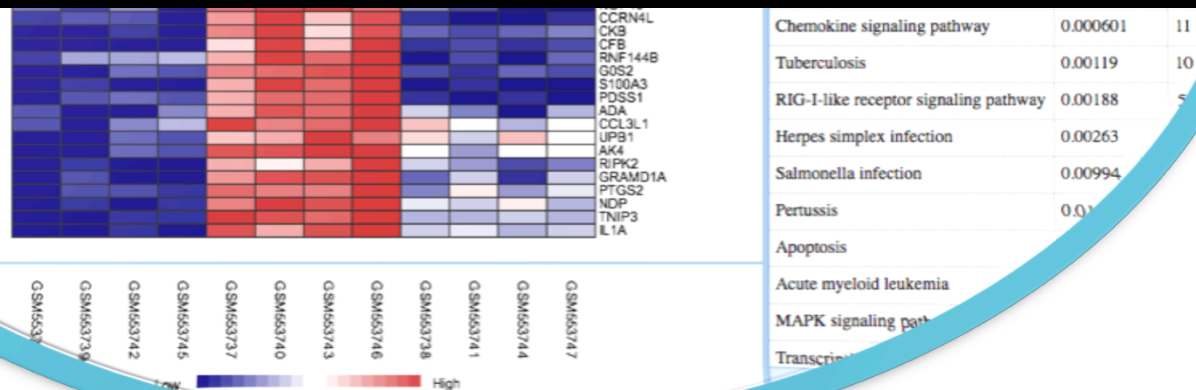


Visual Analytics with Heatmaps



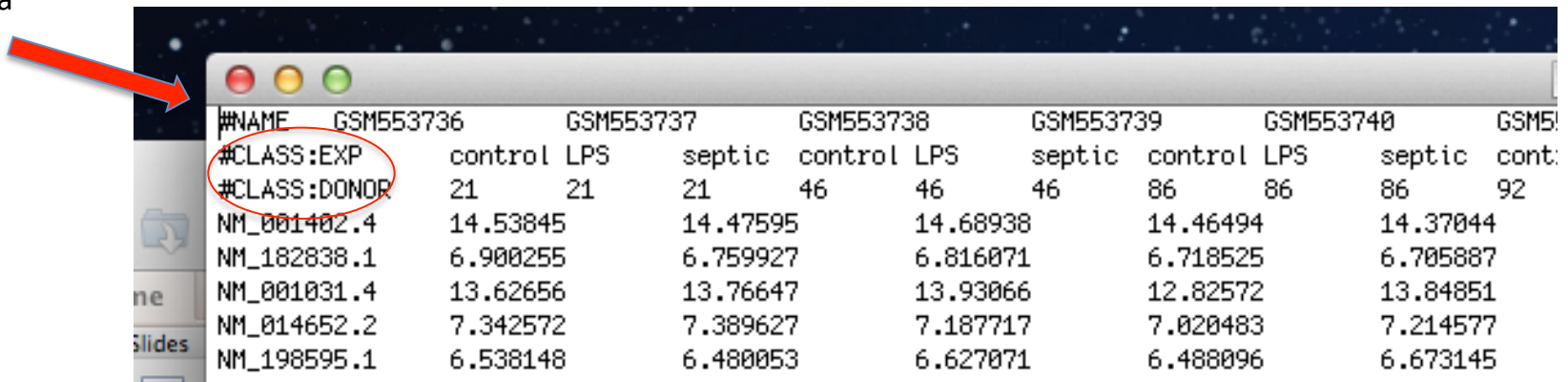
Computer and Browser Requirements

- For good performance and visualization, we recommend the following:
 - Latest version of Google Chrome (version 39+)
 - A computer with at least 4GB of physical RAM
 - A 15-inch screen or bigger (larger is better)

Data Formatting

- Manipulate data headings in a spreadsheet program like MS Excel
- Save as a tab delimited .txt file
- The headings #NAME and #CLASS: (all capital letters) must be used
 - #NAME is for sample names (first row in your data)
 - #CLASS is for the clinical metadata. The screenshot below shows the labels for experimental condition (EXP) and GENDER
 - #CLASS:EXP
 - #CLASS:GENDER

