Lipid Mini-On Installation and User Manual

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Go to https://omicstools.pnnl.gov/shiny/lipid-mini-on/

A. Using the Query/Universe function:

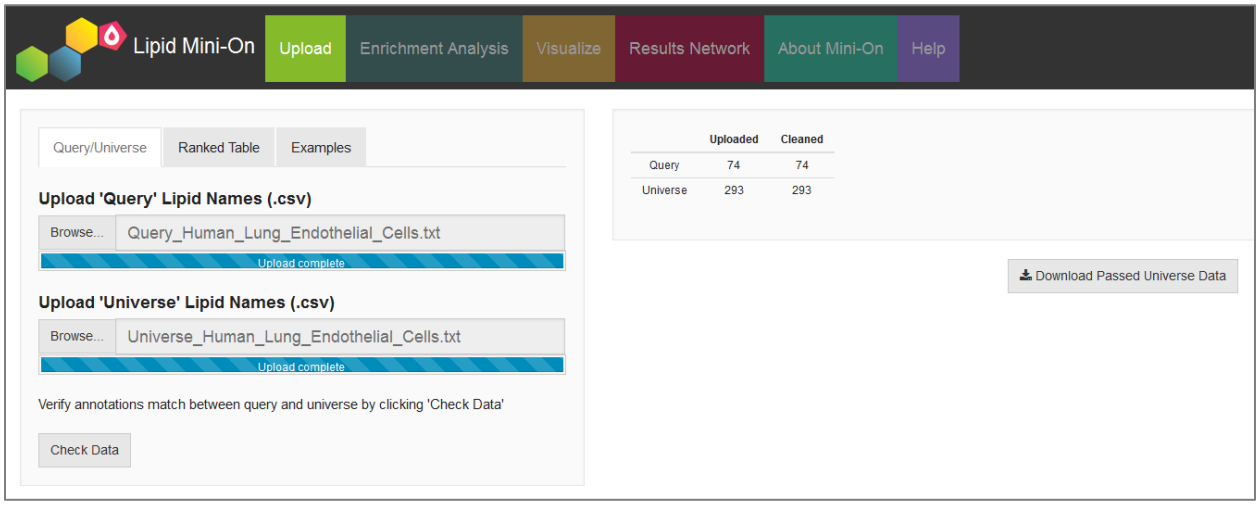
1. File upload

1a. Query/Universe tab: To load query (i.e., list of lipids of interest, such as those found to be statistically significant) and universe (i.e., list of all lipids identified) files ('Upload' tab is the default tab when software opens) use File Upload tab or Text Upload tab. Examples files are provided for reference and for testing the software.

i. Click 'Browse' to upload query file (.csv only)
ii. Click 'Browse' to upload universe file (.csv only)

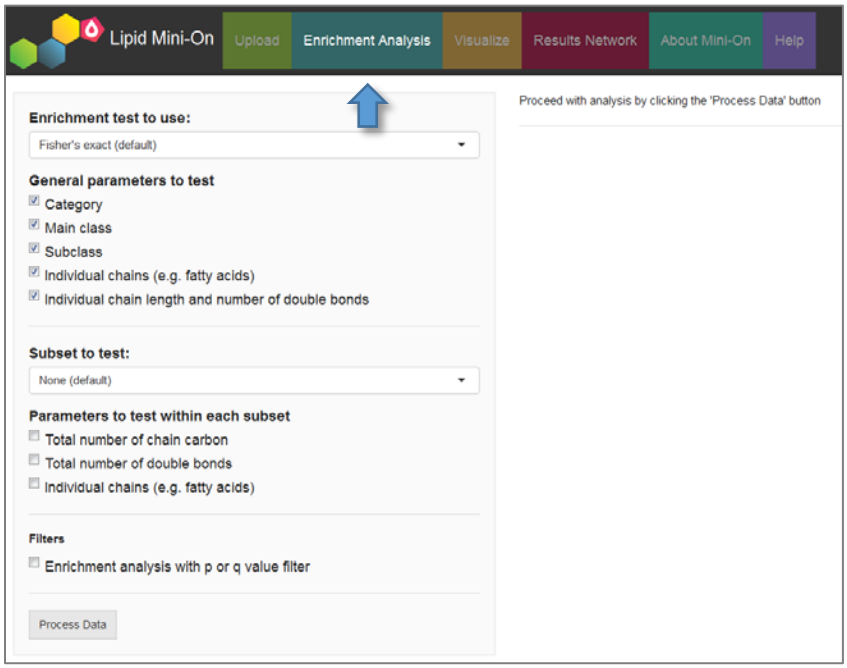
b) Click ‘Check Data’ and wait for confirmation to proceed

- Clicking this button checks that the lipid annotations are present in each file and removes duplicate annotation within the same file as well as other inaccuracies within and between the query and universe files. If an inconsistency is detected an error message will be displayed over the table. The final number of lipids uploaded in each file before and after the checks are listed on the report. These ‘cleaned’ files (after the data checks) can be downloaded after the data has been successfully processed.



