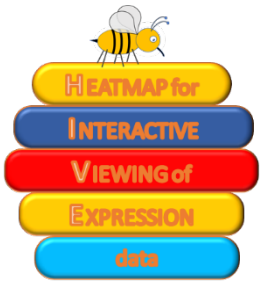
# HEATMAP FOR INTERACTIVE VIEWING OF GENE EXPRESSION: HIVE BROWSER

A USER GUIDE

*July, 2023*

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## INTRODUCTION

To demonstrate how to upload expression data to the HIVE browser, explore it to identify genes of interest, and submit gene lists to secondary tools for further analysis, we use two exemplar single cell RNA-Seq datasets. The first has changes in expression in the cell types of the mouse retina in the Akita model of diabetes. The second is from single human islet cells from Type 2 diabetics and non-diabetics generated by Xin *et al* (Xin, Kim et al. 2016), with expression differences in diabetes according to gender. Further details of the example datasets are available in the 'Example datasets' section.

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## ACCESSING THE HIVE BROWSER

**Quick start**

Click on this link to open the browser tab with preloaded data. You can start to explore the example data and then input your own data.

**Download HIVE browser** file from GitHub (https://qub-simpson-lab.github.io/HIVE_browser/ or https://github.com/QUB-Simpson-lab/HIVE_browser ).

Click on the file to run locally

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## LOADING GENE EXPRESSION DATA

**Download example datasets.** There are 3 formats for you to explore. Example A is the most common starting point and comprises individual files for each comparison. It is also possible to import differential gene expression for multiple conditions from a single file (example B). Any gene expression data organized in columns separated by comma, tab or semi-colon can be imported into the HIVE browser. Once you have your data loaded you can create a custom HIVE format file (example C) suitable for data sharing by clicking on the 'Download your data as a single data file' link. For more information about file formats and two-level inputs see Combined multi-sample and multi-level input files section below.

A. Individual differential expression files (eg output from Seurat)

Diabetic retina: Amacrine.txt, Bipolar.txt, Cone.txt, endothelial.txt, Microglia.txt, Muller.txt, Pericyte.txt, RGC.txt, Rod.txt

B. Single file

Diabetic islet cells: File_name

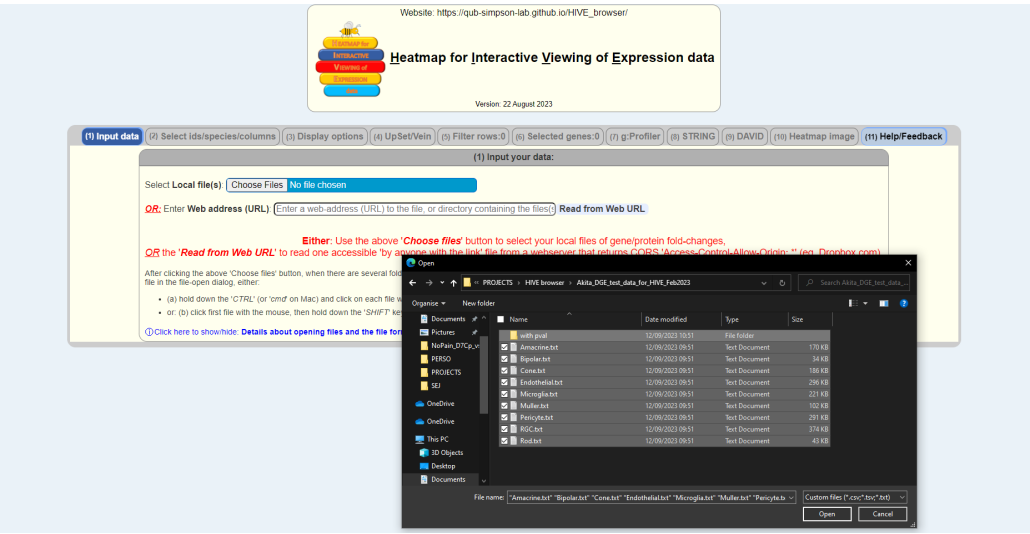
C. HIVE custom format

Diabetic islet cells: File_name

**Choose files**

Navigate to box (1) in the browser, entitled ‘Input your data’. In this case we are using individual differential expression files (example dataset A)

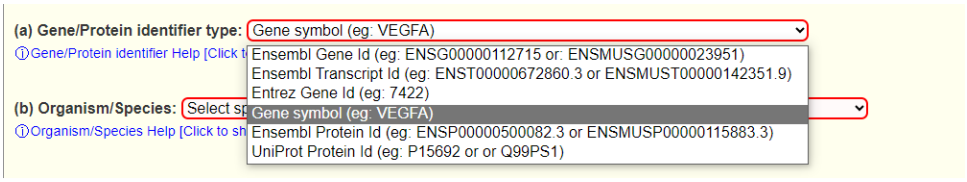
Click on ‘Choose files’ and select the individual files (hold down the ‘CTRL’ key (‘cmd’ on Mac) and click on each file with the mouse, or click on first file, then hold down ‘SHIFT’ key and click on the last file in list).



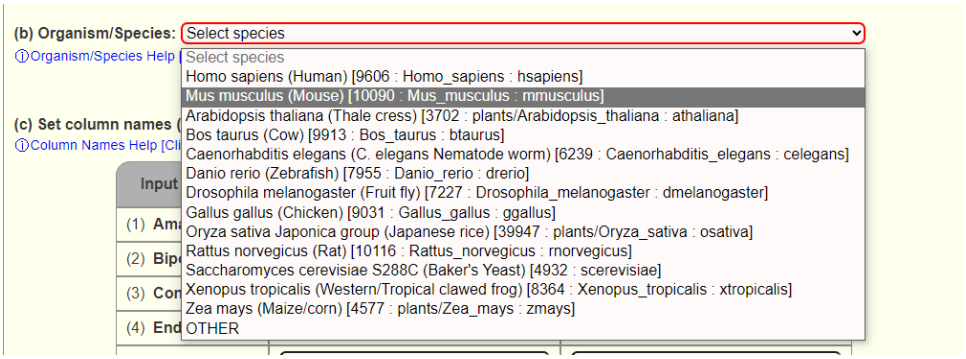

Once the files have been selected click on ‘OPEN’. The files may take few seconds to load (depending on size)

Navigate to box (2) in the browser, entitled ‘Select columns’.

