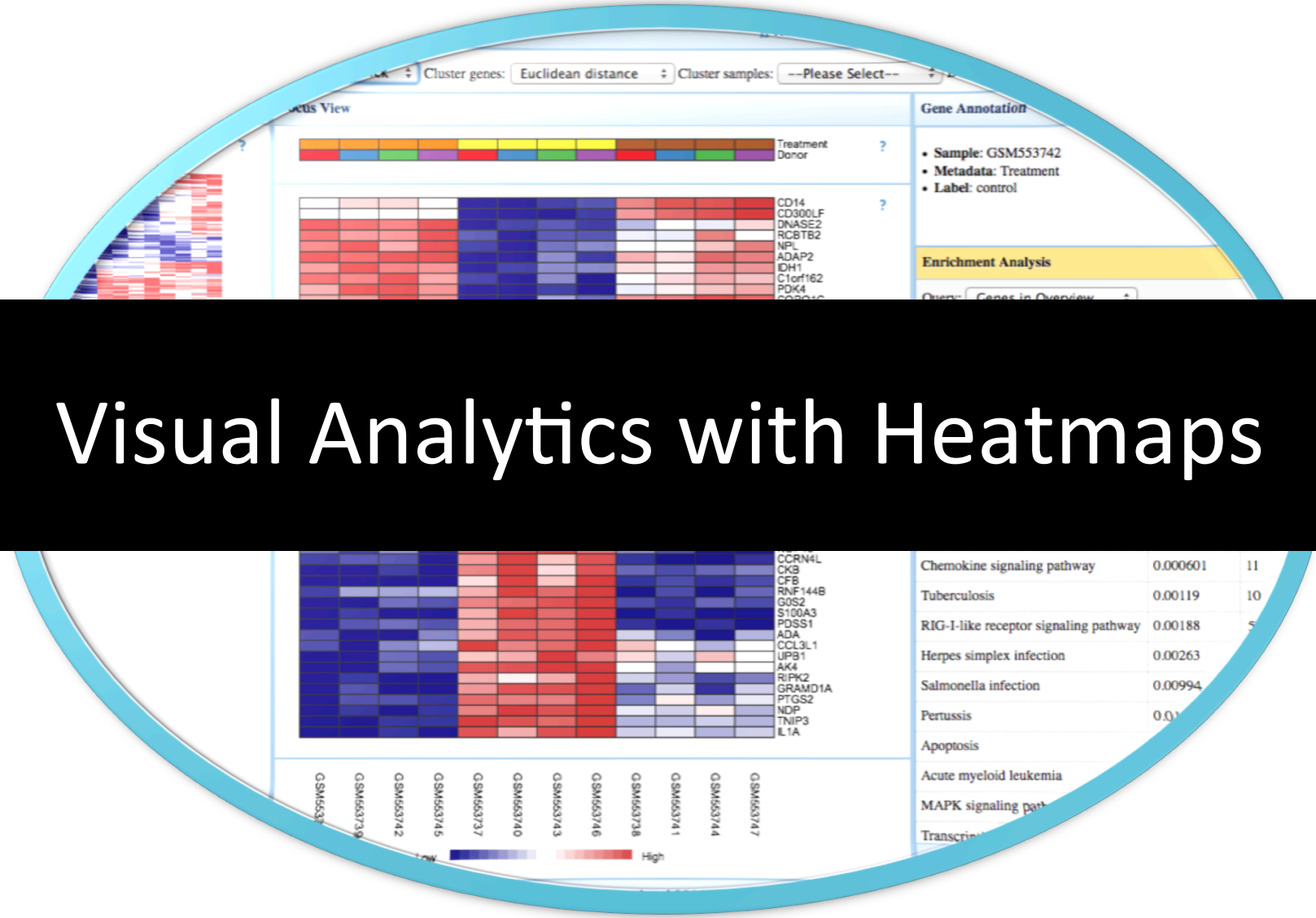


Visual Analytics with Heatmaps

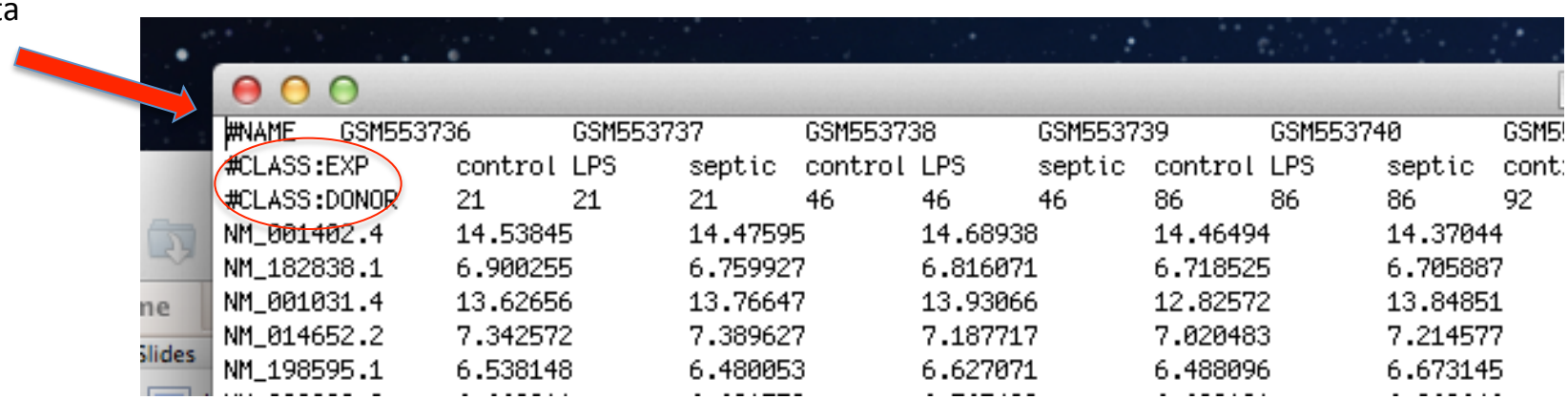
Computer and Browser Requirements

- 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

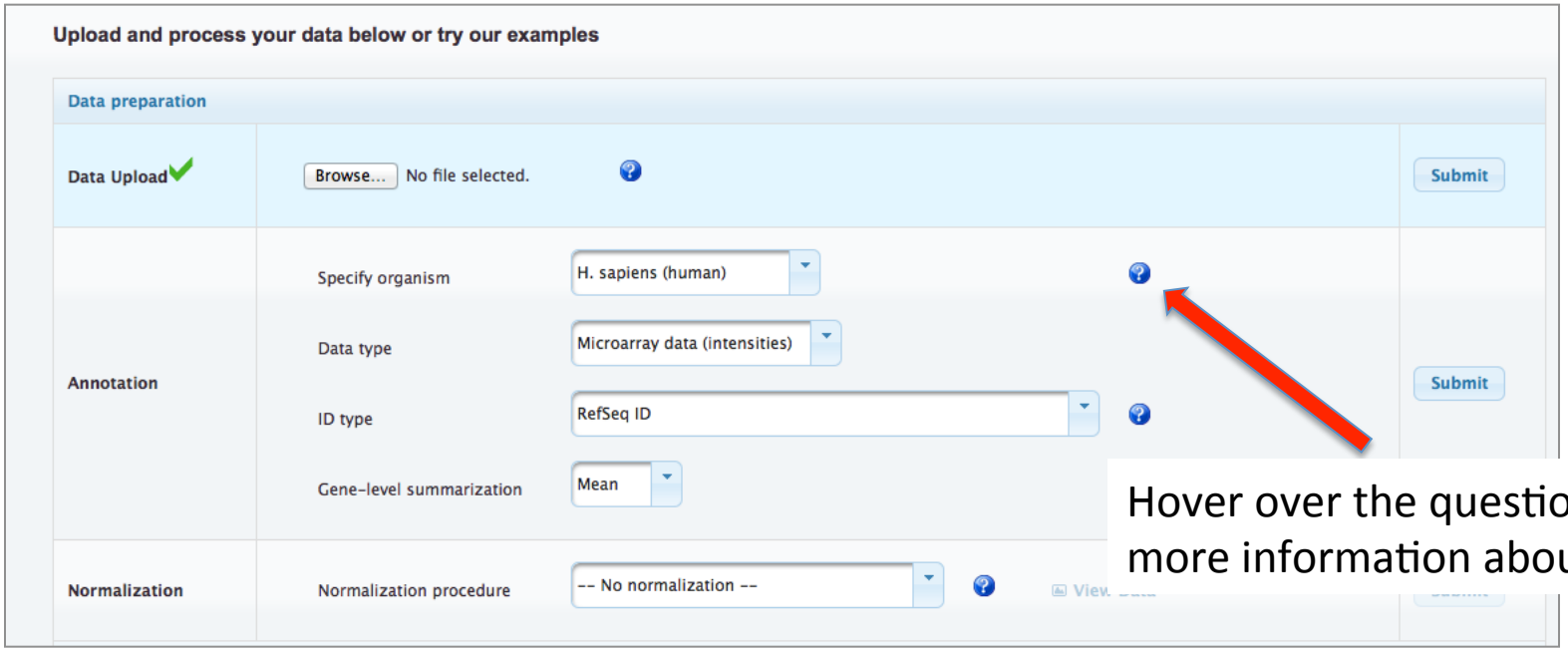
- Manipulate data headings in a spreadsheet program like MS Excel
- Save as a tab delimited .txt file
- The headings #NAME and #CLASS: (all capital letters) must be used
  - #NAME is for sample names (first row in your data)
  - #CLASS is for the clinical metadata. The screenshot below shows the labels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