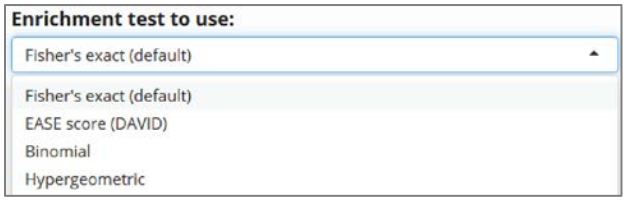
2. Enrichment Analysis

2a. Click ‘Enrichment Analysis’

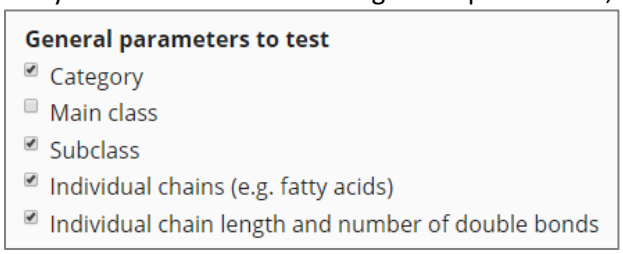


2b. All dropdown menus provide additional options besides the default shown.
Select the 'Enrichment test to use' if you do not want to use the default test (Fisher's exact).

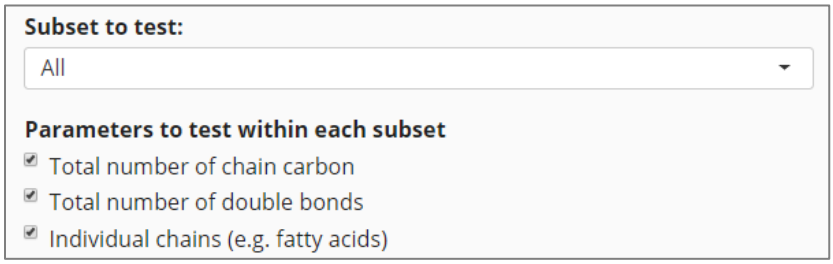
The other options available are the EASE score, Binomial test, and Hypergeometric test.



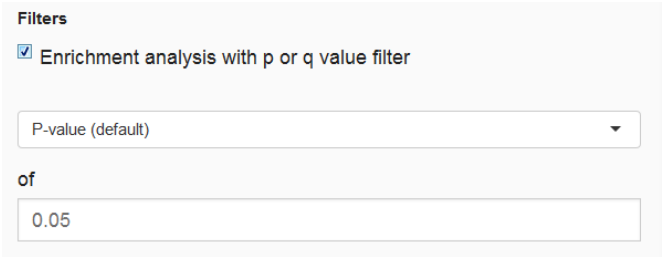
All lipid comparisons are selected by default (checked boxes) so if you do not want to perform an analysis on one or more of the general parameters, uncheck that specific parameter.



2c. If you would like to perform additional analyses within all or within a particular classification (e.g., lipid subclass), click on drop down menu under 'Subset to test' and choose the option you want to select, as well as the parameters within each subset you would like to further analyze (via the check boxes)



2d. Select 'Filters' to highlight results with a p-value(default) or FRD q-value at a user set threshold (p-value < 0.05 is default). P-value adjustment, when selected, is done via the Benjamini-Hochberg method.



2e. Click 'Process Data'

2f. When the analysis is complete (approximately 10 seconds, although this could be slightly longer depending on the size of your lipid lists) a table will be displayed with the number of results in the upper left side of the window. A .txt file will also be generated for download. In the table, the statistically significant (highlighted red in the p-value columns) classification parameters are displayed in the first (Test.performed) and second (Classifier) column. The number of statistically significant lipids with each parameter are shown in columns 'Count.query' with the total number of lipids within that classification from the universe shown in column 'Count.universe'. For example, the first line of results reveals that the lipid category sphingolipids is statistically significant with 18 sphingolipids (with a total of 74 lipids that are classified as a category) in the query out of a total of 37 sphingolipids listed in the universe (with a total of 293 lipids that are classified as a category). The percentage of the result parameter with the query (%.query column) and universe (%.universe column) is also listed. The fold change of enrichment is provided in the last column (Fold.change; green indicates a positive enrichment and bolded indicates it is statistically significant). Each column can be filtered (box above each column; showing 'All' results is the default) and organized (up/down arrows beside the column header).

Fisher output table (12 pvals < 0.05)

Download Results Table

Show 100 entries

Search:

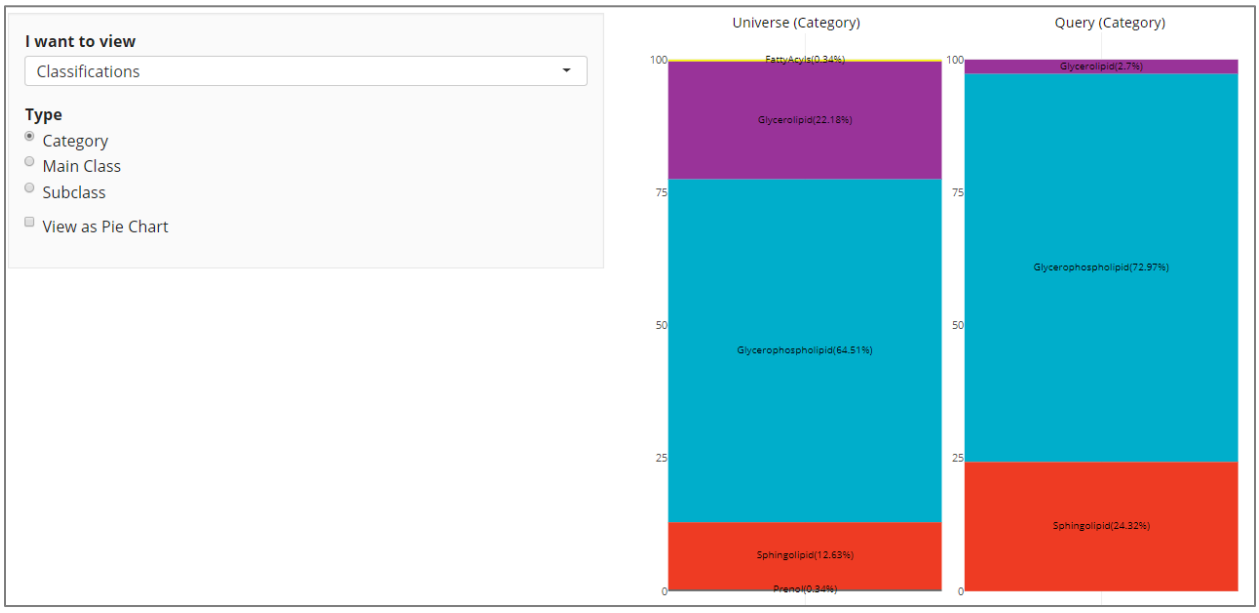
Test.performed	Classifier	Count.query	Count.universe	%.query	%.universe	p-value	FDR.q-value	Fold.change
All	All	All	All	All	All	All	All	All
Category	Sphingolipid	18/74	37/293	24.32	12.63	0.0171	0.0257	**1.93**
Main class	Cer	8/74	8/293	10.81	2.73	**0.0062**	0.0247	**3.96**
Main class	PS	11/74	13/293	14.86	4.44	**0.0029**	0.0235	**3.35**
Sub class	Cer(d	8/74	8/293	10.81	2.73	**0.0062**	0.0370	**3.96**
Sub class	PS(	11/74	13/293	14.86	4.44	**0.0029**	0.0352	**3.35**
Chain characteristics	VLCFA	27/145	61/521	18.62	11.71	0.0371	0.2964	**1.59**
Specific chain	d18:0	5/145	5/521	3.45	0.96	0.0447	0.5269	**3.59**
Total chain carbon by all	Glycerophospholipid with a total number of chain carbon of 38	24/54	47/189	44.44	24.87	**0.0068**	0.0610	**1.79**
Total chain carbon by all	Glycerophospholipid with a total number of chain carbon of 40	14/54	23/189	25.93	12.17	0.0181	0.0814	**2.13**
Total chain carbon by all	PC with a total number of chain carbon of 38	7/18	12/74	38.89	16.22	0.0496	0.3475	**2.40**
Specific chains by all	PI with the chain 20:4	3/6	5/42	50.00	11.90	0.0497	0.1492	**4.20**
Specific chains by all	PI(with the chain 20:4	3/6	5/42	50.00	11.90	0.0497	0.1492	**4.20**