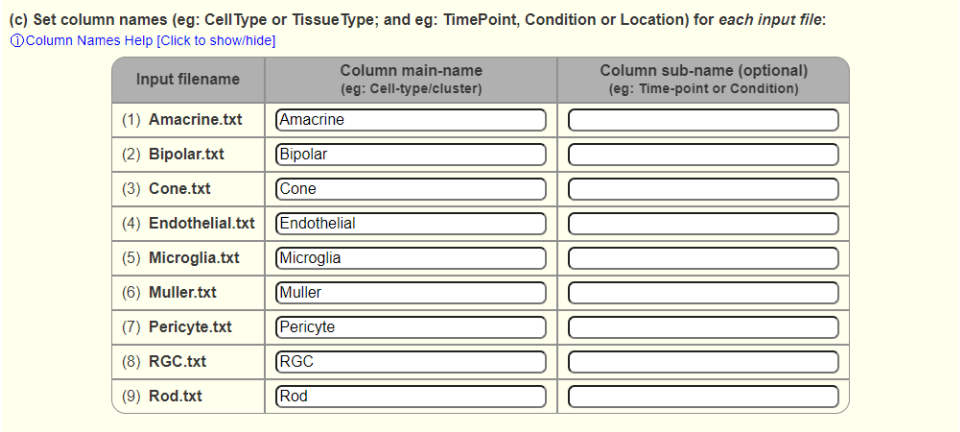
a. Select the gene or protein identifier used in your data from the drop-down menu (it will normally be correctly pre-populated based on your input data)



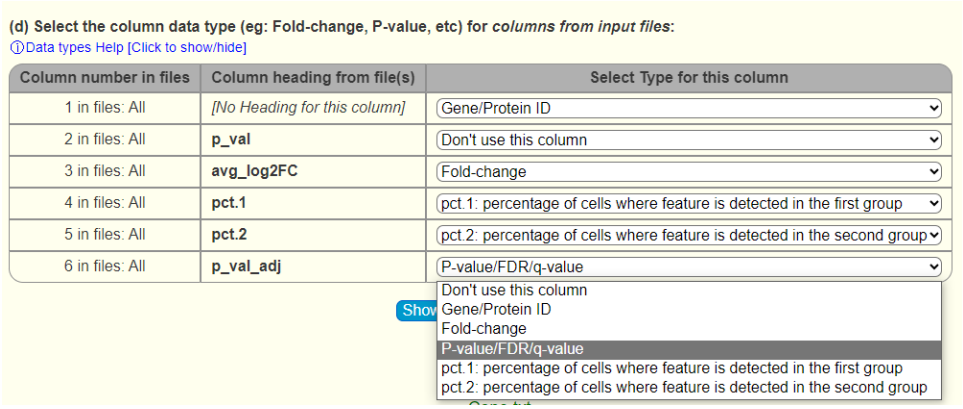

b. Select the organism from the drop-down menu



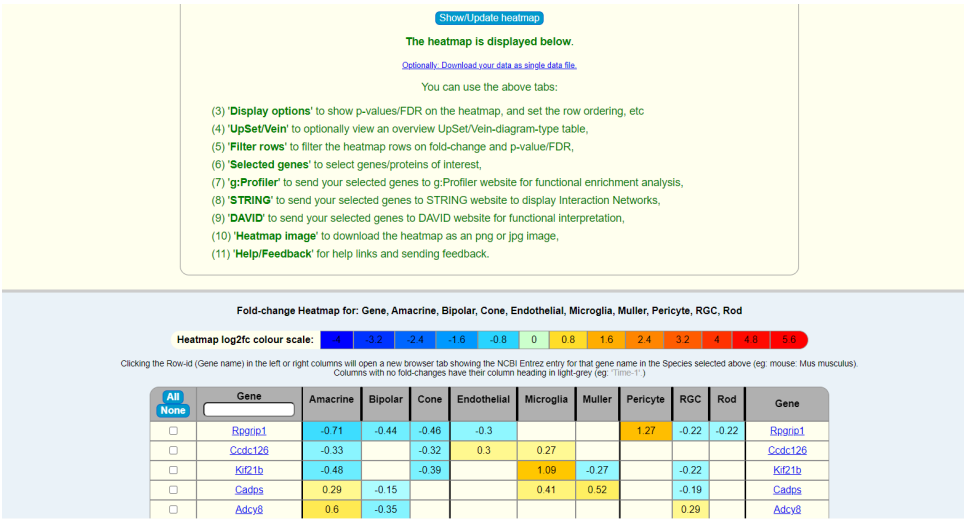

c. Set names for columns in the heatmap. These will be pre-populated based on your input files, but can be amended if required (eg of Main-name: CellType or TissueType; and of sub-name: TimePoint, Condition or Location). In this example we have just the main column for each file.




d. Select the column data type for each column in your input files




View the heatmap by clicking on the 'Show/Update heatmap' button

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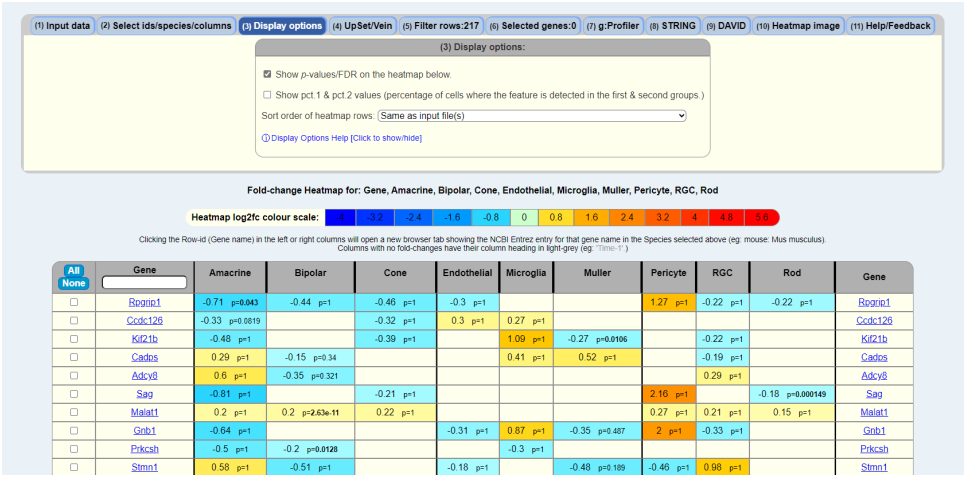
## DISPLAY OPTIONS & FILTERING BY FOLD CHANGE, P VALUE, CELL TYPE/CONDITION