Clinical or experimental metadata



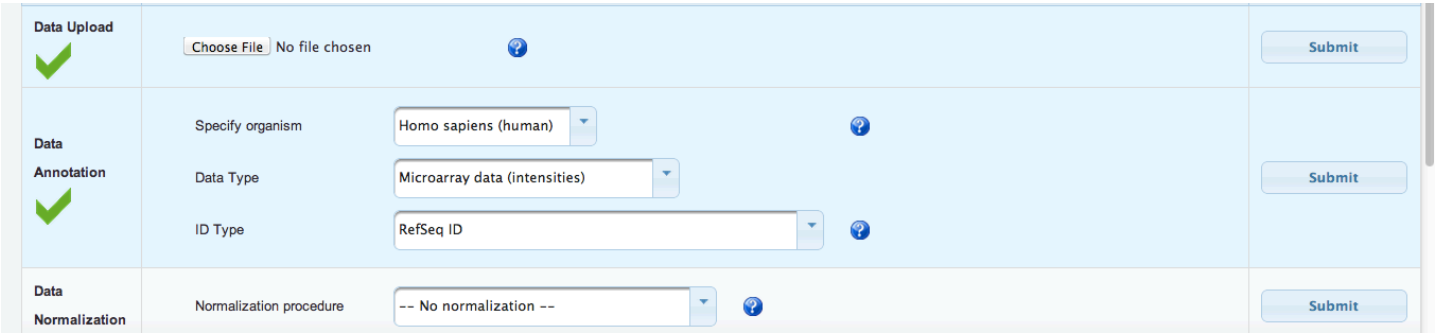
#NAME	GSM553736		GSM553737		GSM553738		GSM553739		GSM553740		GSM5
#CLASS:EXP	control	LPS	septic	control	LPS	septic	control	LPS	septic	cont	
#CLASS:DONOR	21	21	21	46	46	46	86	86	86	92	
NM_001402.4	14.53845		14.47595		14.68938		14.46494		14.37044		
NM_182838.1	6.900255		6.759927		6.816071		6.718525		6.705887		
NM_001031.4	13.62656		13.76647		13.93066		12.82572		13.84851		
NM_014652.2	7.342572		7.389627		7.187717		7.020483		7.214577		
NM_198595.1	6.538148		6.480053		6.627071		6.488096		6.673145		