Showing 1 to 12 of 12 entries

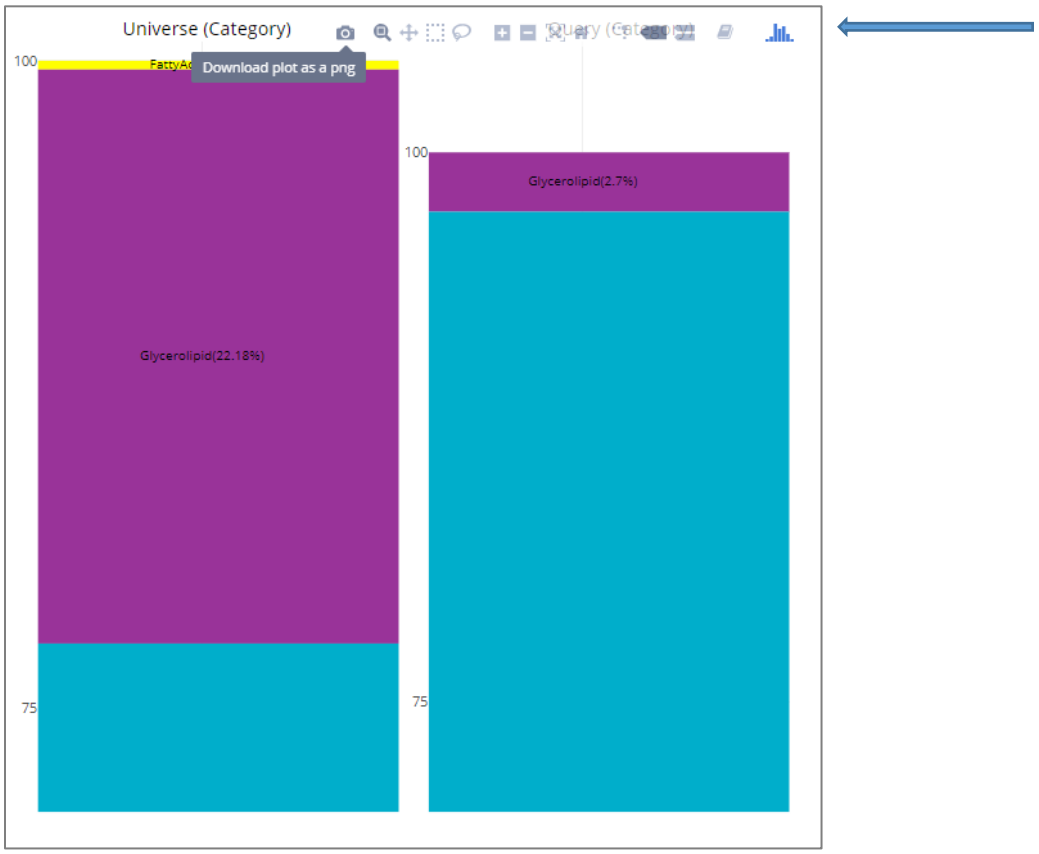
Previous 1 Next

3. Visualize

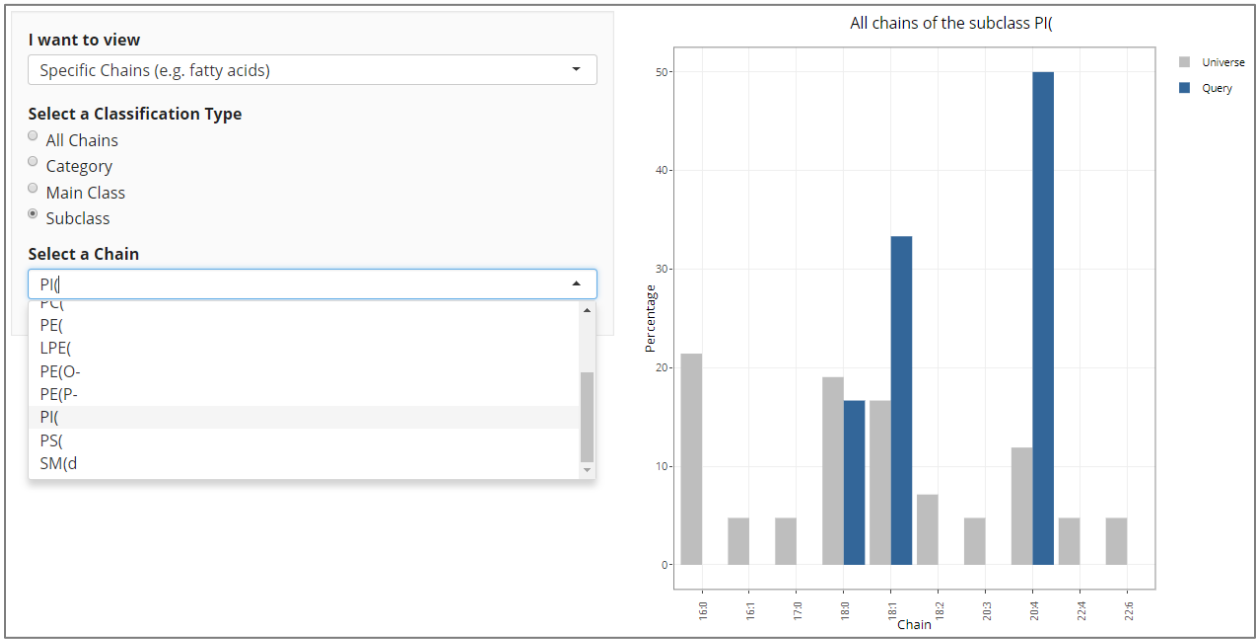
3a. Click on the 'Visualize' tab for bar and / pie graph representation of the enrichment results.



3b. To adjust (e.g., zoom in) and download the figures, hover the mouse over the graphs at which time the options bar graph will appear above the figure (arrow). Below is an example of zooming into the figure. Double click on the figure to zoom back out.

