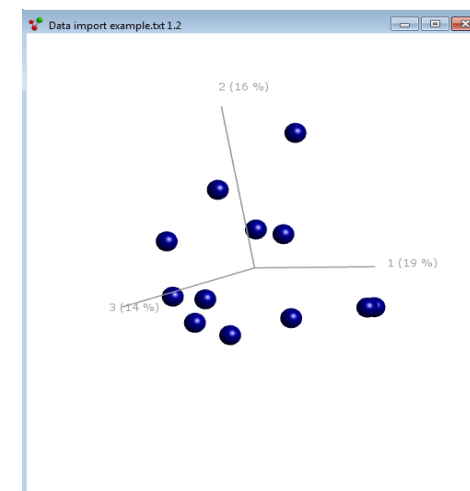
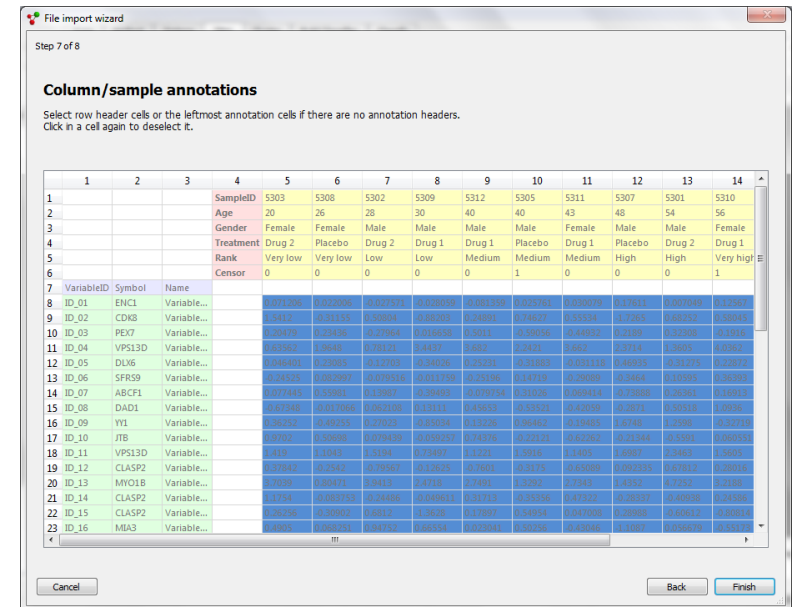


Exercises

1. Import data with Wizard
2. User interface
3. Heatmap
4. ANOVA (F-test) – Export Variable list
5. t-test – Work with synchronized plots
6. Templates
7. Box plot, Volcano plot
8. Import and analyze GEO data
9. Exploratory Data Analysis
10. t-SNE plot