Clinical or
experimental
metadata



#NAME	GSM553736	GSM553737	GSM553738	GSM553739	GSM553740	GSM553741
#CLASS:EXP	control	LPS	septic	control	LPS	septic
#CLASS:DONOR	21	21	21	46	46	46
NM_001402.4	14.53845	14.47595	14.68938	14.46494	14.37044	14.37044
NM_182838.1	6.900255	6.759927	6.816071	6.718525	6.705887	6.705887
NM_001031.4	13.62656	13.76647	13.93066	12.82572	13.84851	13.84851
NM_014652.2	7.342572	7.389627	7.187717	7.020483	7.214577	7.214577
NM_198595.1	6.538148	6.480053	6.627071	6.488096	6.673145	6.673145

Data Upload


Upload and process your data below or try our examples

Data preparation	
Data Upload ✓	<div>Browse... No file selected. ?</div> <div>Submit</div>
Annotation	<div>Specify organism</div> <div>H. sapiens (human) ?</div>
	<div>Data type</div> <div>Microarray data (intensities) ?</div>
	<div>ID type</div> <div>RefSeq ID ?</div>
	<div>Gene-level summarization</div> <div>Mean</div>
Normalization	<div>Normalization procedure</div> <div>-- No normalization -- ?</div> <div>View</div>

Hover over the question marks for more information about each step


- A green check appears to indicate when each step is successfully completed

Data Annotation

Data Upload ✓	<input type="button" value="Choose File"/> No file chosen 	<input type="button" value="Submit"/>
Data Annotation ✓	<div>Specify organism <input <img="" alt="help icon" data-bbox="1072 592 1097 611" type="text" value="Homo sapiens (human)"/></div> <div>Data Type <input <img="" alt="help icon" data-bbox="1072 649 1097 668" type="text" value="Microarray data (intensities)"/></div> <div>ID Type <input <img="" alt="help icon" data-bbox="1072 706 1097 725" type="text" value="RefSeq ID"/></div>	<input type="button" value="Submit"/>
Data Normalization	Normalization procedure <input <img="" alt="help icon" data-bbox="966 771 991 789" type="text" value="-- No normalization --"/>	<input type="button" value="Submit"/>

- Specifying organism, data type and ID type allows INVEX to annotate your data
- Use the “not-specified” option for non-supported organisms/platforms
 - You can still analyze and visualize your data, but no gene annotation or functional enrichment analysis will be performed.

Data Normalization

	ID type	RefSeq ID	
Data Normalization 	Normalization procedure	-- No normalization --	Submit
	Metadata of interest:	Primary: --- Not Available ---	

- This example dataset is already normalized and needed no normalization
- If raw data is uploaded normalization can be applied
 - Log2 scale normalization is recommended
 - Further quantile normalization is optional

Data Analysis

Up to two metadata can be selected

The screenshot shows the 'Differential Analysis' interface. A red arrow points to the 'Primary' dropdown menu, which is set to 'Treatment'. Another red arrow points to the 'Secondary' dropdown menu, which is set to 'Donor' and is circled in red. To the right of the 'Secondary' dropdown, there is a checkbox labeled 'This is a blocking factor' which is checked. Below the dropdowns, there are radio buttons for 'Pairwise comparisons', 'Time series', 'Specific comparison' (which is selected), and 'Use a common control'. Below these, there are more radio buttons for 'Nested comparisons'. The 'Specific comparison' section shows a dropdown set to 'LPS vs. LPS_LPS' and a 'control' dropdown. At the bottom, there are two dropdowns both set to 'control vs. LPS' with a 'versus' label between them. Below these are radio buttons for 'Interaction only' (selected) and 'Full Model'. On the right side, there is a table titled 'Analysis Results:' with columns 'Name', 'DE#', and a trash icon. The table contains the following data:

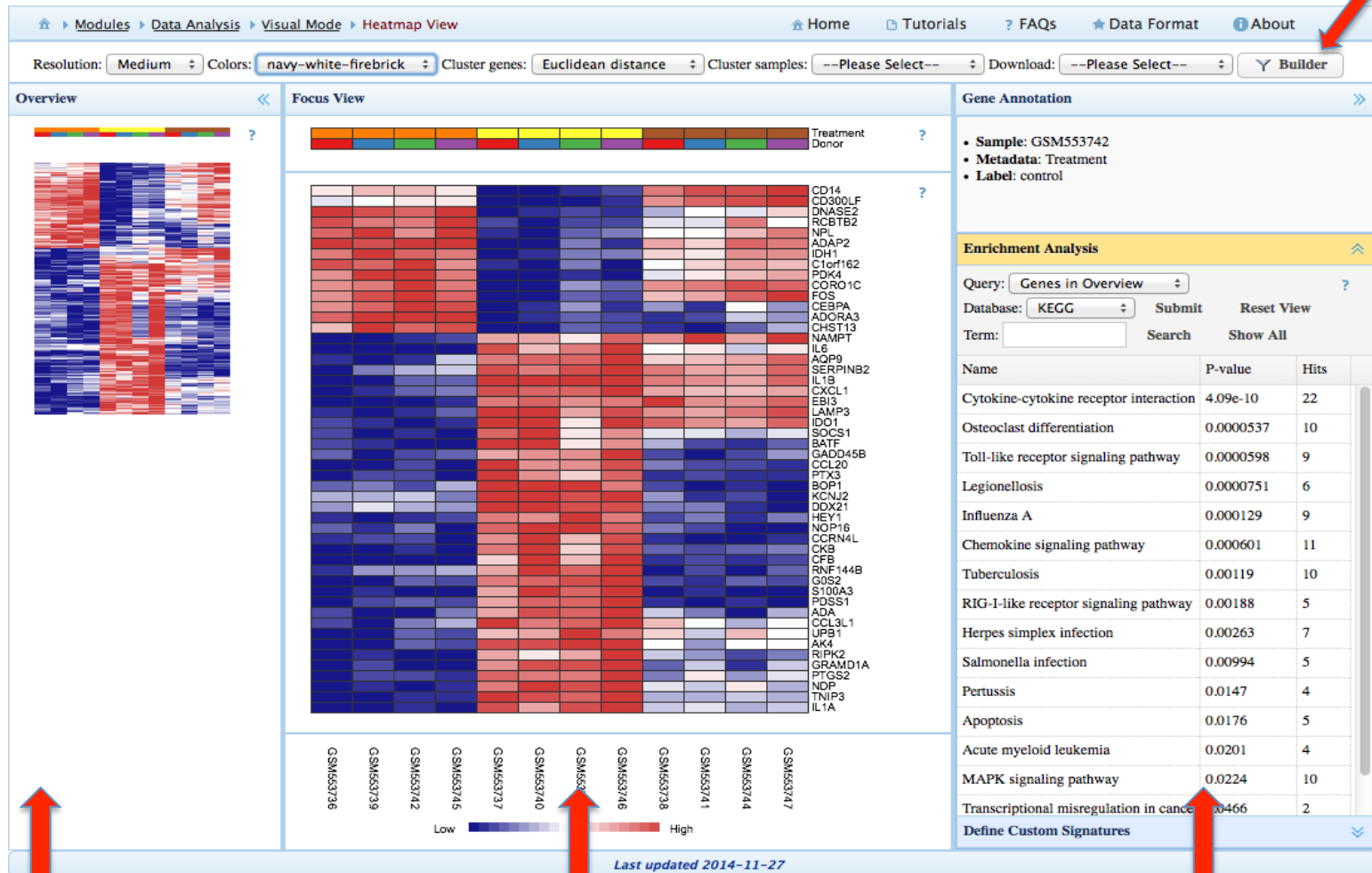
Name	DE#	
± LPS	206	
± LPS2	135	
± LPS_LPS2	236	
± LPS_D	215	
± LPS2_D	153	
	251	

A text box on the right side of the interface states: 'The second metadata can be treated as a blocking factor to account for batch effects'.