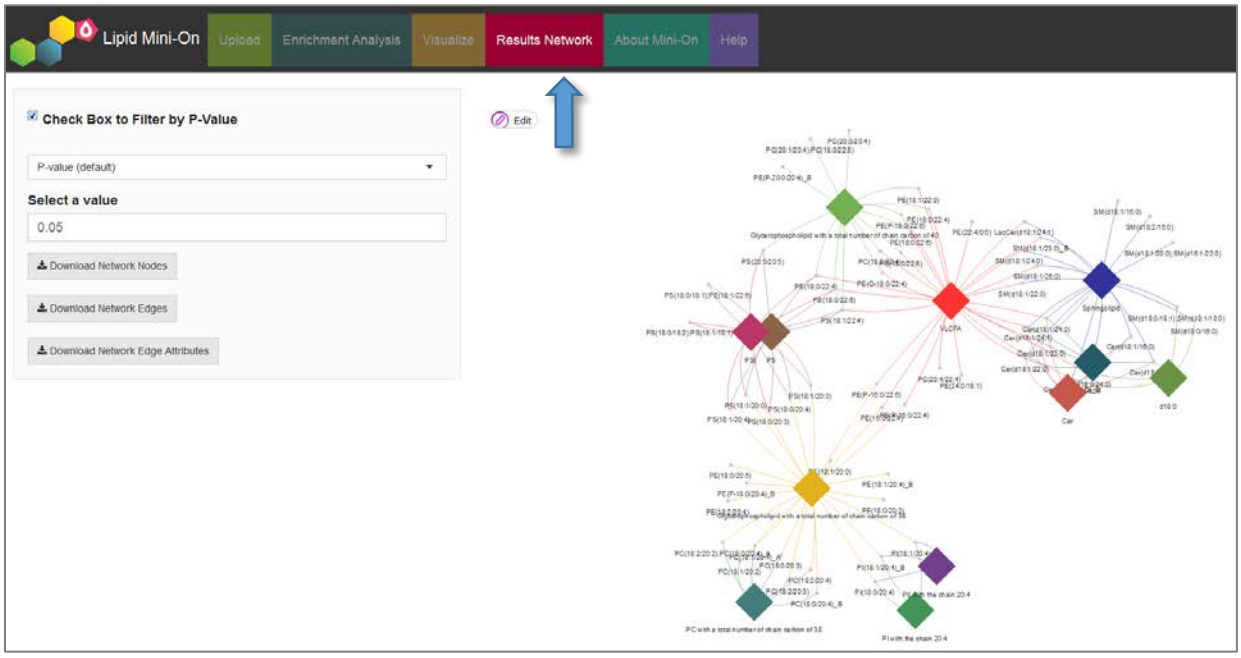


3c. Click on the drop down menu to change the information presented. Depending which parameter you want to visualize, the subset options (e.g., category, subclass) will alter accordingly. Here is an example of viewing results of 'specific chains' then 'subclass' and the subclass 'PI'. Hovering the cursor over the bars in the graph will provide additional information.