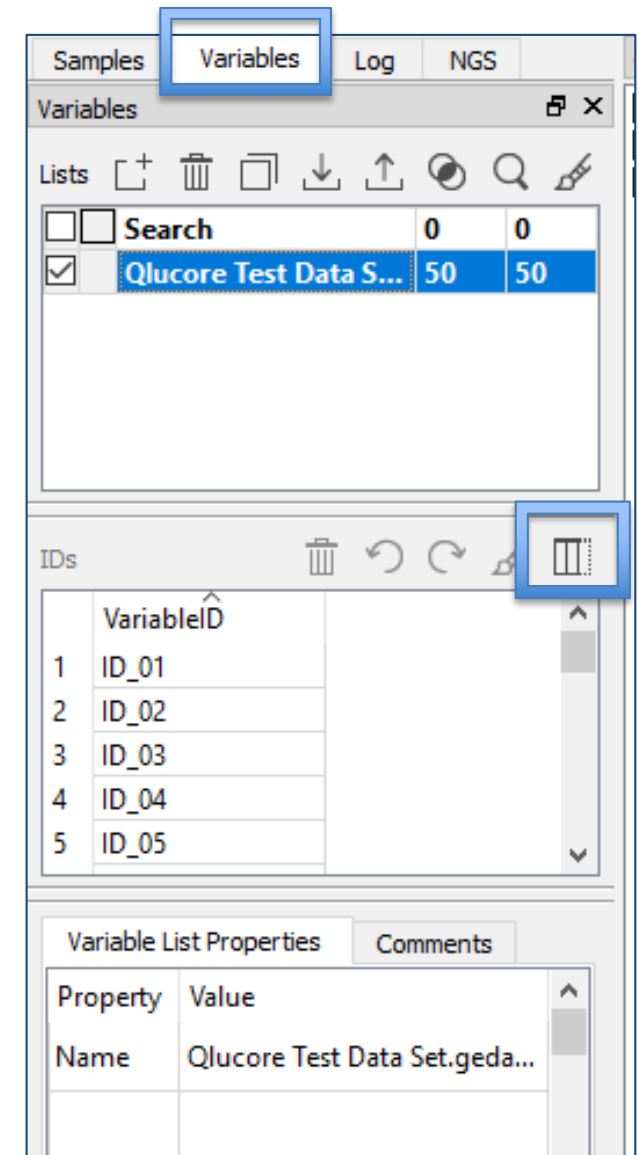
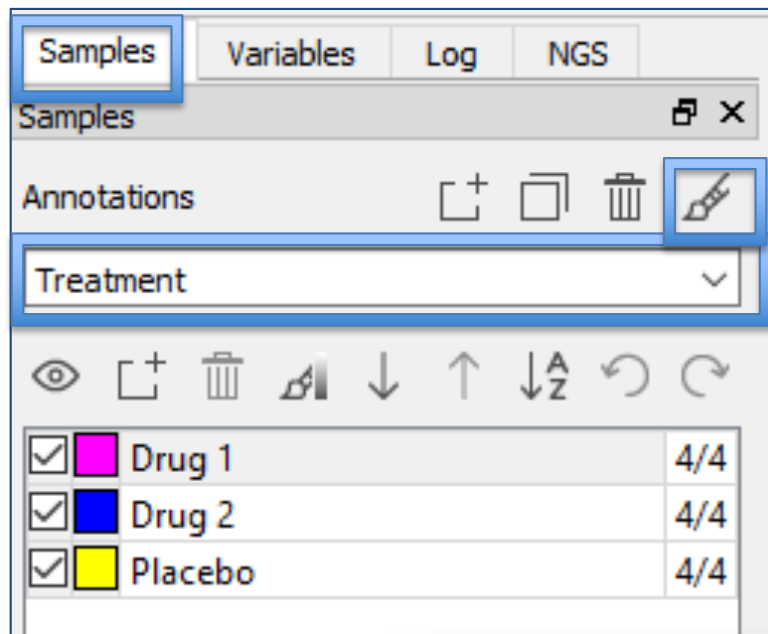
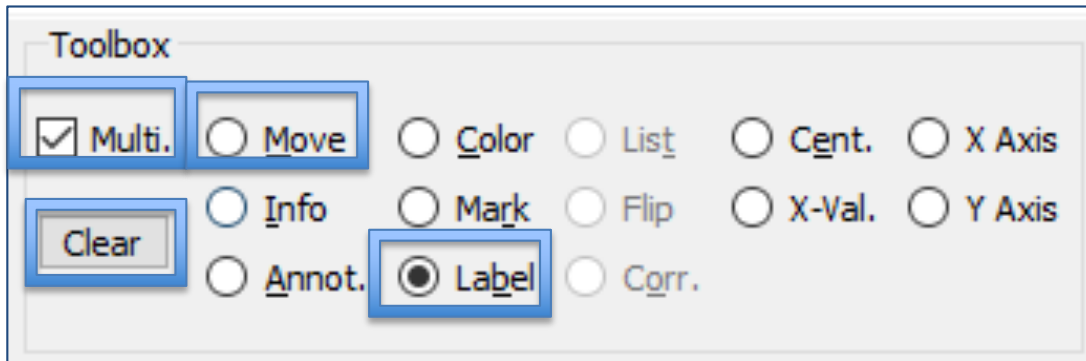
Exercise 1 – Import data with the Wizard

1. Select File/Open Wizard
2. Select the file Data Import example.txt
3. Select Wizard
4. Step through the Wizard
5. The dataset is loaded and a Sample PCA plot is displayed
6. Select Method/Table to view the imported table of values.
7. Save the data set as a gedata-file (File/Save as) on your Desktop
8. Close the dataset