Data Upload



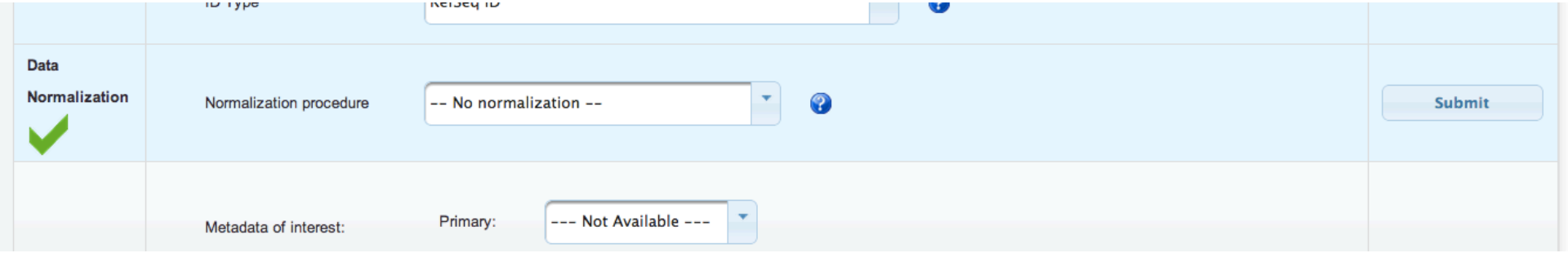
- A green check appears to indicate when each step is successfully completed

Data Annotation



- 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.

