4x
- //
- Cd3g
- Slc11a1
- Ciita
- Eif4e3
- Rnase6
- H2-DMb2
- Csf1r
- Ccr5
- Gimap7
- Wipf1
- Dennd1c

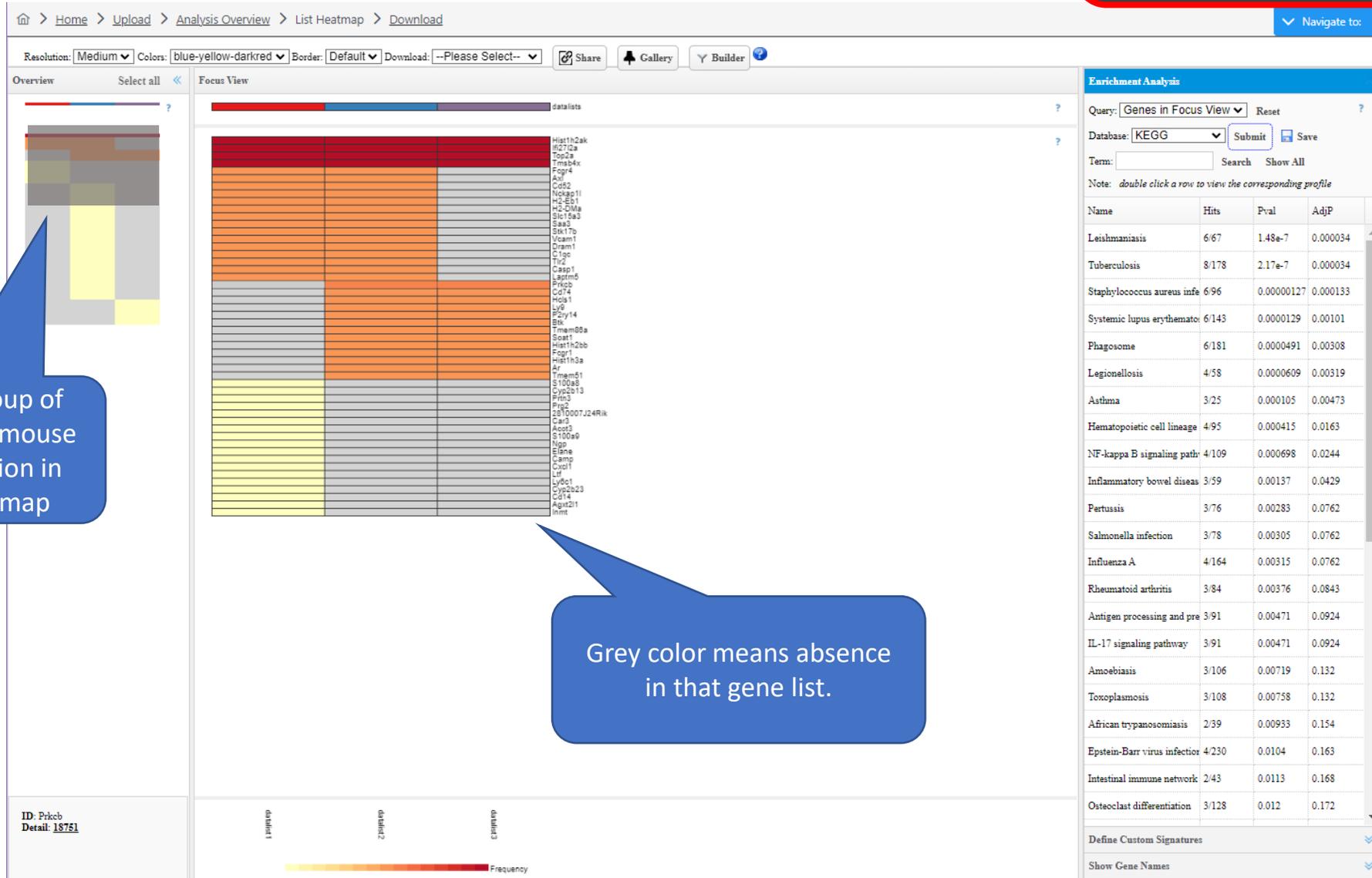
Upload

<< Previous      >> Proceed

“//” is used to separate different gene lists

# ORA Heatmap

ORA heatmap is useful to visualize shared/unique genes across different datasets and perform enrichment analysis



You can select a group of genes by using your mouse to drag select a region in the overview heatmap