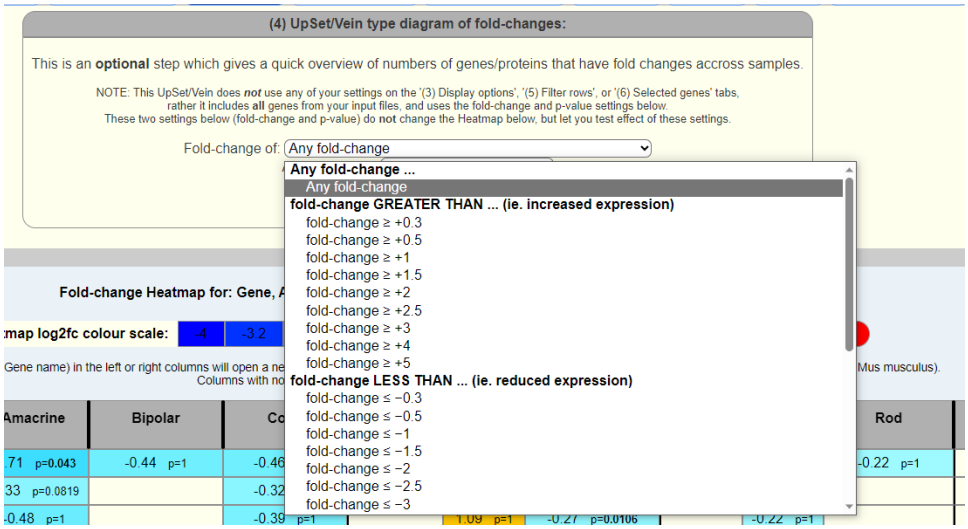
Navigate to box (3) at the top of the webpage entitled ‘Display options’. You can toggle display of *p*-values and pct1 & 2 in the heatmap by selecting the boxes as required.




Navigate to box (4)

Default filter options are; one or more columns having fold-change values, p<0.5 in at least one column in the row, and any fold change. The users can change these values as shown below.

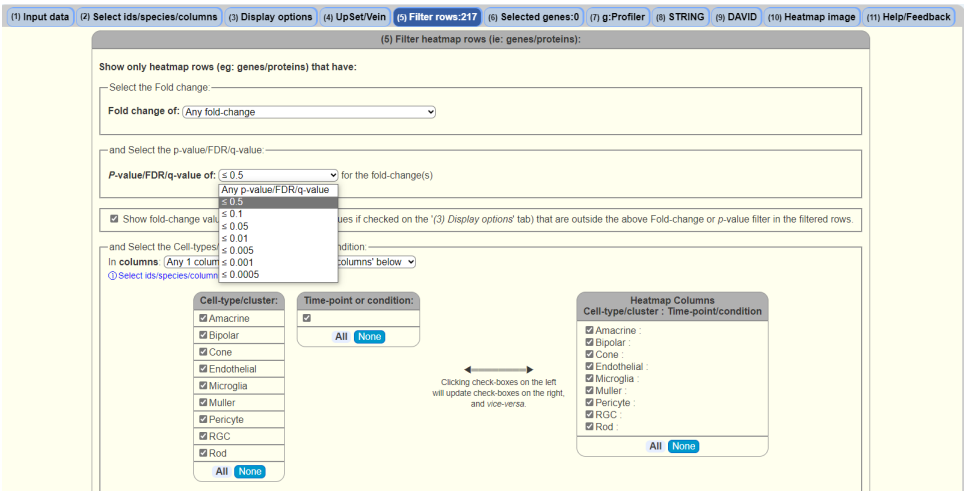
a. Select how many rows must have the chosen fold change




b. Select the desired fold change range and cell type of interest.

If your data has two levels, for example several time-points, age groups or sexes at which each of the cell types were assessed, it is possible to select both (more than one group can be selected by holding down the CTRL button).

c. Select the P-value (this refers to the adjusted P-value or raw P-value as selected above). All rows that contain at least one value below this threshold will be displayed. The fold changes with p-values below this threshold can be hidden by checking the box.

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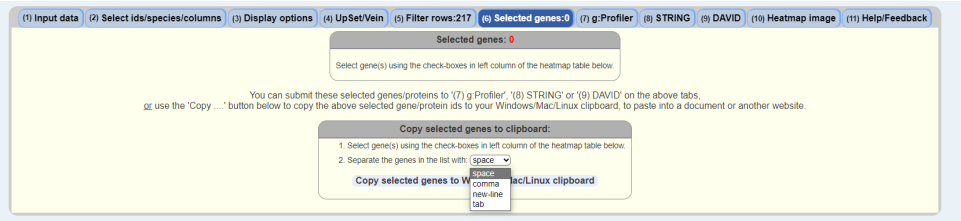
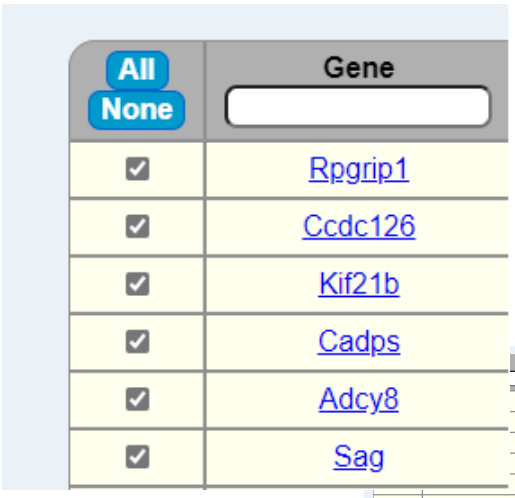
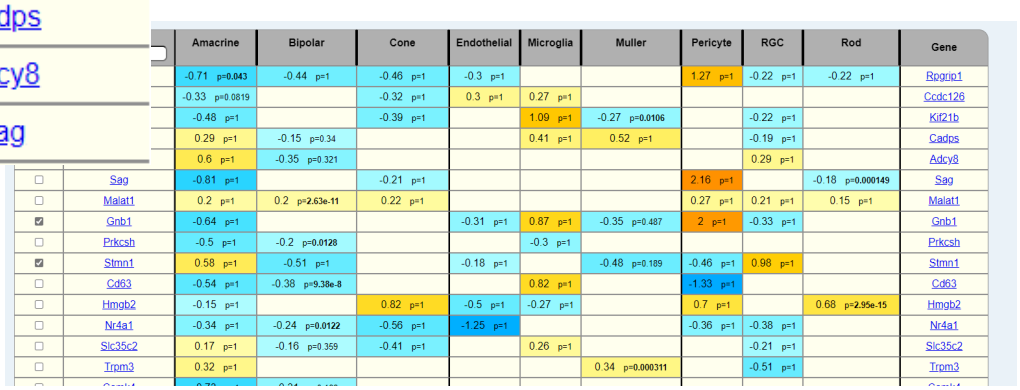
## SELECTION OF GENES OF INTEREST

Navigate to box (6) ‘Selected genes’