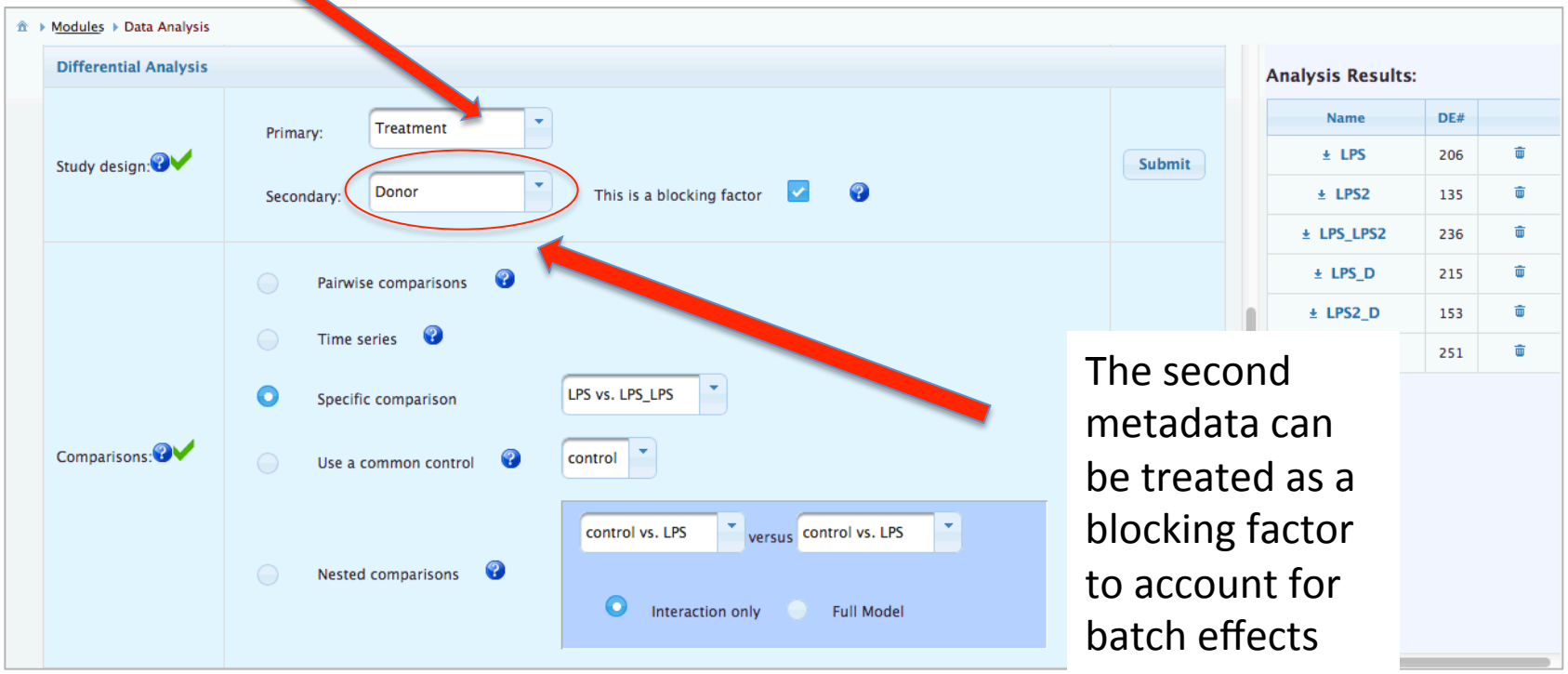
Data Normalization



- 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

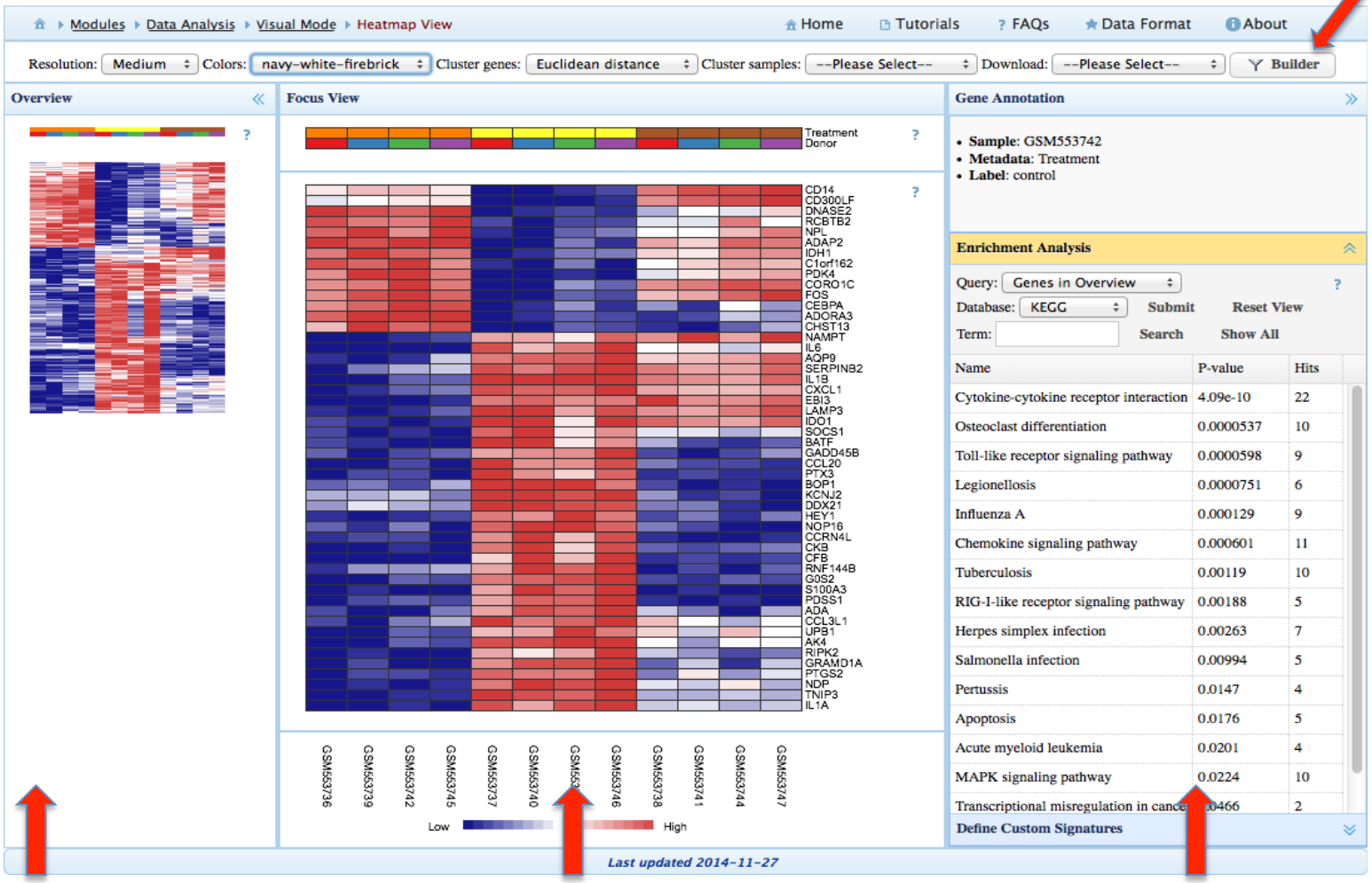


The second metadata can be treated as a blocking factor to account for batch effects