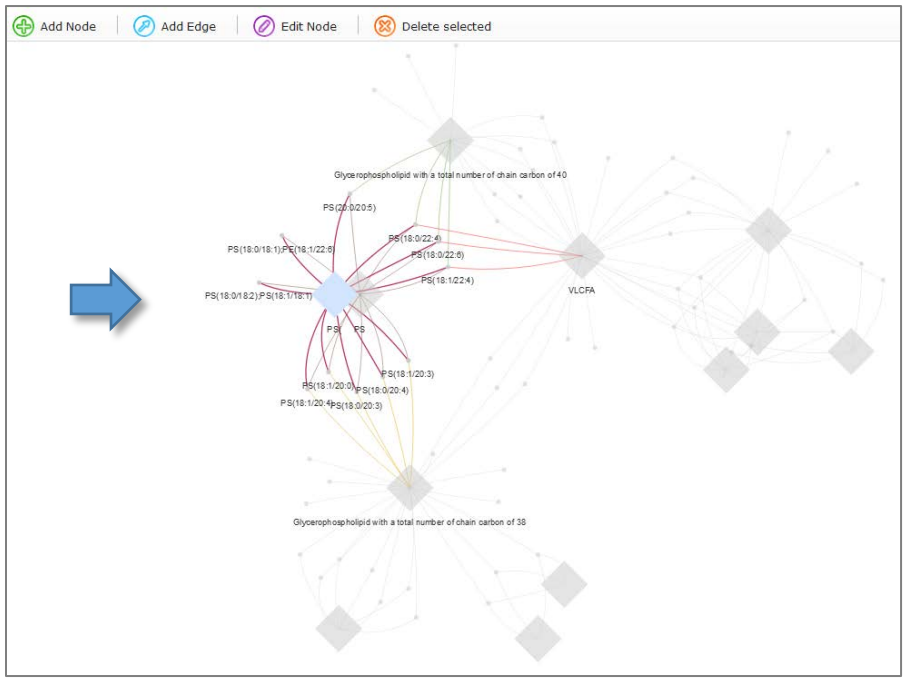


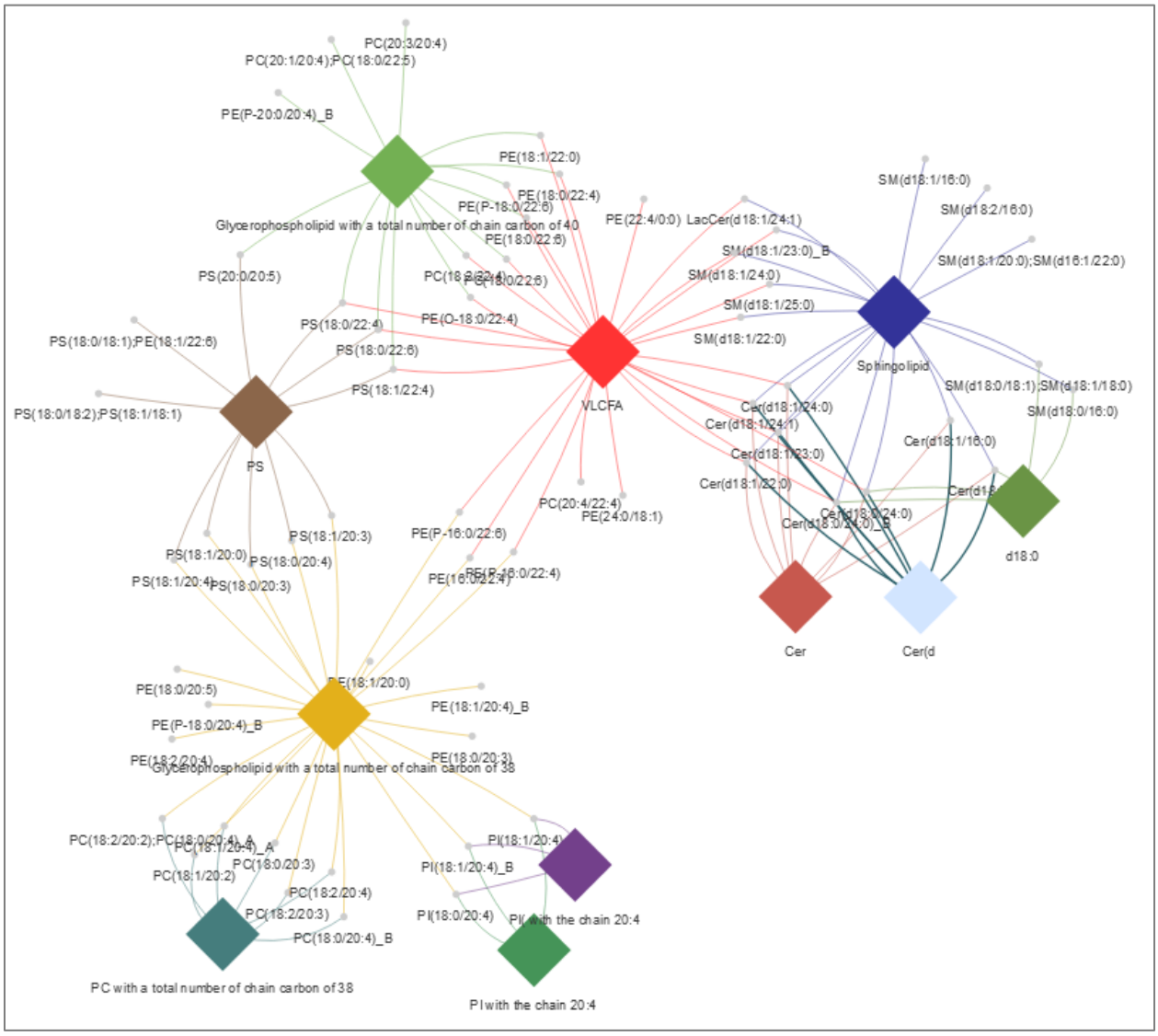
4. Results Network

4a. Click on the 'Results Network' tab to transfer the enrichments into a network figure. Downloadable network nodes, edges, and edge attributes are also generated and are readily usable for representation in other network representation software (e.g. Cytoscape).



4b. To edit the network, click on 'edit' above the network then click on the node you want to remove. For the example below, you can remove the main class 'PS' node as the subclass PS(contains all of the same lipids. Removing the main class does not break or change the network. This visualization enables you to focus on the connections between statistically significant parameters.



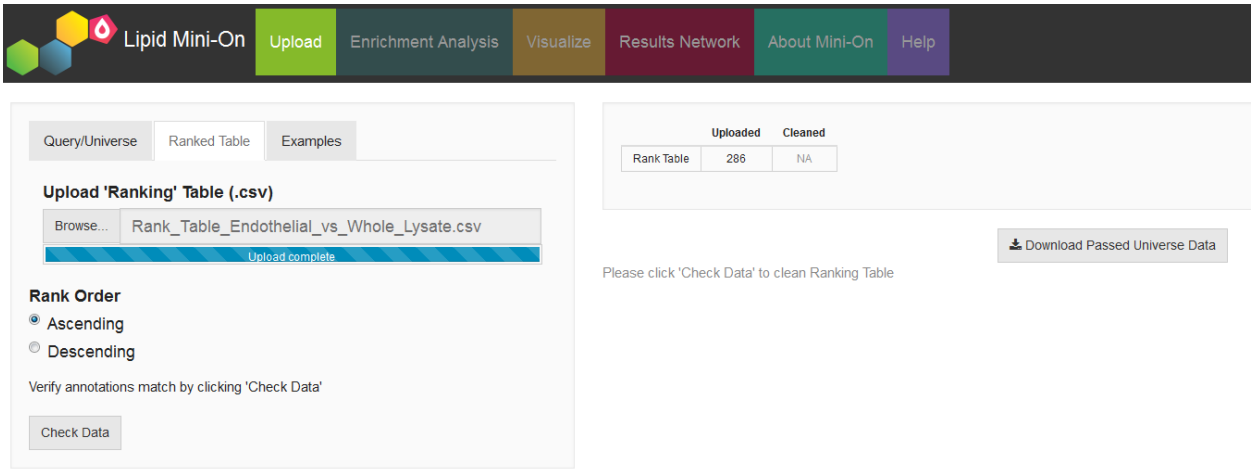


4c. To regenerate the original network map, if edited, redo the enrichment analysis then click back to the 'Results Network' tab.