Your genes of interest can be chosen simply by using the filtering criteria above and then clicking on ‘All.’ It is also possible to select a subset of genes by manually clicking on genes of interest. This is particularly helpful for preliminary investigation of genes of interest identified while browsing the heatmap.



| | | Amacrine | Bipolar | Cone | Endothelial | Microglia | Muller | Pericyte | RGC | Rod | Gene |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | -0.71 p=0.043 | -0.44 p=1 | -0.46 p=1 | -0.3 p=1 | | | 1.27 p=1 | -0.22 p=1 | -0.22 p=1 | Rpgrip1 |
| | | -0.33 p=0.0819 | | -0.32 p=1 | 0.3 p=1 | 0.27 p=1 | | | | | Ccdc126 |
| | | -0.48 p=1 | | -0.39 p=1 | | 1.09 p=1 | -0.27 p=0.0106 | | -0.22 p=1 | | Kif21b |
| | | 0.29 p=1 | -0.15 p=0.34 | | | 0.41 p=1 | 0.52 p=1 | | -0.19 p=1 | | Cadps |
| | | 0.6 p=1 | -0.35 p=0.321 | | | | | | 0.29 p=1 | | Adcy8 |
| ☐ | Sag | -0.81 p=1 | | -0.21 p=1 | | | | 2.16 p=1 | | -0.18 p=0.000149 | Sag |
| ☐ | Malat1 | 0.2 p=1 | 0.2 p=2.63e-11 | 0.22 p=1 | | | | 0.27 p=1 | 0.21 p=1 | 0.15 p=1 | Malat1 |
| ☑ | Gnb1 | -0.64 p=1 | | | -0.31 p=1 | 0.87 p=1 | -0.35 p=0.487 | 2 p=1 | -0.33 p=1 | | Gnb1 |
| ☐ | Prkcsh | -0.5 p=1 | -0.2 p=0.0128 | | | -0.3 p=1 | | | | | Prkcsh |
| ☑ | Stmn1 | 0.58 p=1 | -0.51 p=1 | | -0.18 p=1 | | -0.48 p=0.189 | -0.46 p=1 | 0.98 p=1 | | Stmn1 |
| ☐ | Cd63 | -0.54 p=1 | -0.38 p=9.38e-8 | | | 0.82 p=1 | | -1.33 p=1 | | | Cd63 |
| ☐ | Hmgb2 | -0.15 p=1 | | 0.82 p=1 | -0.5 p=1 | -0.27 p=1 | | 0.7 p=1 | | 0.68 p=2.95e-15 | Hmgb2 |
| ☐ | Nr4a1 | -0.34 p=1 | -0.24 p=0.0122 | -0.56 p=1 | -1.25 p=1 | | | -0.36 p=1 | -0.38 p=1 | | Nr4a1 |
| ☐ | Slc35c2 | 0.17 p=1 | -0.16 p=0.359 | -0.41 p=1 | | 0.26 p=1 | | | -0.21 p=1 | | Slc35c2 |
| ☐ | Trpm3 | 0.32 p=1 | | | | | 0.34 p=0.000311 | | -0.51 p=1 | | Trpm3 |

As you type a gene name into the box at the top of the ‘Gene’ column in the heatmap, the genes will be filtered to retain those with a matching name.

When you have chosen your genes of interest they can be copied to the clipboard with the chosen separator.

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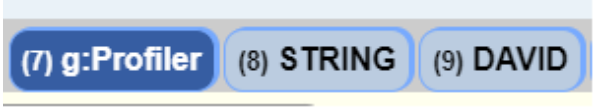
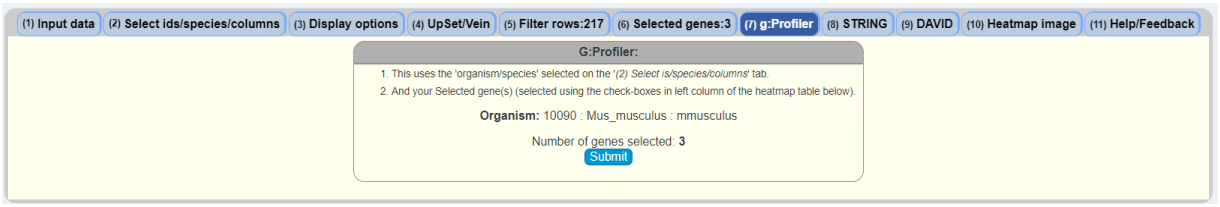
## SUBMITTING GENE LISTS FOR SECONDARY ANALYSIS

To submit selected genes directly to an external tool for network analysis and detection of enriched gene categories and pathways navigate to box 6, 7 or 8.



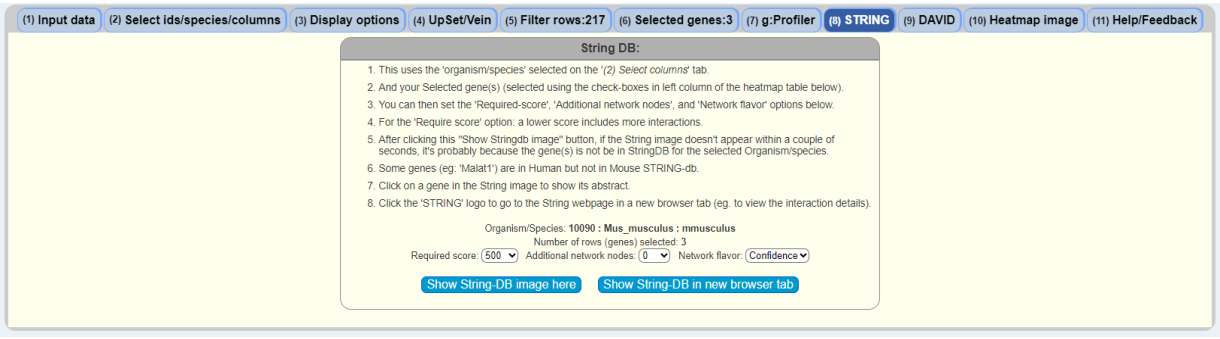
## G:PROFILER: A WEB SERVER FOR FUNCTIONAL ENRICHMENT ANALYSIS

For g:Profiler, simply click on the blue button to open the g:Profiler web server with your list of selected genes



