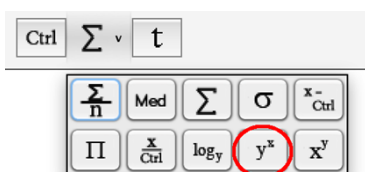


## Drug repurposing based in the functional context


You can find the sample files for this tutorial in the Downloads. We strongly recommend you to start with the “Data Import and Processing” and “NetWalk and EF Tables” tutorials first.

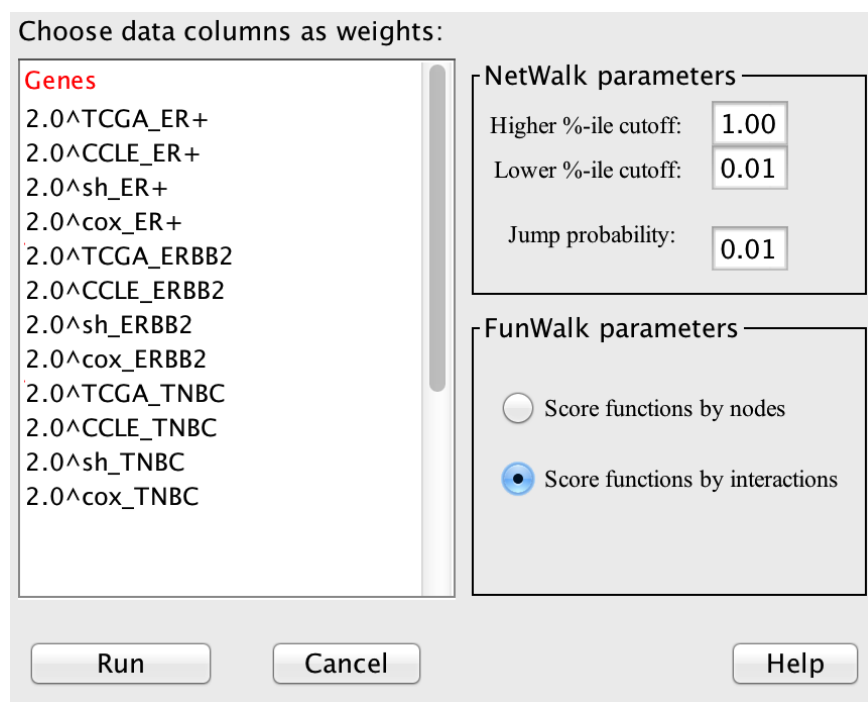
- 1.- Start NetWalker and import sample dataset according to the 1 and 2 steps of the “Data Import and Processing” tutorial.
- 2.- Select the 12 columns and take log<sub>2</sub>



Then, click OK and you will have log<sub>2</sub>-transformed row data. You should have 26 total columns now: 1 column of row names, 1 column of rows type, 12 columns of original data, and 12 columns of log<sub>2</sub> transformed values. You can remove the 12 columns of original raw untransformed data now. Retain the log<sub>2</sub> columns, as these will be used later for NetWalk analyses.

- 3.- Select the Names column by clicking on  button to set this column as gene names.

- 4.- Click “ Run NetWalk” button to display the Run NetWalk dialog.




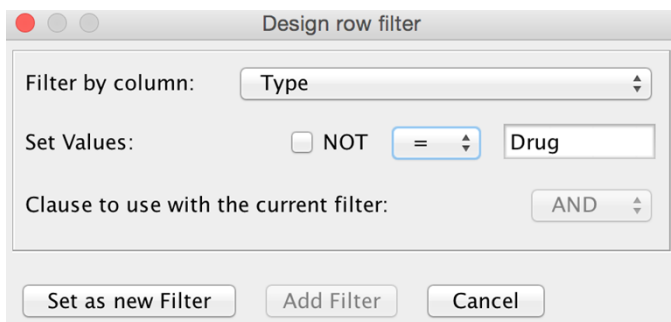
The list on the left shows data columns of the TableView. These can be selected to use as weights for NetWalk analysis, and a separate NetWalk will be run for each selected data column to generate EF Table. The data columns to be used, as weights for NetWalk should be ratio-like values centered around 1, and should not contain negative values. Those data columns unsuitable for NetWalk analysis are colored red in the Run NetWalk dialog. Values to the right show cutoff percentages for data in the data columns to use as higher and lower cutoff of weights for NetWalk (see Manual for details). In most cases, these should not be modified. While EF Tables have nodes and interactions in each row and EF values as their scores, respectively, FunTable contains functional terms (GO Terms) in each row of the table and their FunWalk scores in the rest of columns (see “FunWalk and FunTables” tutorial).

5.- Select the 12 columns for Ratio values (those whose names start with “2.0” number). Click “Run” button to execute NetWalk analysis. Click OK again when prompted. Wait till NetWalk run finishes, it may take a while.


6.- After several seconds, three new TableView windows will be created, one containing EF values for each node, a EF Table for interactions and the other containing FunTable containing functional terms (GO Terms) in each row of the table and their FunWalk scores for the 12 columns in the sample dataset.


An EF Table can be processed just like a primary dataset (see Dataset import and processing tutorial). For example, we will show how to create Heatmaps for comparative analyses of drugs between breast cancer subtypes. First, we will filter the NV Table to only show rows that correspond to drugs.