B. Using the Ranked Table:

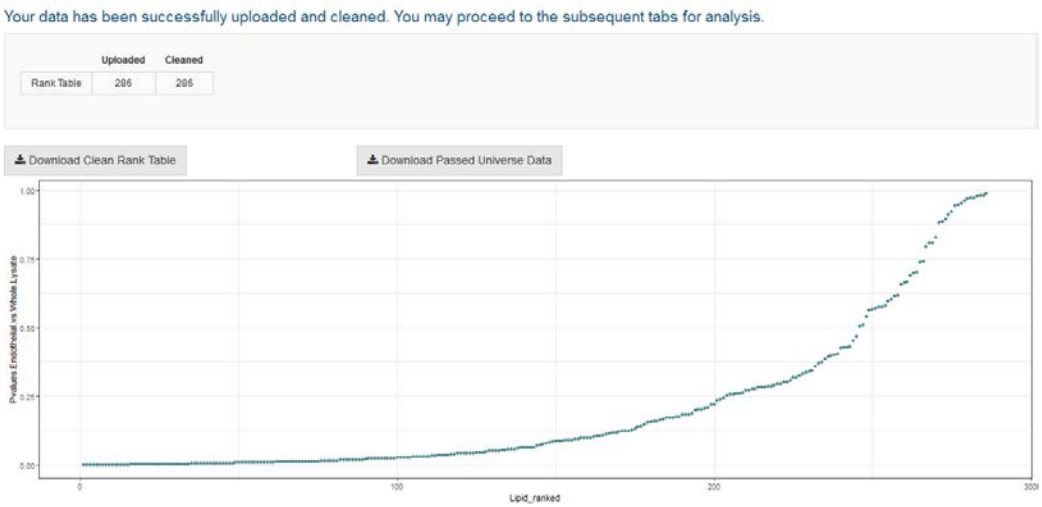
1. File upload

1a. Ranked Table tab: Click on 'Browse' and upload the file. Examples files are provided for reference and for testing the software. Choose 'Rank Order' (ascending or descending).



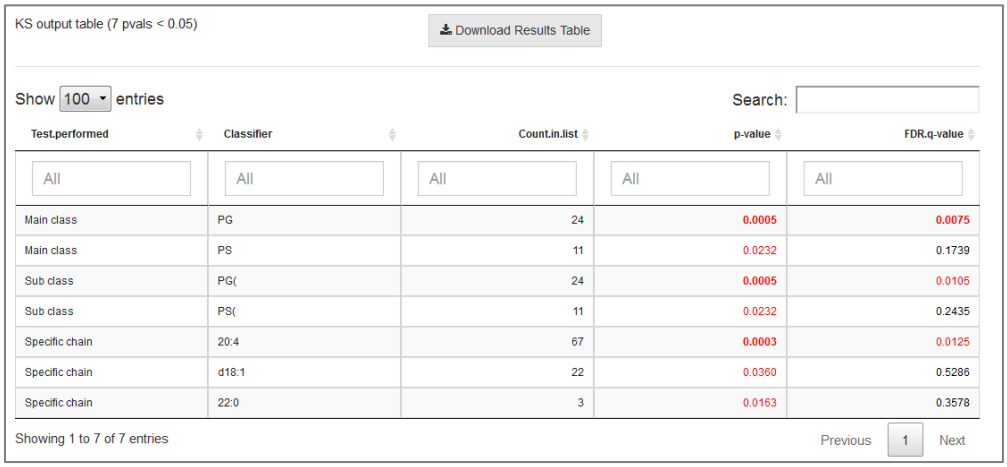
b) Click 'Check Data' and wait for confirmation to proceed

- Clicking this button checks that the lipid annotations acceptable and removes duplicate annotation within the same file as well as other inaccuracies. If an inconsistency is detected an error message will be displayed over the table. The final number of lipids uploaded in each file before and after the checks are listed on the report. These 'cleaned' files (after the data checks) can be downloaded after the data has been successfully processed. The 'Passed Universe Data' can also be downloaded.



2. Enrichment Analysis

2. Click ‘Enrichment Analysis to use’. The Weighted Kolomogorov-Smirnov Test is the only option. All remaining options are the same as displayed above in (A) Query/Universe Function ‘Enrichment Analysis’ section. Please see above section for details.


KS output table (7 pvals < 0.05)

Download Results Table

Show 100 entries

Search:

Test.performed	Classifier	Count.in.list	p-value	FDR.q-value
All	All	All	All	All
Main class	PG	24	0.0005	0.0075
Main class	PS	11	0.0232	0.1739
Sub class	PG(	24	0.0005	0.0105
Sub class	PS(	11	0.0232	0.2435
Specific chain	20:4	67	0.0003	0.0125
Specific chain	d18:1	22	0.0360	0.5286
Specific chain	22:0	3	0.0163	0.3578