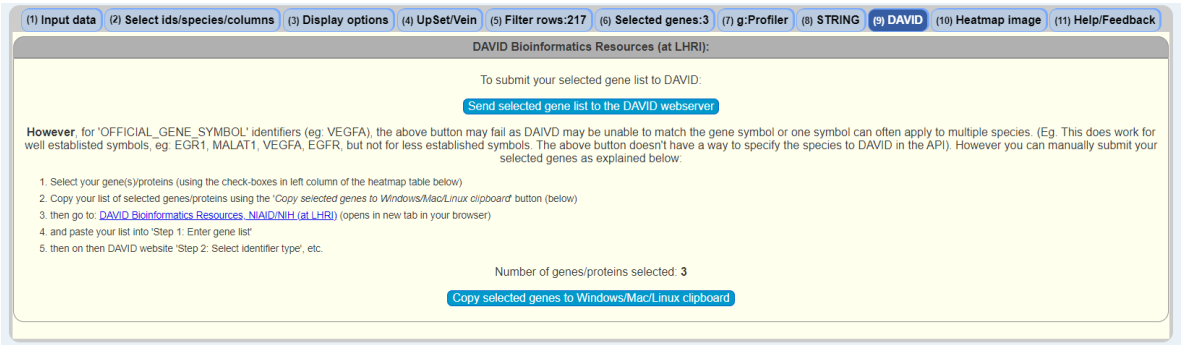

## STRING: FUNCTIONAL PROTEIN ASSOCIATION NETWORKS

For StringDB you can choose some basic parameters for the network and then view the image within the HIVE bowser by clicking the ‘Show StringDB image here’ button. For more in depth analyses you can view your selected gene list on the String server by clicking the ‘Show StringDB in new tab’ button.




## DAVID BIOINFORMATICS RESOURCES

For DAVID Bioinformatics Resources copy the selected genes to the clipboard using the tool provided, follow the DAVID link, and paste in your gene list.

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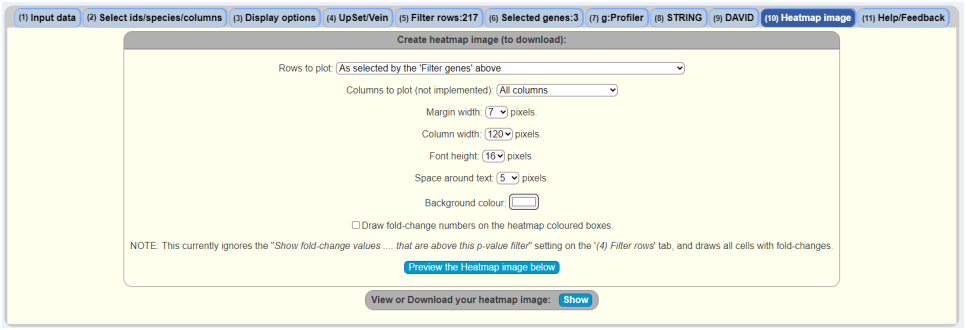
## CREATING A HEATMAP IMAGE FOR DOWNLOAD

Navigate to box 9




Choose options for customizing the output

Preview

Select format

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## DATA FORMATS

The only requirement of your data is that it contains gene id and differential expression ("avg_log2FC") columns, which can be selected during the input process. It should be in comma, semicolon, or tab, separated format and can include optional p value ("p_val", "p_val_adj") and percent of cells ("pct.1" "pct.2") columns (see example input files). You can import the expression data from multiple individual files or from a single file containing multiple measurements.

### Individual input files

The input data must be in tabular format as shown above. This is the typical column order, but specific columns can be selected during import. The first column is expected to contain gene/protein identifiers, such as gene symbols. It is possible to use any gene identifier, but for the 'additional gene information' links in the HIVE browser to be functional, it must be one of those types available from the drop-down list. Subsequent columns contain

information about the differential expression of each gene. The 'avg_log2FC' or 'avg_logFC' column is the fold-change value. The 'p_val_adj' is the P=value (if there is no 'p_val_adj' column, then 'p_val' [unadjusted p-value] column is used instead). The ‘pct’ is the percentage of cells in which the gene is detected.

A full dataset follows the format generated by analysis of scRNA-Seq data using the Seurat pipeline and contains the following columns: "" "p_val" "avg_log2FC" "pct.1" "pct.2" "p_val_adj". Only the "avg_log2FC" is essential, but omission of the other columns will reduce the functionality of the browser. Whatever the source of your data, the column headings must have these names enclosed within double quotation marks (fold change can be "avg_log2FC" or "avg_logFC").

The columns in the input file(s) should be separated by either: comma, semicolon, tab, or space. Typically an individual fold-change file is provided for each comparison eg differential expression in a specific cell type.

Example of data in the required format:

```
""          "p_val"     "avg_log2FC"      "pct.1"     "pct.2"  "p_val_adj"
"Rpgrip1"   2.49e-06    -0.71             0.025       0.186    0.043
"Ccdc126"   4.74e-06    -0.33             0.006       0.137    0.081
"Epha6"     3.56e-05    0.78              0.46        0.288    0.615
...etc...
```

These columns typically have the following meaning (from: Differential expression testing in Seurat):

- *p_val*: p_val (unadjusted)
- *avg_logFC*: log fold-change of the average expression between the two groups. Positive values indicate that the feature is more highly expressed in the first group
- *pct.1*: The percentage of cells in which the feature is detected in the first group
- *pct.2*: The percentage of cells in which the feature is detected in the second group
- *p_val_adj* : Adjusted p-value, based on Bonferroni correction using all features in the dataset

Each filename (without any '.csv' or ".tsv" ending) will be used as the Heatmap column heading.

## Combined multi-sample and two-level input files

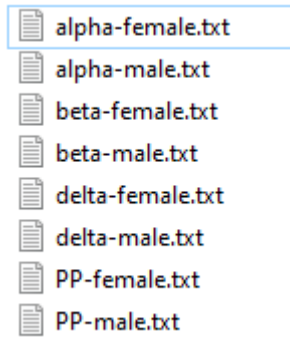
It is possible to upload a single CSV file containing differential (eg. between test and control samples) fold changes for all the samples. The HIVE browser requires a column with an identifier (gene or protein) and a column for $\log_2$fold change for each condition (additional columns can include p value, adjusted p value and percent of cells with expression). If the

column headings have the standard format (ie. P_val, avg_log2FC... ) they will be imported automatically. If your file has different column names you can select manually which contains each value). You can also load individual gene expression files by selecting them manually – once these are loaded you can download the input data as a single multi-sample file using the 'Optionally: Download your data as single data file' link. This single file makes subsequent loading of data easier.

Combined file

If your data has multiple levels, for example differential expression for each cell type was recorded in several conditions, groups or timepoints, it is possible to reflect this in either the title of individual file or the column name in a multi-sample file. If a '-' is included in the filename of an individual file, for example between the CellType and Condition this is reflected in the heatmap column titles. Alternatively, the same samples within a single file could be specified using a colon in the column names as follows CellType:Condition.