- Please refer to our FAQs “Differential Expression Analysis” section for detailed explanations about different comparisons and study designs

Overview

Toolbar

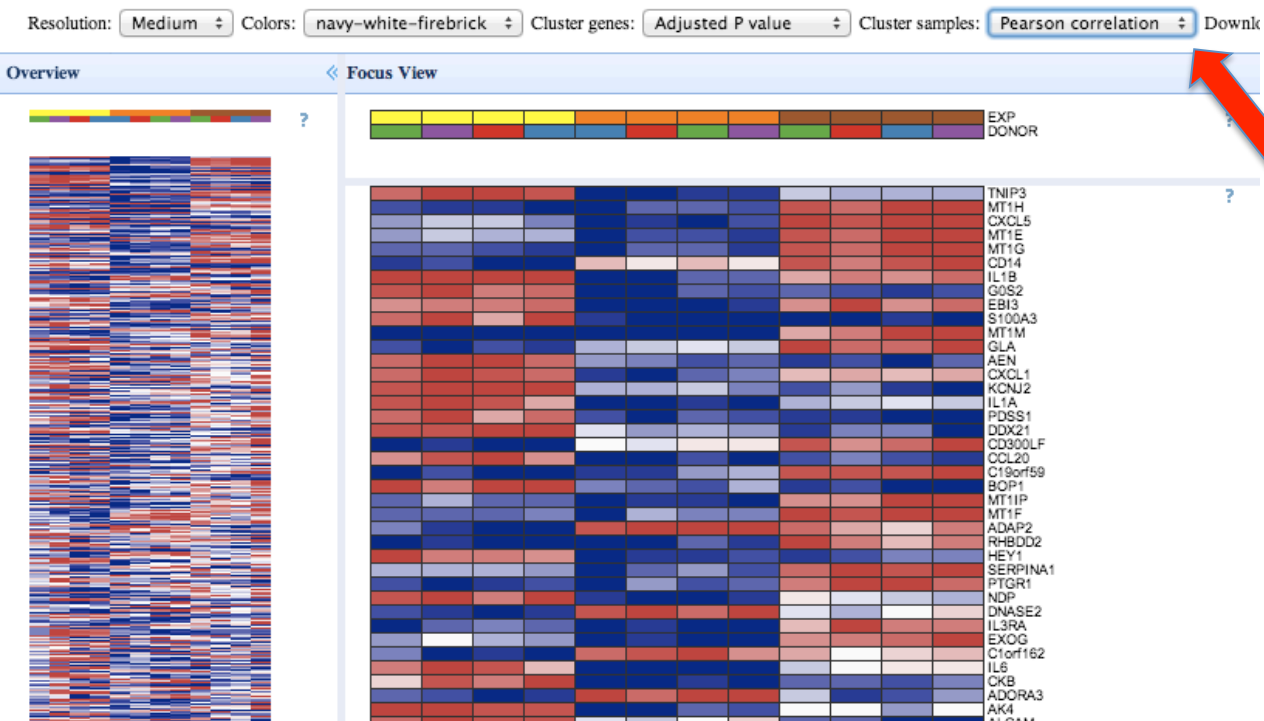


Global View

Focus View

Functional Analysis

Explorer Mode: Cluster Genes



Clustering genes can reveal more striking patterns of differential gene expression

Explorer Mode: Focus View

Drag-and-select
here!



Double
click here!

- Focus view shows current genes/metabolites of interest:
 - Drag and select subsets of the global view to display in the focus view
 - Double click on a functional annotation name to display associated genes in the focus view