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

Overview

Toolbar

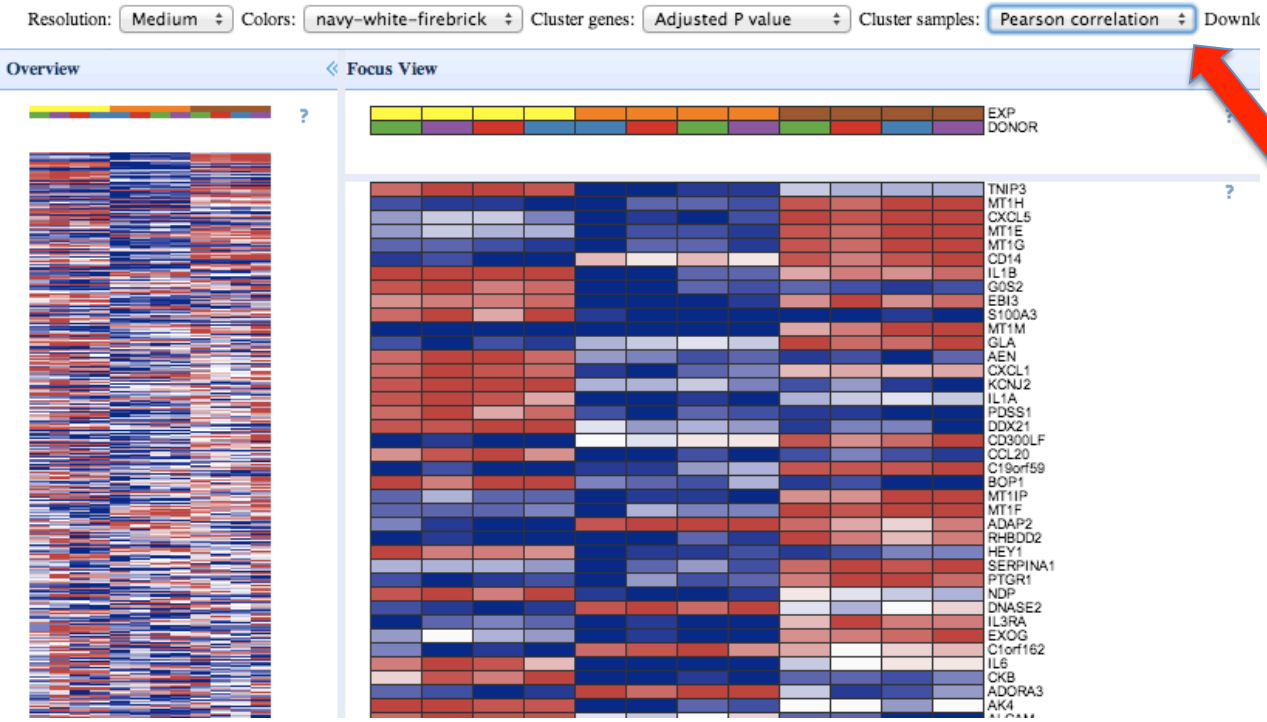


Global View

Focus View

Functional Analysis

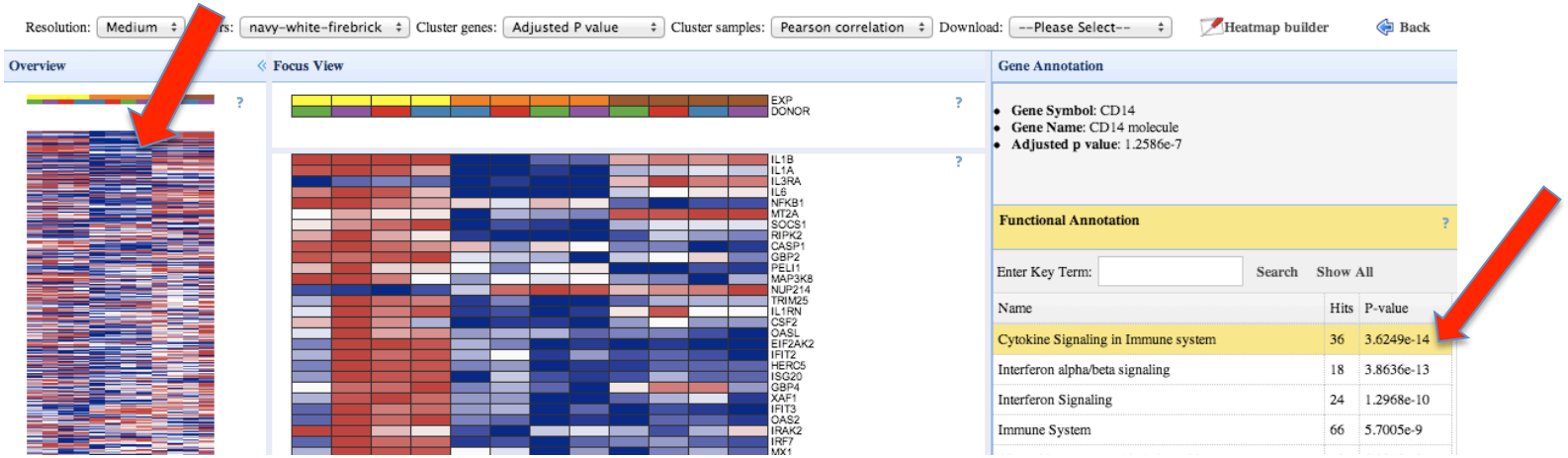
Explorer Mode: Cluster Genes



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

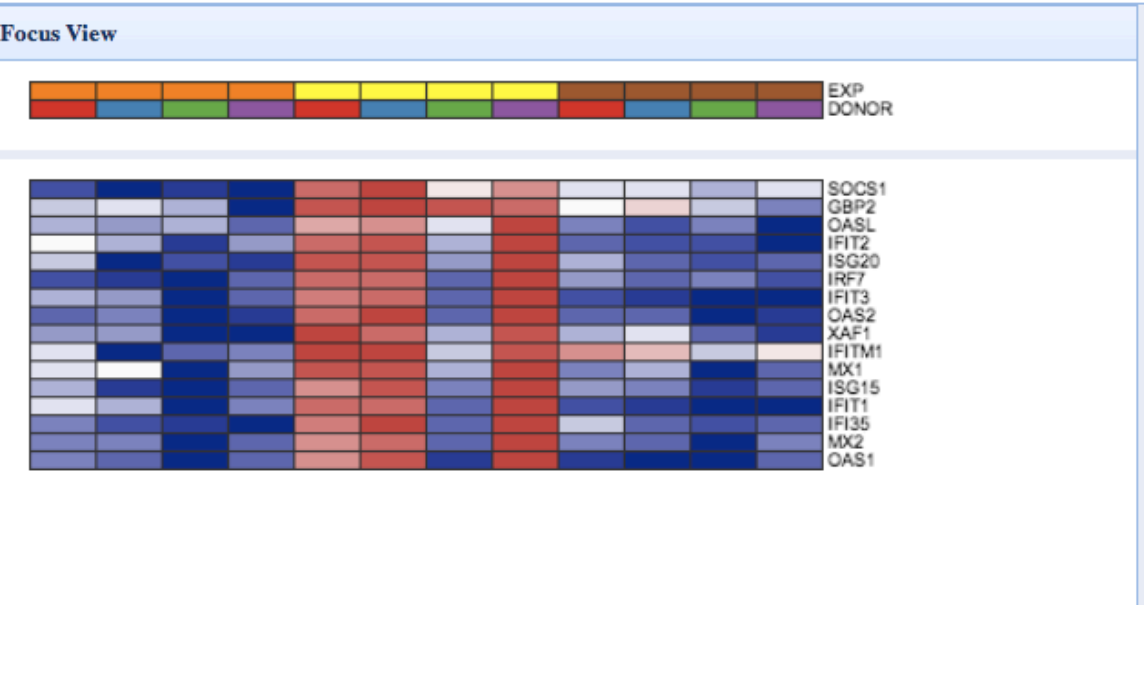
Explorer Mode: Cluster Samples by Metadata