Add image of file with Celltype:Condition...




| | p_val | avg_log2FC | pct.1 | pct.2 | p_val_adj |
|---|---|---|---|---|---|
| 54453 | 5.65E-24 | 1.33 | 0.996 | 0.919 | 9.13E-20 |
| 5967 | 0.063374 | 1.21 | 0.353 | 0.496 | 1 |

| Gene | alpha | | beta | | delta | | PP | |
|---|---|---|---|---|---|---|---|---|
| | female | male | female | male | female | male | female | male |
| 54453 | 1.33 | | 1.22 | 0.19 | 0.23 | 1.13 | 0.82 | -0.44 |

**OR:**
**(2)**

```
Gene,Amacrine:EARLY,Amacrine:MID,Amacrine:LATE,Bipolar:EARLY,B
ipolar:MID,Bipolar:LATE,Cone:EARLY,Cone:MID,Cone:LATE
Aak1,0,0.6,0,0,-2.1,0,0,0,0.4
Aamp,0,0.3,0,0,-0.6,0,0,0.3,0
```

```
Aars,0,0.4,0,0,-0.3,0,1.2,0,0
...etc....
```

where:

- first column is 'Gene' name
- then several columns with heading format: CellType:TimePoint (or CellType:Condition/Location, etc)
- then gene/protein fold-change rows in format: GeneName, Fold-change:Optional p-value:Optional pct.1: Optional pct2, .....

**Multilevel Explain format:'s names**

**Eg Islet**

Can be used for other datatypes

For an explanation of the format required for combined multi-sample and multi-level input files see below.

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## EXAMPLE DATASETS

**Dataset 1:** Mouse retina. This was generated by our group from the Akita mouse model of diabetes and has been deposited in GEO, accession number xxxx. The major retinal cell types were identified and differential expression between Akita and control mice performed using the Seurat package v4(Hao, Hao et al. 2021). The output files used for analysis in the HIVE browser are available from as 'Example dataset 1' from https://github.com/QUB-Simpson-lab/HIVE_browser.

Dataset 2: (Xin, Kim et al. 2016)4 Human islet cells. The original data was generated by Xin et al (Xin, Kim et al. 2016), and is available from GEO, accession number GSE81608. The samples were divided into male and female and differential expression for each cell type and sex determined using the Seurat package v4 (Hao, Hao et al. 2021). The output files used for analysis in the HIVE browser are available as a single multi-sample file (Example dataset 2) and in the cutom HIVE format (Example dataset 3) from https://github.com/QUB-Simpson-lab/HIVE_browser.

**Dataset 2:** Human islet cells. The original data was generated by Xin et al (Xin, Kim et al. 2016), and is available from GEO, accession number GSE81608. The samples were divided into male and female and differential expression for each cell type and sex determined using

the Seurat package v4 (Hao, Hao et al. 2021). The output files used for analysis in the HIVE browser are available as a single multi-sample file (Example dataset 2) and in the cutom HIVE format (Example dataset 3) from https://github.com/QUB-Simpson-lab/HIVE_browser.

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## BIBLIOGRAPHY

Hao, Y., et al. (2021). "Integrated analysis of multimodal single-cell data." Cell **184**(13): 3573-3587 e3529.

Xin, Y., et al. (2016). "RNA Sequencing of Single Human Islet Cells Reveals Type 2 Diabetes Genes." Cell Metab **24**(4): 608-615.

.

Extra stuff

Combined file

Optionally a '-' in the filename between the CellType and TimePoint (or Condition, etc), eg: "Amacrine-EARLY.csv"

**OR:**
**(2) One CSV file containing differential (eg. between test and control samples) fold changes for all the samples**,
which can be downloaded using the 'Optionally: Download your data as single data file' link after selecting your differenital
expression files. This single file can make future loading of data easier, as just need to open one file then.
It's format is, eg:

```
Gene,Amacrine:EARLY,Amacrine:MID,Amacrine:LATE,Bipolar:EARLY,B
ipolar:MID,Bipolar:LATE,Cone:EARLY,Cone:MID,Cone:LATE
Aak1,0,0.6,0,0,-2.1,0,0,0,0.4
Aamp,0,0.3,0,0,-0.6,0,0,0.3,0
Aars,0,0.4,0,0,-0.3,0,1.2,0,0
...etc....
```

where:

- first column is 'Gene' name
- then several columns with heading format: CellType:TimePoint (or CellType:Condition/Location, etc)
- then gene/protein fold-change rows in format: GeneName, Fold-change:Optional p-value:Optional pct.1:Optional pct2, .....

**Multilevel Explain format:'s names**

**Eg Islet**

Can be used for other datatypes

For this vignette we will use a dataset of single cell RNA-Seq from human islet cells from diabetic and non-diabetic individuals [REF] We have