Explorer Mode: Cluster Samples by Metadata

Click anywhere on the metadata row to order the samples by that metadata

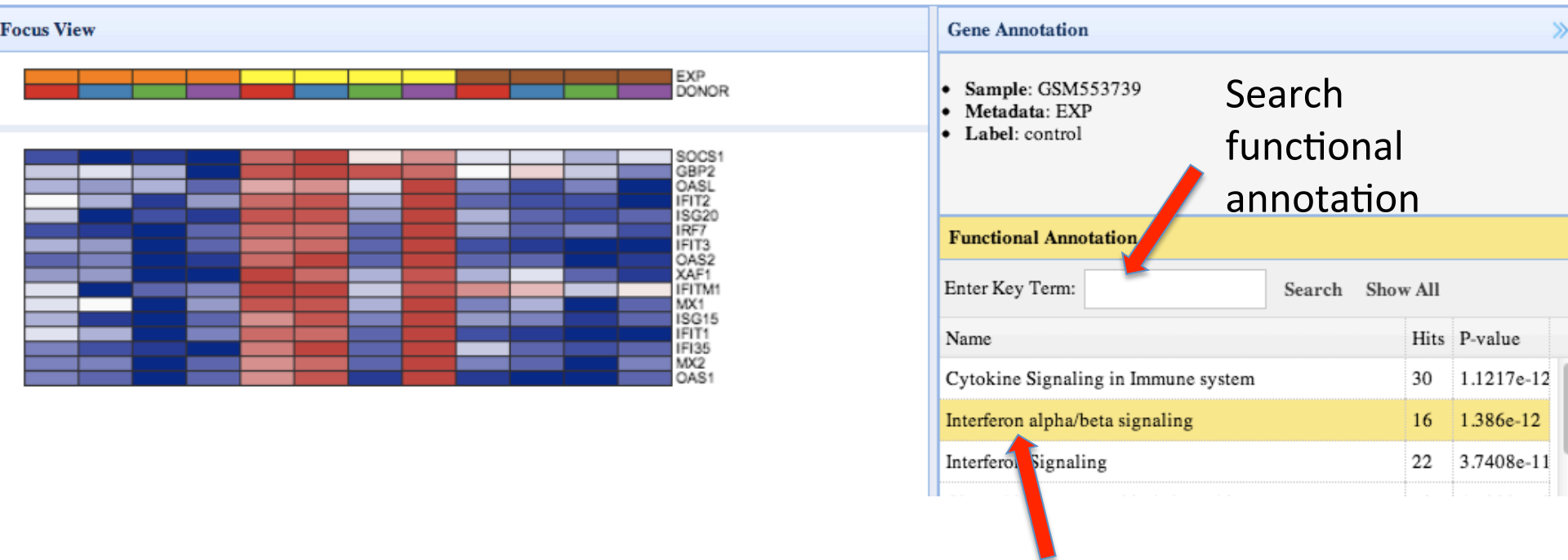


Experimental conditions (EXP) are ordered alphabetically



Visualize the differential gene expression between different experimental conditions

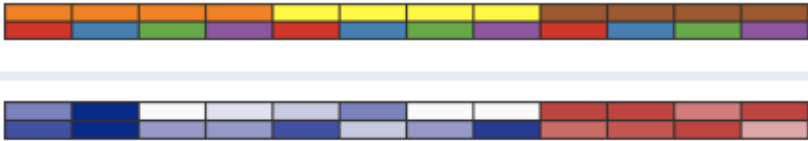
Explorer Mode: Visualize Predefined Functional Groups



Double click to view differential expression of 16 genes associated with interferon alpha and beta signaling

Explorer Mode: Visualize a Custom Molecular Signature

Focus View



Gene Annotation

- Sample: GSM553744
- Metadata: EXP
- Label: septic

Functional Annotation

Custom Signature

Enter ID list (same type as displayed, one per row):