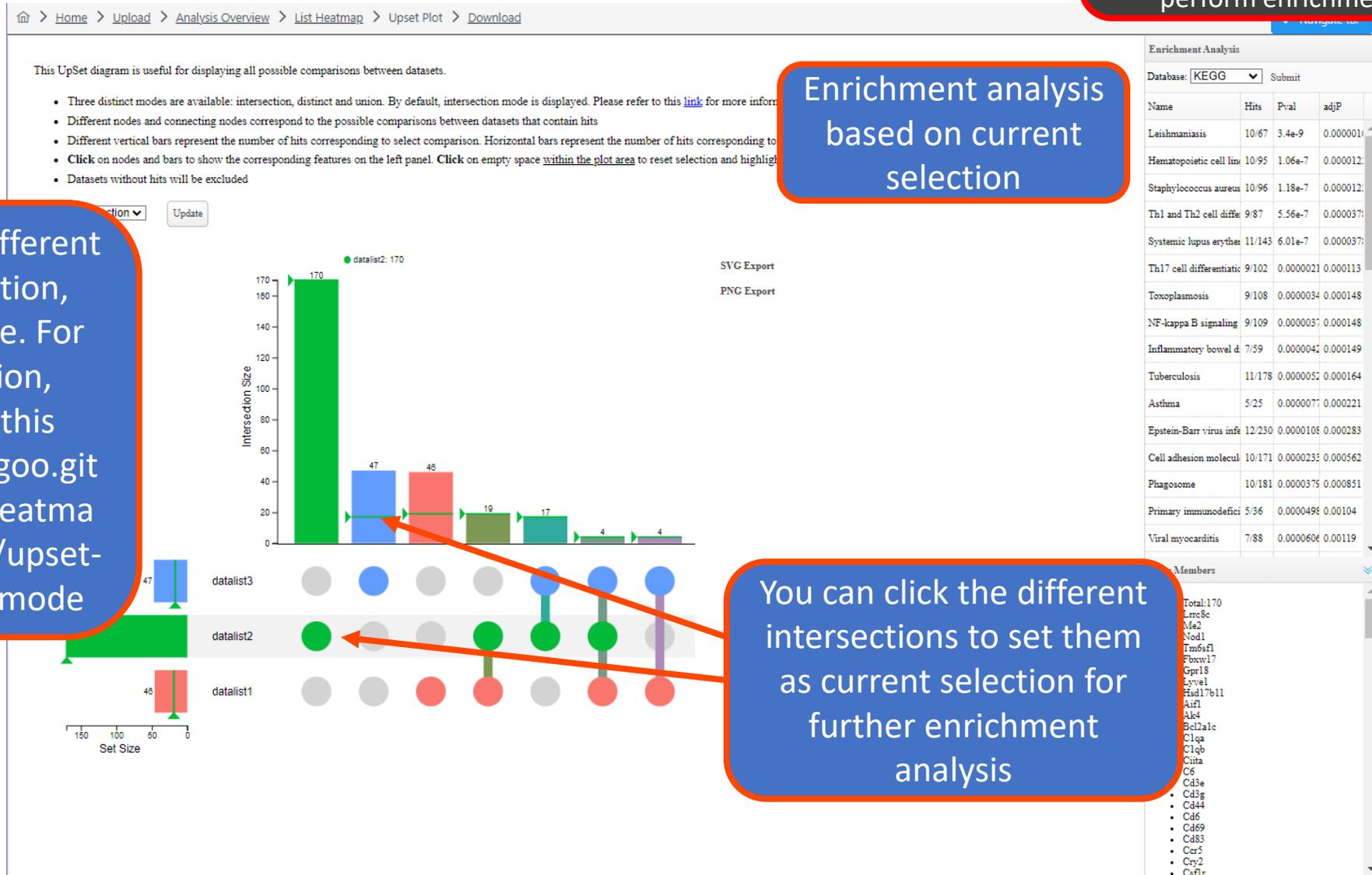
Grey color means absence in that gene list.

ID: Pricb  
Detail: 18751



# Upset Diagram

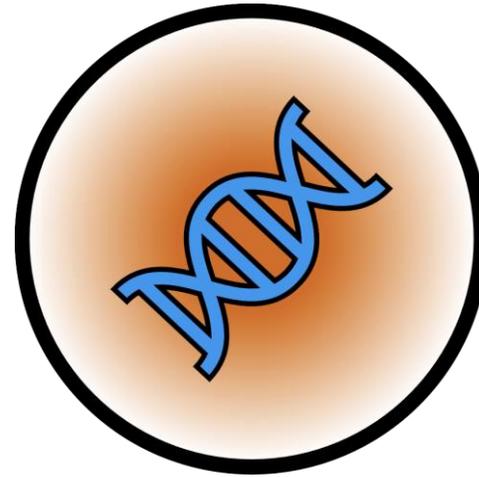
Upset diagram is an alternative to venn diagram and our heatmap viewer, to visualize shared/unique genes across different datasets and perform enrichment analysis



Enrichment analysis based on current selection

There are three different modes: Intersection, Union and Unique. For more information, please refer to this link: <https://jokergoo.github.io/ComplexHeatmap-reference/book/upset-plot.html#upset-mode>

You can click the different intersections to set them as current selection for further enrichment analysis



# The End

*For more information, visit Tutorials, Resources  
and Contact pages on [www.expressanalyst.ca](http://www.expressanalyst.ca)  
Also visit our forum for FAQs on [www.omicsforum.ca](http://www.omicsforum.ca)*