Import data into HIVE

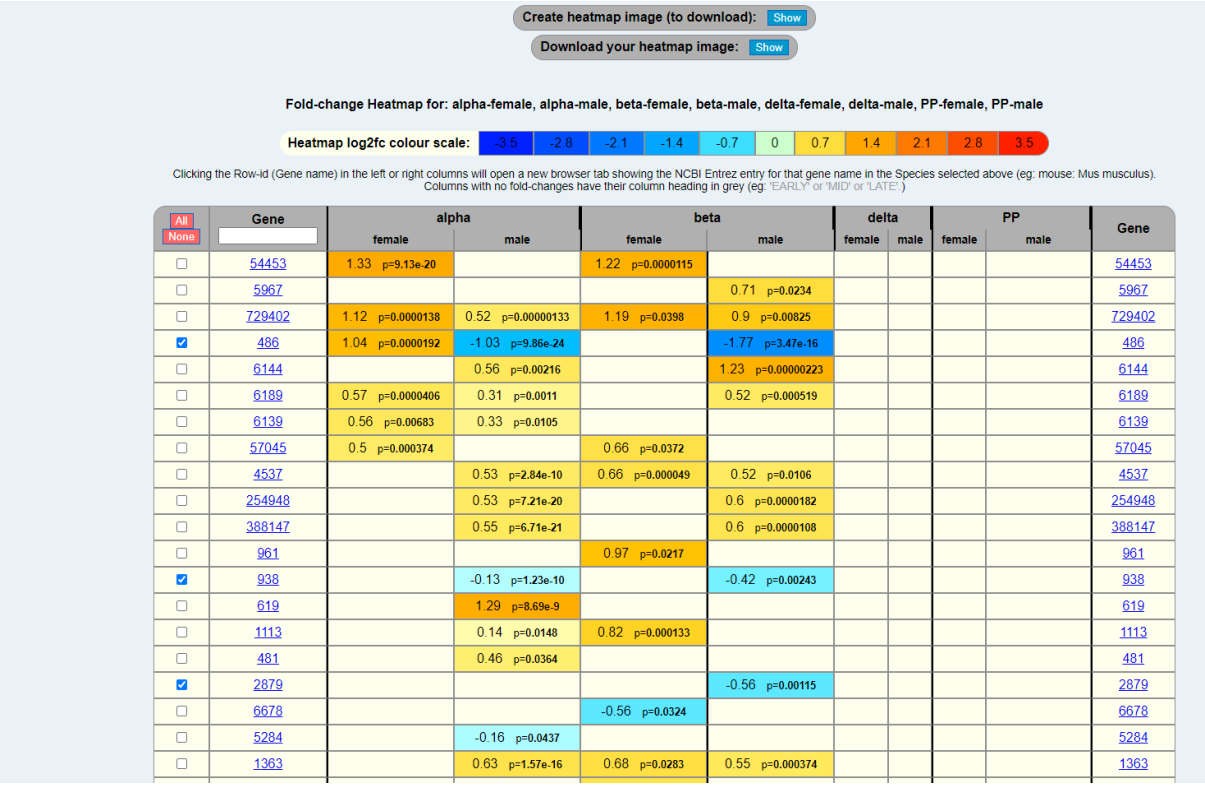
Select genes of interest

For example, genes which are not significantly changed in females but go down in males

Enriched in secretory vesicles

Other way round – genes high in females, uncganged in males - Chromogranin A (in vesicle) and INS-IGF2 readthrough (723961) and RIN2 Ras and Rab interactor 2, a small GTPase involved in membrane trafficking in the early endocytic pathway.

Similarly in alpha cells – genes higher in females (including down in male) enriched in extracellular vesicle



Create heatmap image (to download): Show

Download your heatmap image: Show

**Fold-change Heatmap for: alpha-female, alpha-male, beta-female, beta-male, delta-female, delta-male, PP-female, PP-male**

Heatmap log2fc colour scale: -3.5 -2.8 -2.1 -1.4 -0.7 0 0.7 1.4 2.1 2.8 3.5

Clicking the Row-id (Gene name) in the left or right columns will open a new browser tab showing the NCBI Entrez entry for that gene name in the Species selected above (eg: mouse: Mus musculus).
Columns with no fold-changes have their column heading in grey (eg: 'EARLY' or 'MID' or 'LATE'.)

| All / None | Gene | alpha | | beta | | delta | | PP | | Gene |
|---|---|---|---|---|---|---|---|---|---|---|
| | | female | male | female | male | female | male | female | male | |
| ☐ | 54453 | 1.33 p=9.13e-20 | | 1.22 p=0.0000115 | | | | | | 54453 |
| ☐ | 5967 | | | | 0.71 p=0.0234 | | | | | 5967 |
| ☐ | 729402 | 1.12 p=0.0000138 | 0.52 p=0.00000133 | 1.19 p=0.0398 | 0.9 p=0.00825 | | | | | 729402 |
| ☑ | 486 | 1.04 p=0.0000192 | -1.03 p=9.86e-24 | | -1.77 p=3.47e-16 | | | | | 486 |
| ☐ | 6144 | | 0.56 p=0.00216 | | 1.23 p=0.00000223 | | | | | 6144 |
| ☐ | 6189 | 0.57 p=0.0000406 | 0.31 p=0.0011 | | 0.52 p=0.000519 | | | | | 6189 |
| ☐ | 6139 | 0.56 p=0.00683 | 0.33 p=0.0105 | | | | | | | 6139 |
| ☐ | 57045 | 0.5 p=0.000374 | | 0.66 p=0.0372 | | | | | | 57045 |
| ☐ | 4537 | | 0.53 p=2.84e-10 | 0.66 p=0.000049 | 0.52 p=0.0106 | | | | | 4537 |
| ☐ | 254948 | | 0.53 p=7.21e-20 | | 0.6 p=0.0000182 | | | | | 254948 |
| ☐ | 388147 | | 0.55 p=6.71e-21 | | 0.6 p=0.0000108 | | | | | 388147 |
| ☐ | 961 | | | 0.97 p=0.0217 | | | | | | 961 |
| ☑ | 938 | | -0.13 p=1.23e-10 | | -0.42 p=0.00243 | | | | | 938 |
| ☐ | 619 | | 1.29 p=8.69e-9 | | | | | | | 619 |
| ☐ | 1113 | | 0.14 p=0.0148 | 0.82 p=0.000133 | | | | | | 1113 |
| ☐ | 481 | | 0.46 p=0.0364 | | | | | | | 481 |
| ☑ | 2879 | | | | -0.56 p=0.00115 | | | | | 2879 |
| ☐ | 6678 | | | -0.56 p=0.0324 | | | | | | 6678 |
| ☐ | 5284 | | -0.16 p=0.0437 | | | | | | | 5284 |
| ☐ | 1363 | | 0.63 p=1.57e-16 | 0.68 p=0.0283 | 0.55 p=0.000374 | | | | | 1363 |

G profiler

**Selected genes: 15** Hide

**486, 938, 2879, 1191, 100533181, 2495, 7276, 3326, 2950, 5502, 28958, 100873254, 6319, 5950, 146456**

Select gene(s) using the check-boxes in left column of the heatmap table below.

**Copy selected genes to clipboard:** Show

**G:Profiler:** Hide

1. Select the organism/species (above)
2. Select gene(s) (using the check-boxes in left column of the heatmap table below).

**Organism:** 9606 : Homo_sapiens : hsapiens

Number of genes selected: **15**

Open G:Profiler with this selected gene-list

Redirecting to G:Profiler in a new tab ...

**String DB:** Hide

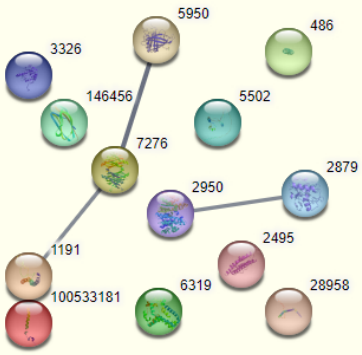
1. Select the 'Organism/Species' from the drop-down menu above.
2. Select gene(s) (using the check-boxes in left column of the heatmap table below).
3. The Stringdb button, Species and Required-score selection will then be enabled (red).
4. For 'Require score' option: lower score includes more interactions.
5. After clicking this "Show Stringdb image" button, if the String image doesn't appear within a couple of seconds, it's probably because the gene(s) is not be in StringDB for the selected Organism/species.
6. Some genes (eg: 'Malat1') are in Human but not in Mouse Stringdb.
7. Click on a gene in the String image to show its abstract.
8. Click the 'STRING' logo to go to the String webpage in a new browser tab (eg. to view the interaction details).