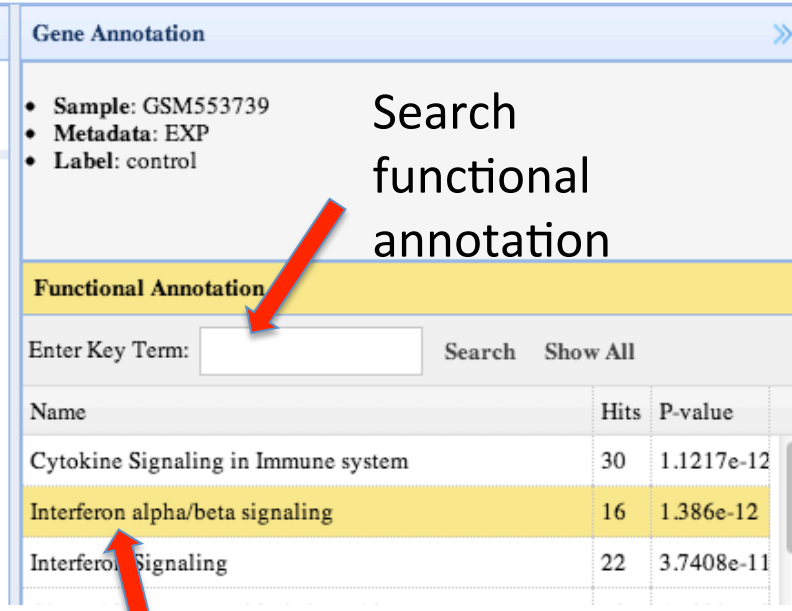
Click anywhere on the metadata row to order the samples by that metadata

Experimental conditions (EXP) are ordered alphabetically

Visualize the differential gene expression between different experimental conditions

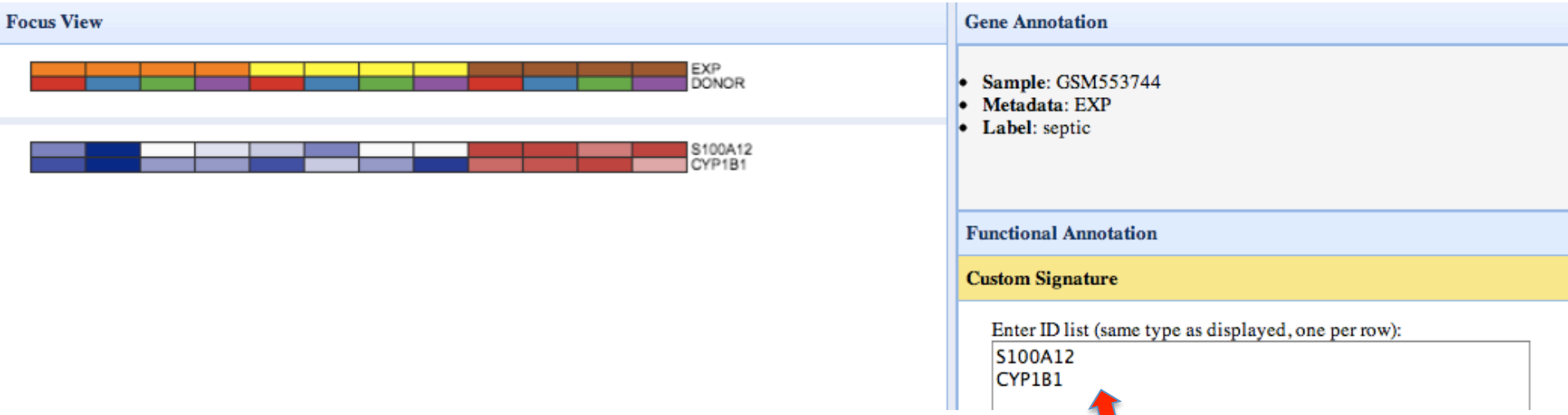
Explorer Mode: Visualize Predefined Functional Groups