7.- For this, we will use the “Filter rows” feature in TableView (note that this feature is present in all TableView windows, whether they contain primary datasets). Click on “ Filter rows” button, this will bring up the dialog for designing row filters.

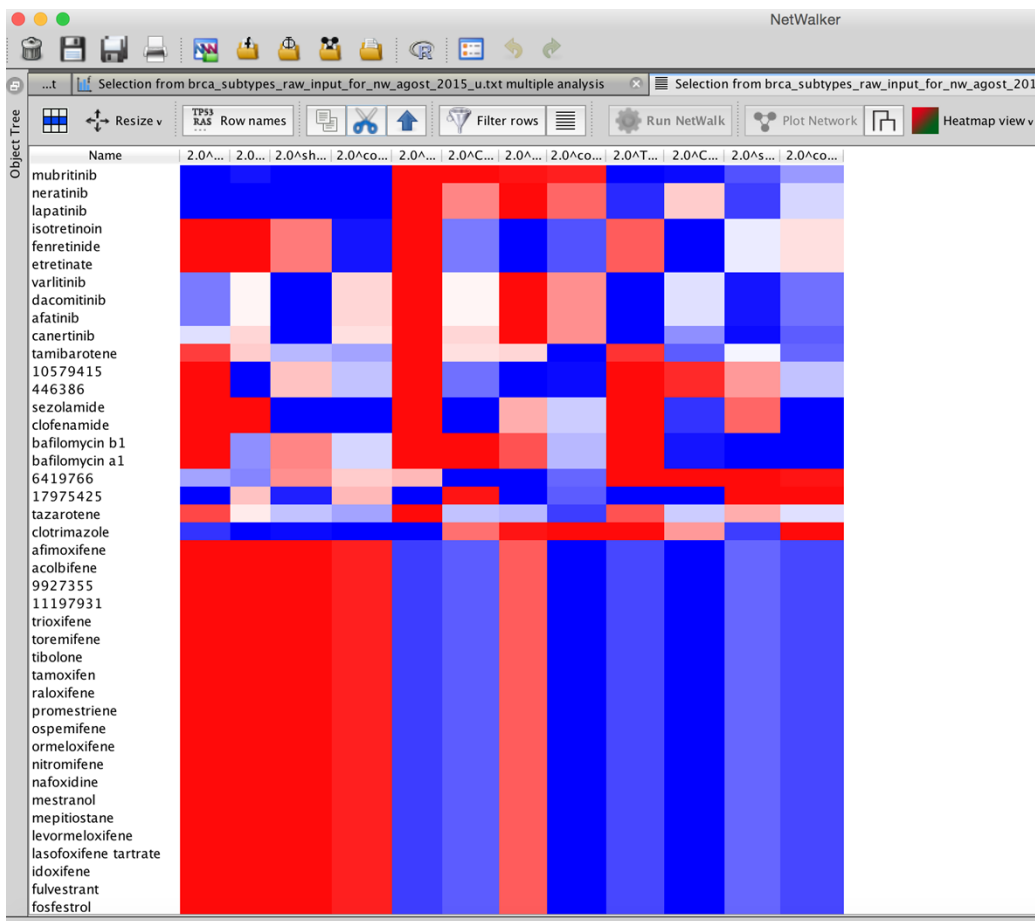


The drop-down menu on top (next to “Filter by column:”) is to select the column according to the values of which rows will be sorted. Select the column for “Type”. You will see the drop-down box next to “Set Values” get activated. Select “=”, and enter “Drug” in the text field next to it. Press “Set as new Filter” button. Now, only rows where “Type: nodes” column is a Drug are shown.

8.- Select all the columns (14 total) of filtered NV table, and click on  to make a new table for a clustering heatmap. Note that each time we want to make a clustering heatmap, we generate a new table of rows and columns that we wish to cluster. This is important, as the original table, though only showing the desired rows and columns, still contains the whole data table, therefore they are not suitable for clustering. Clustering analysis should only be done on an unfiltered dataset.

9.- Select all columns (you should only have node visitation probabilities columns now as data columns) except for the Names and Type columns. Click on the heatmap button () and select blue-red coloring. You can manually enter data range for color mapping here (by default: [-1,1], meaning that all data  $<-1$  will be colored as brightest blue, and all values  $>1$  will be colored by brightest red, intermediate values will be colored according to the gradient). You can adjust column widths by moving the ends of the column as we do in a excel table to make the view prettier.

10.- Next, click on “cluster” button () and select “Re-order rows”. Click OK.



This is how the clustered heatmap view will look like.

11.- Now you have a clustered heatmap of drugs in the context of different datasets for molecular subtypes of breast cancer. If you resize all rows so that you can see the whole heatmap, you will notice that there are subtype-specific drugs.

It is worth noting that this platform is aimed to enable to experimental users to prioritize drugs or targets from genome-wide data (RNAseq, shRNA, siRNA, etc) and drug screens, respectively.

For example, the user can use a table (matrix) from a set of in-house experiments (genomics or drug screens) or can start by using a GEO or Pubchem database-derived file, processing and analyze it according to the “Data Import and Processing” tutorial. Thus, the user can gain insights on candidate drugs or targets for a condition specific disease, respectively.