Showing 1 to 7 of 7 entries

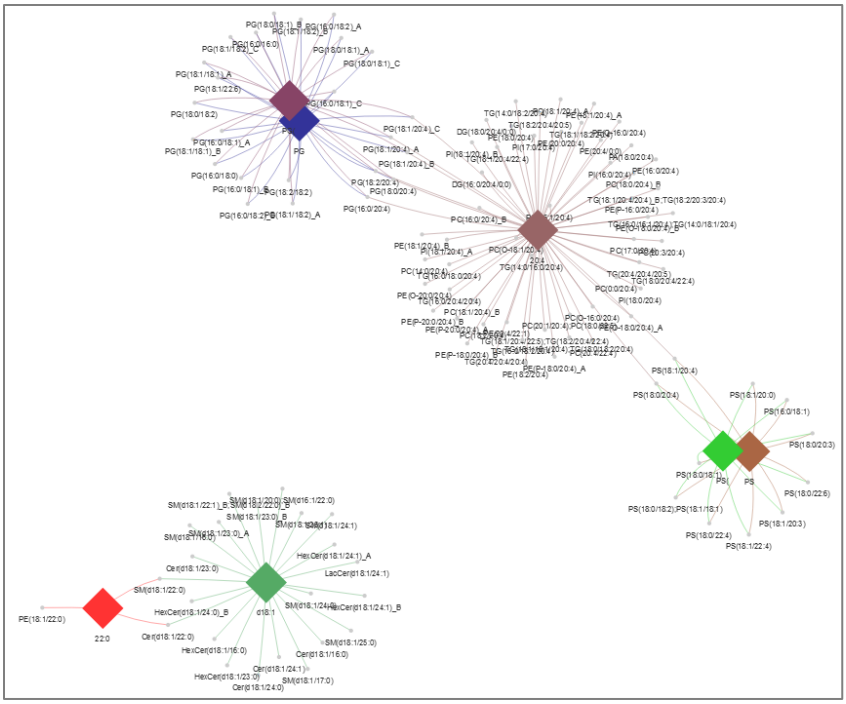
Previous 1 Next

3. Visualize

3. Click on the ‘Visualize’ tab for bar and / pie graph representation of the enrichment results. All remaining options are the same as displayed above in (A) Query/Universe Function ‘Visualize’ section. Please see above section for details.

4. Results Network

4. Click on the ‘Results Network’ tab to transfer the enrichments into a network figure. Downloadable network nodes, edges, and edge attributes are also generated and are readily usable for representation in other network representation software (e.g. Cytoscape). All options are the same as displayed above in (A) Query/Universe Function ‘Results Network’ section. Please see above section for details.


PG(18:0/18:1)_B
PG(16:0/18:2)_A
PG(18:1/18:2)_B
PG(16:0/16:0)
PG(18:1/18:2)_C
PG(18:0/18:1)_A
PG(16:0/18:1)_C
PG(18:1/18:1)_A
PG(18:1/22:6)
PG(16:0/18:1)_C
PG(18:0/18:2)
PG
PG(18:1/20:4)_C
PG(16:0/18:1)_A
PG(18:1/18:1)_B
PG(18:1/20:4)_A
PG(18:1/20:4)_B
PG(16:0/18:0)
PG(16:0/18:1)_B
PG(18:2/18:2)
PG(18:2/20:4)
PG(18:0/20:4)
PG(16:0/18:2)_B
PG(18:1/18:2)_A
PG(16:0/20:4)
TG(14:0/18:2/20:4)
TG(18:2/20:4/20:5)
DG(18:0/20:4/0:0)
PE(18:1/20:4)_A
PE(18:0/20:4)
TG(18:1/18:2/20:4)
PE(16:0/20:4)
PI(17:0/18:1)
PE(20:4/20:4)
PI(18:1/20:4)_B
TG(16:1/20:4/22:4)
PI(18:0/20:4)
PE(16:0/20:4)
PI(16:0/20:4)
PG(18:0/20:4)_B
DG(16:0/20:4/0:0)
TG(18:1/20:4/20:4)_B
TG(18:2/20:3/20:4)
PE(P-16:0/20:4)
PG(16:0/20:4)_B
TG(20:5/18:1/20:4)
TG(14:0/18:1/20:4)
PE(O-16:0/22:4)_B
PE(18:1/20:4)_B
PE(18:1/20:4)_A
PC(O-16:1/20:4)
P-C(17:0/20:4)
TG(14:0/16:0/20:4)
PC(18:1/20:4)
TG(16:0/18:0/20:4)
TG(20:4/20:4/20:5)
TG(18:0/20:4/22:4)
PC(O-20:0/20:4)
PG(0:0/20:4)
TG(16:0/20:4:0/20:4)
PI(18:0/20:4)
PC(18:1/20:4)_B
PE(P-20:0/20:4)_B
PDG-16:0/20:4)
PE(P-2:0/20:4)_A
PC(O-18:0/20:4)_A
PC(20:1/20:4)
PE(P-18:0/20:4)_A
TG(20:4/22:4/22:4)
PE(P-18:0/20:4)_B
PE(18:2/20:4)
PS(18:1/20:4)
PS(18:0/20:4)
PS(18:1/20:0)
PS(16:0/18:1)
PS(18:0/20:3)
PS(18:0/18:1)
PS
PS(18:0/22:6)
PS(18:0/18:2)
PS(18:1/18:1)
PS(18:1/20:3)
PS(18:0/22:4)
PS(18:1/22:4)
SM(d18:1/22:1)_B
SM(d18:1/20:0)
SM(d16:1/22:0)
SM(d18:1/23:0)_B
SM(d18:1/24:1)
SM(d18:1/23:0)_A
SM(d18:1/16:0)
HexCer(d18:1/24:1)_A
Cer(d18:1/23:0)
LacCer(d18:1/24:1)
SM(d18:1/22:0)
PE(18:1/22:0)
HexCer(d18:1/24:0)_B
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SM(d18:1/24:0)
HexCer(d18:1/24:1)_B
22:0
Cer(d18:1/22:0)
SM(d18:1/25:0)
HexCer(d18:1/16:0)
Cer(d18:1/16:0)
Cer(d18:1/24:1)
HexCer(d18:1/23:0)
SM(d18:1/17:0)
Cer(d18:1/24:0)