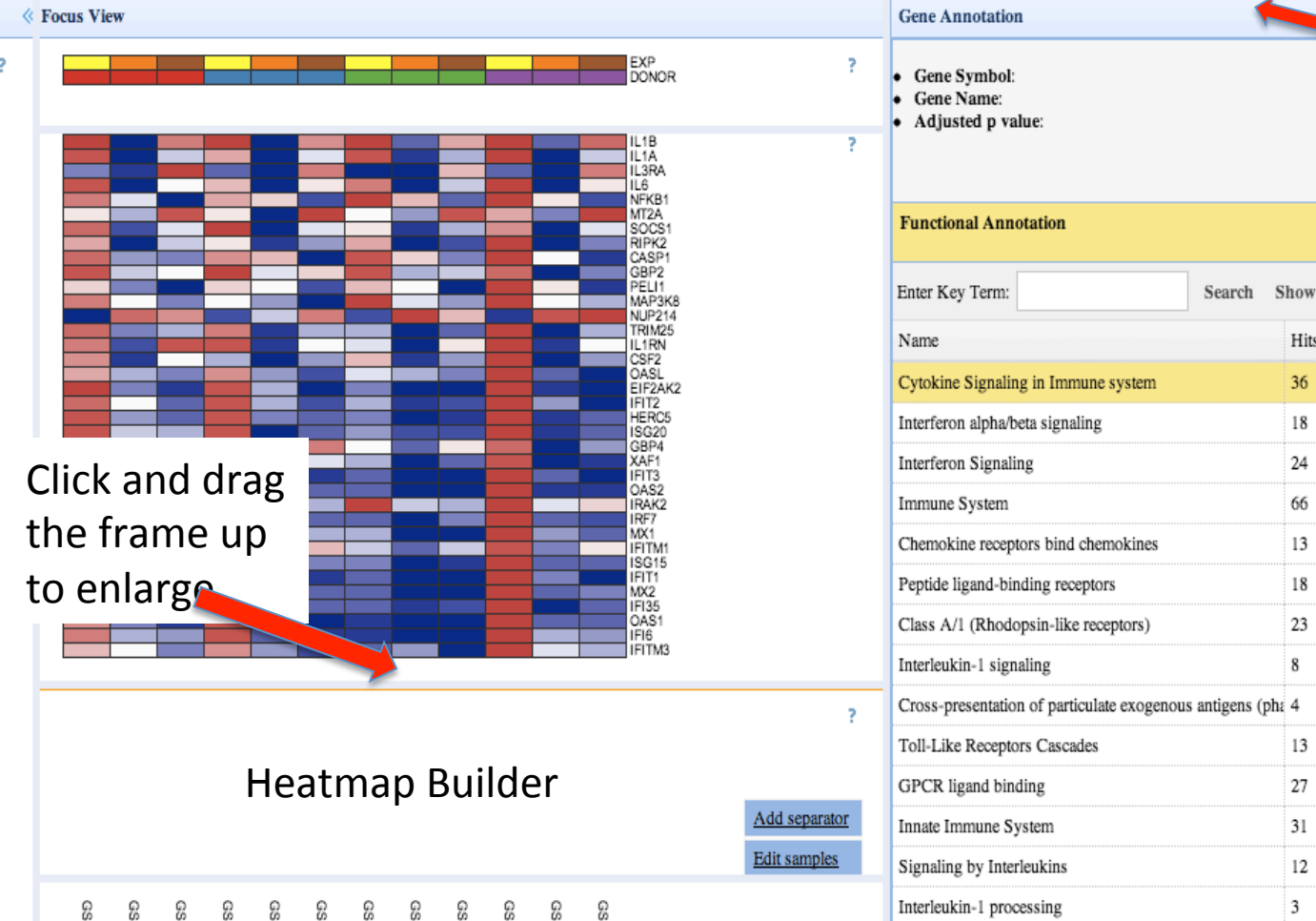
S100A12

CYP1B1

If you know which genes you are interested in, enter one or more in a list to display in the Focus View

Heatmap Builder

navy-white-firebrick Cluster genes: Adjusted P value Cluster samples: Pearson correlation Download: --Please Select-- Heatmap builder



Build your own heatmap!

Gene Annotation

- Gene Symbol:
- Gene Name:
- Adjusted p value:

Functional Annotation

Enter Key Term: Search Show

Name	Hits
Cytokine Signaling in Immune system	36
Interferon alpha/beta signaling	18
Interferon Signaling	24
Immune System	66
Chemokine receptors bind chemokines	13
Peptide ligand-binding receptors	18
Class A/1 (Rhodopsin-like receptors)	23
Interleukin-1 signaling	8
Cross-presentation of particulate exogenous antigens (ph	4
Toll-Like Receptors Cascades	13
GPCR ligand binding	27
Innate Immune System	31
Signaling by Interleukins	12
Interleukin-1 processing	3

Build a Custom Heatmap



Focus View

Double click on any row on to add to editor

Select and drag to add multiple rows to add to editor

Heatmap Builder:

Double click to remove any row;

Add separator to separate groups of genes;

Drag and drop any row or separator!

If you want to edit samples, first make sure all genes of interest are selected and grouped, then click “Edit samples”.