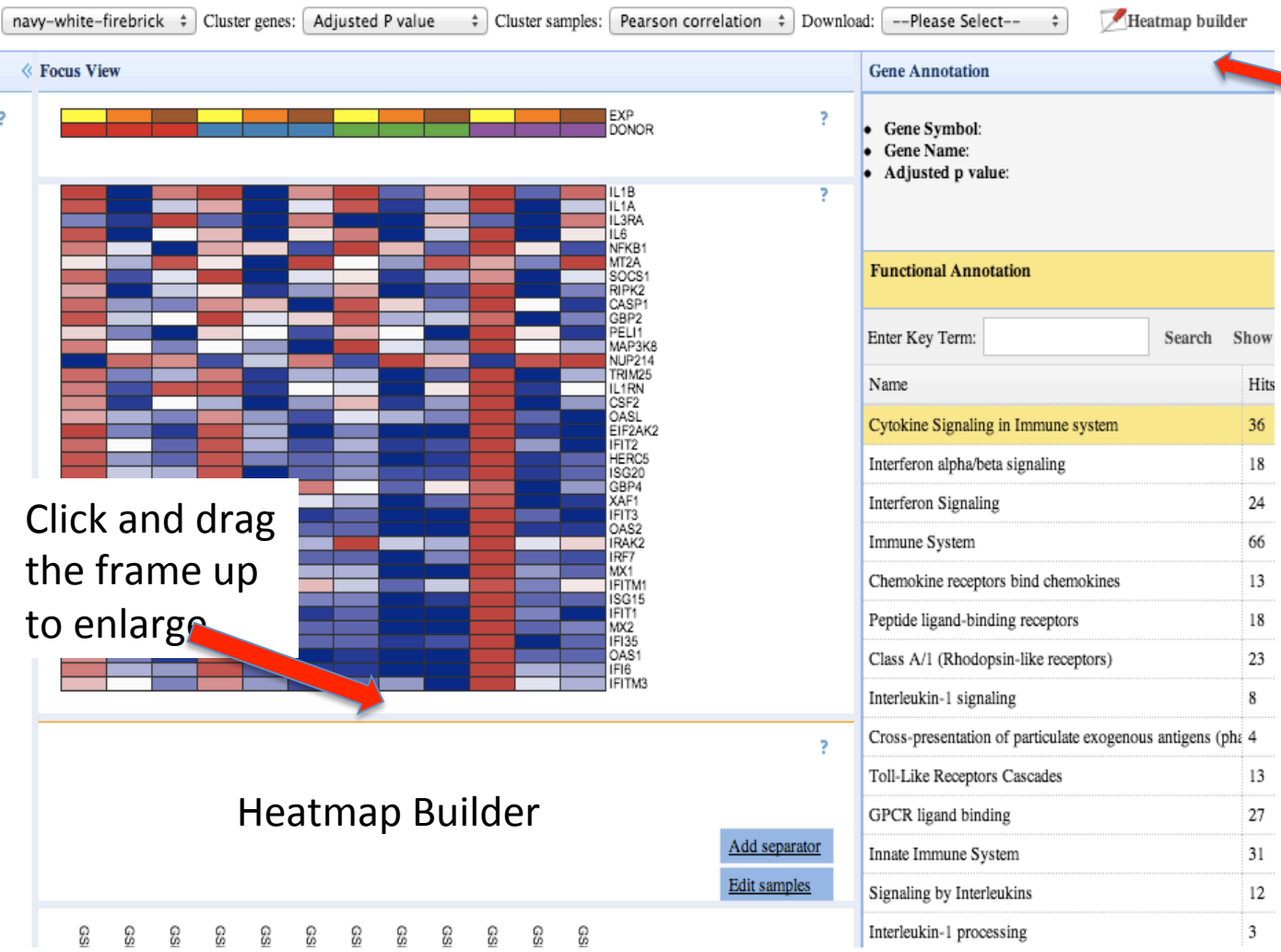
Double click to view differential expression of 16 genes associated with interferon alpha and beta signaling

Explorer Mode: Visualize a Custom Molecular Signature



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

Heatmap Builder



Click and drag the frame up to enlarge

Build your own heatmap!

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:

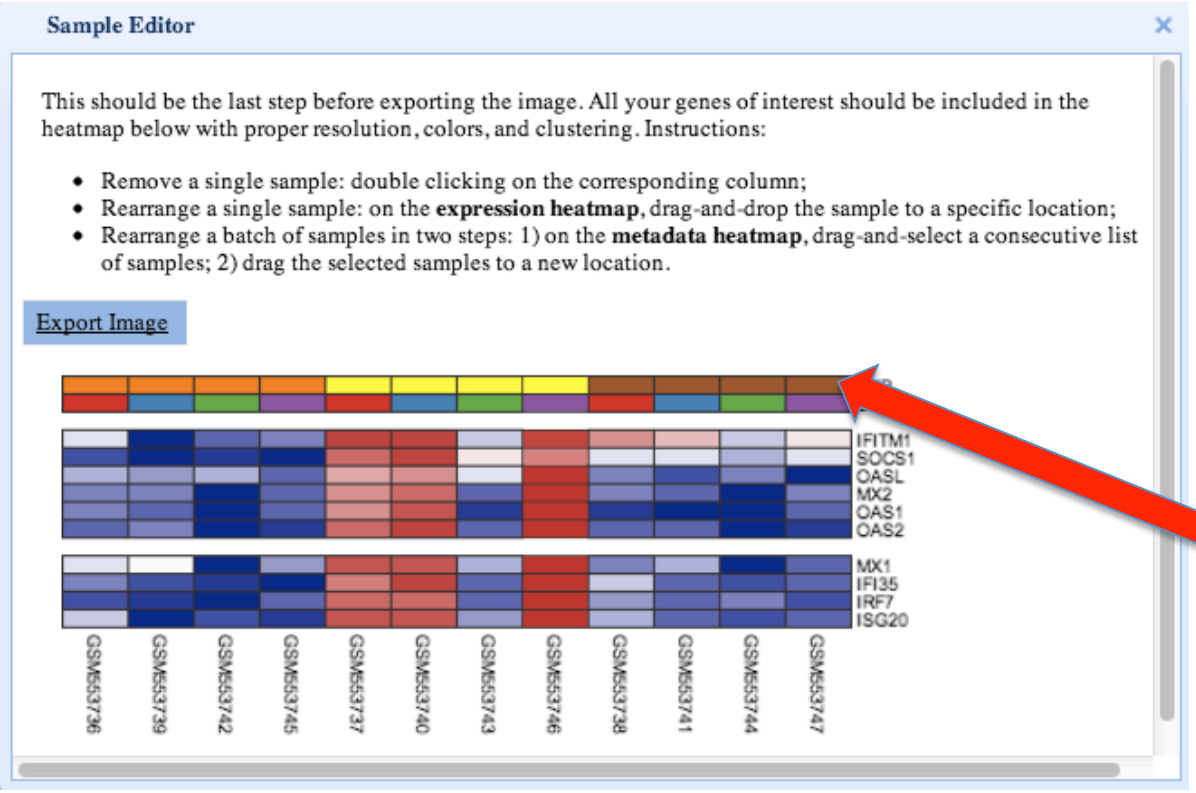
Double click to remove any row;

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

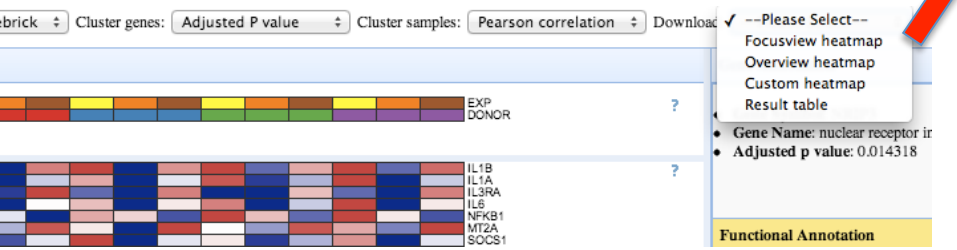


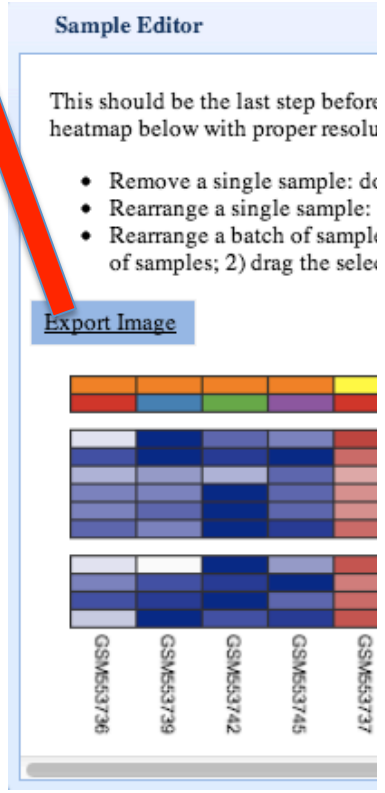
Final step before image export

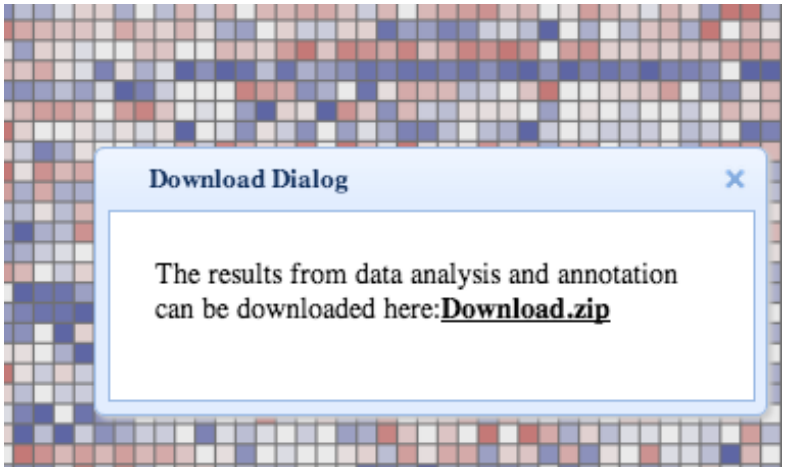
- Double click sample column to remove
- Drag and drop one or more samples to rearrange

Getting Images and Results

Many options for image export







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

=== END ===