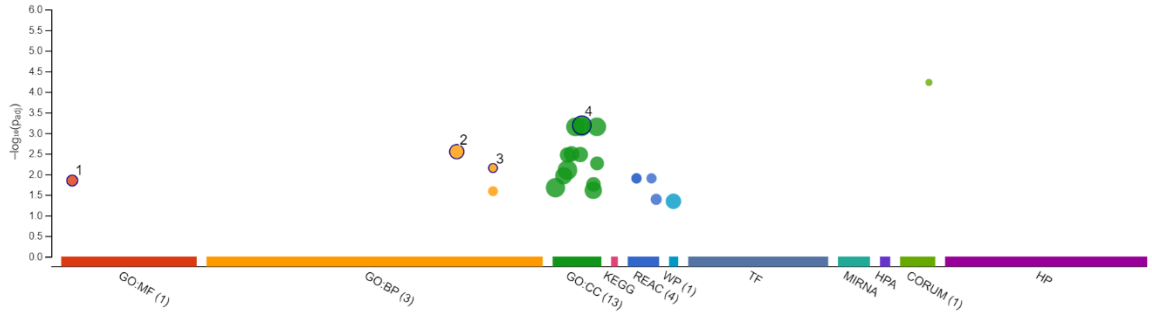
Organism/Species: **9606 : Homo_sapiens : hsapiens**
Number of rows (genes) selected: **15**
Required score: 500 Additional network nodes: 0 Network flavor: Confidence

Show StringDB image here | Show StringDB in new browser tab

post onload(): Retrieved the StringDB link okay.
If your browser doesn't automatically open String in a new tab, then click this liink: https://string-db.org/cgi/link?to=744075EE90BB817B

STRING






| ID | Source | Term ID | Term Name | $p_{adj}$ (query_1) |
|---|---|---|---|---|
| 1 | GO:MF | GO:0004602 | glutathione peroxidase activity | $1.422\times10^{-2}$ |
| 2 | GO:BP | GO:0120254 | olefinic compound metabolic process | $2.830\times10^{-3}$ |
| 3 | GO:BP | GO:1902949 | positive regulation of tau-protein kinase activity | $7.074\times10^{-3}$ |
| 4 | GO:CC | GO:0070062 | extracellular exosome | $6.474\times10^{-4}$ |

| | |
|---|---|
| **version** | e108_eg55_p17_0254fbf |
| **date** | 10/03/2023, 18:15:05 |
| **organism** | hsapiens |

g:Profiler

| GO:CC | | stats | |
|---|---|---|---|
| Term name | Term ID | $p_{adj}$ | $-\log_{10}(p_{adj})$ |
| cytoplasmic vesicle lumen | GO:0060205 | $3.325\times10^{-3}$ | |
| extracellular exosome | GO:0070062 | $6.474\times10^{-4}$ | |
| extracellular membrane-bounded organelle | GO:0065010 | $7.077\times10^{-4}$ | |
| extracellular organelle | GO:0043230 | $7.077\times10^{-4}$ | |
| extracellular space | GO:0005615 | $2.125\times10^{-2}$ | |
| extracellular vesicle | GO:1903561 | $7.052\times10^{-4}$ | |
| ficolin-1-rich granule | GO:0101002 | $1.761\times10^{-2}$ | |
| ficolin-1-rich granule lumen | GO:1904813 | $5.456\times10^{-3}$ | |
| secretory granule | GO:0030141 | $1.087\times10^{-2}$ | |
| secretory granule lumen | GO:0034774 | $3.206\times10^{-3}$ | |
| secretory vesicle | GO:0099503 | $2.468\times10^{-2}$ | |
| vesicle | GO:0031982 | $7.928\times10^{-3}$ | |
| vesicle lumen | GO:0031983 | $3.406\times10^{-3}$ | |

1 to 13 of 13 Page 1 of 1

For STRING set additional network nodes to 0 default (keep option to increase)

When have selected genes and looked at STRING DB make easier to select different genes and rerun String. Way to clear the String input?

**Create heatmap image (to download):** Hide

Rows to plot: As selected by the 'Filter genes' above

- As selected by the 'Filter genes' above
- As chosen by the checkboxes in left column below (so are in the genelist above)
- All rows

Column width: 120 pixels.

Font height: 16 pixels.

Space around text: 5 pixels.

Draw fold-change numbers on the heatmap coloured boxes.

NOTE: This currently ignores the "Show all p-values in rows with at least one p-value under the above p-value filter" setting above and therefore draws cells with p-value above the p-value filter, but I can fix this.

Preview the Heatmap image below

**Create heatmap image (to download):** Hide

Rows to plot: As selected by the 'Filter genes' above

- As selected by the 'Filter genes' above
- As chosen by the checkboxes in left column below (so are in the genelist above)
- All rows

Column width: 120 pixels.

Font height: 16 pixels.

Space around text: 5 pixels.

Draw fold-change numbers on the heatmap coloured boxes.

NOTE: This currently ignores the "Show all p-values in rows with at least one p-value under the above p-value filter" setting above and therefore draws cells with p-value above the p-value filter, but I can fix this.

Preview the Heatmap image below

**Download your heatmap image:** Hide

Select Image file type: PNG = Higher quality (Usually the best option)

- PNG = Higher quality (Usually the best option)
- JPEG/JPG = Larger file size for this heatmap (although is smaler for photos, etc)
- WebP = Newer google (more compact) file for webpages

then: Downl

| Gene | alpha female | alpha male | beta female | beta male | delta female | delta male | PP female | PP male |
|---|---|---|---|---|---|---|---|---|
| 938 | | | | | | | | |
| 2879 | | | | | | | | |
| 1191 | | | | | | | | |
| 100533181 | | | | | | | | |
| 2495 | | | | | | | | |
| 7276 | | | | | | | | |
| 3326 | | | | | | | | |
| 2950 | | | | | | | | |
| 5502 | | | | | | | | |
| 28958 | | | | | | | | |
| 100873254 | | | | | | | | |
| 6319 | | | | | | | | |
| 5950 | | | | | | | | |
| 146456 | | | | | | | | |
| | | | | | | | | |

Analysis by age

In beta cells:

Higher in over 45 – mostly mitochondria tRNA genes

Higher in under 45 – various including ribosomal and INS(!)

Down in under 45 mostly mitochondria tRNA genes

Down >0.5 and significant <0.01 in B cell at either age group: Extracellular vesicles

GEO submission

Retinas were dissected from xxx month old control and Akita mice, cells dissociated [REF] and processed on the 10X Genomics platform (gex v2). Sequence data was processed