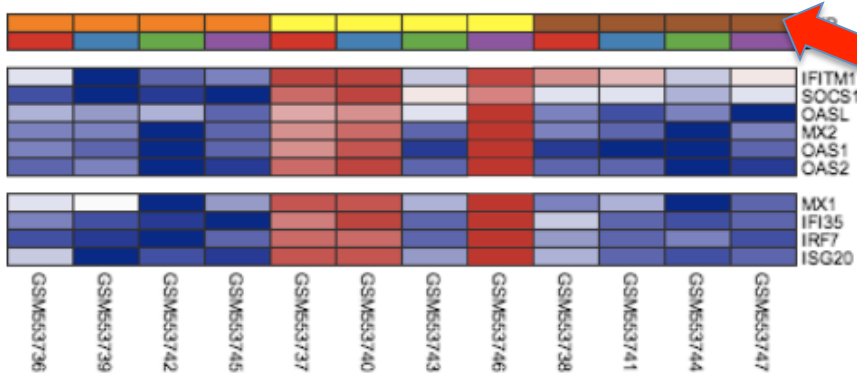
Edit Samples

Sample Editor

This should be the last step before exporting the image. All your genes of interest should be included in the heatmap below with proper resolution, colors, and clustering. Instructions:

- Remove a single sample: double clicking on the corresponding column;
- Rearrange a single sample: on the **expression heatmap**, drag-and-drop the sample to a specific location;
- Rearrange a batch of samples in two steps: 1) on the **metadata heatmap**, drag-and-select a consecutive list of samples; 2) drag the selected samples to a new location.

Export Image

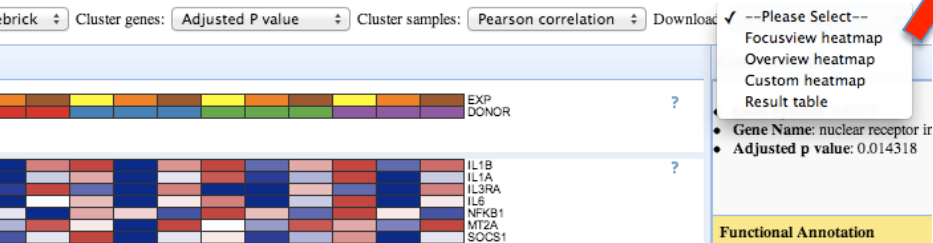


Final step before image export

- Double click sample column to remove
- Drag and drop one or more samples to rearrange

Getting Images and Results

Many options for image export

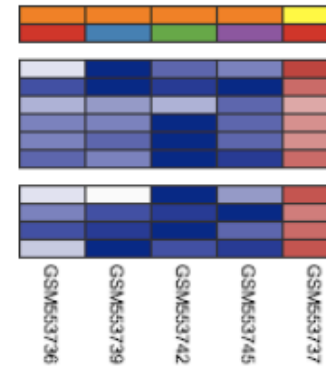


Sample Editor

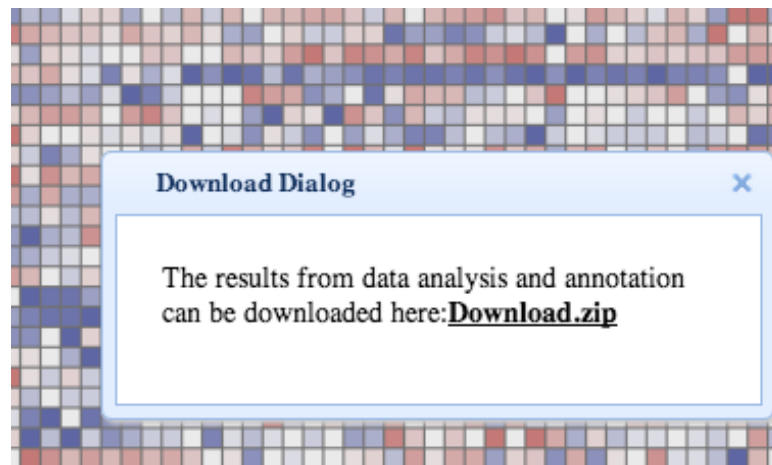
This should be the last step before heatmap below with proper resolution

- Remove a single sample: click on the sample name
- Rearrange a single sample: drag the sample name
- Rearrange a batch of samples: 1) select the samples; 2) drag the selection to the desired position

Export Image



Download result table as a zip file (significant genes and enriched functional groups)



=== END ===