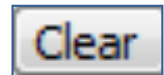
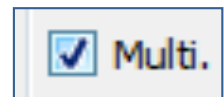
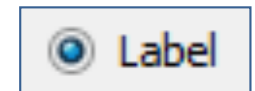
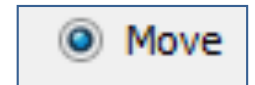
	p-values	q-values	5301	5302	5303	5304	5305	5306	5307	5308	5309	5310
ID 01			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 02			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 03			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 04			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 05			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 06			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 07			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 08			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 09			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 10			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 11			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 12			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 13			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 14			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 15			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 16			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 17			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 18			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 19			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 20			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 21			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 22			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 23			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 24			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 25			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 26			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
ID 27			0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000

Exercise 2 Uer Interface: Tools





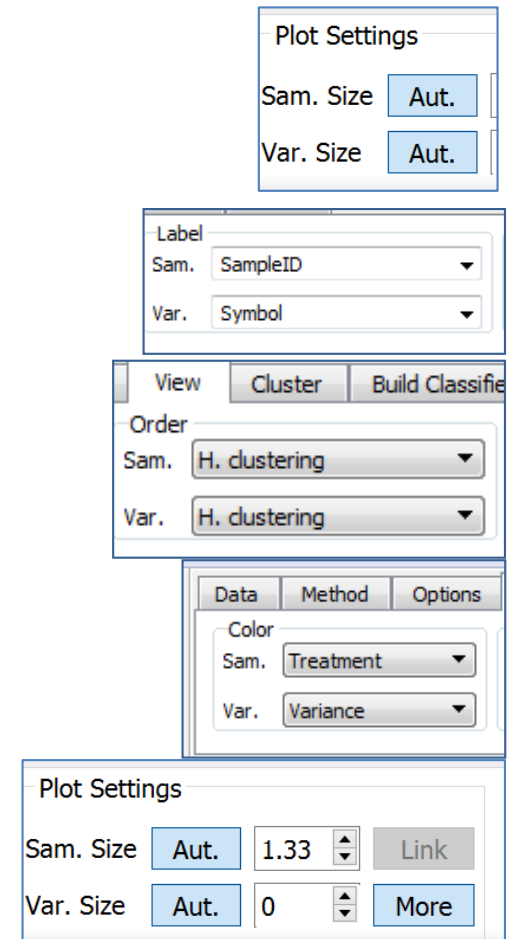
Exercise 2: Tools

1. Open the Data Import Example gedata-file that you saved. *(Either by doubleclicking on the file or by File/Open)*
2. Select Mouse tool Move (default). Rotate the PCA plot with the mouse to look at it from different angles.
3. Select mouse tool Label and label one/several samples by clicking on a sample/ circling several samples with the mouse (clockwise) (Note that the Multi check box in the upper left corner must be marked)
4. Undo by pressing Clear and reset the Mouse tool to Move
5. Go to the Samples tab and colour the samples by Age, Gender and Treatment pressing the colour icon
6. See how the samples seem to cluster based on Treatment in the Sample PCA plot
7. Go to the Variables tab.
8. Click on the active list and see the detailed list of the variables below.
9. Add the column Symbol to the list using the column selector symbol
10. Close the dataset



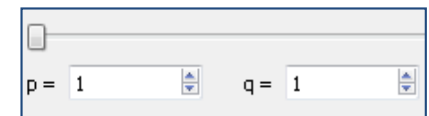
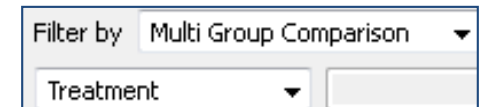
Exercise 3 Heatmap: Steps

1. Open the Data Import Example gedata-file
2. Select Heatmap in the Method tab
3. Select the View Tab and press Size/AUTO to show all samples and variables
4. Then change the variable label to Symbol (Label/Var)
5. Go to the View Tab and change Order to Hierarchical clustering for both the samples and the variables
6. Color the samples according to the Treatment annotation.
7. Color by additional annotations e.g. Age and Gender  (View/Colour/Sam/Additional annotations)
8. Colour the variables by Variance (View/Colour/Var)
9. Use the Flip Mouse tool to flip the clusters 
10. Change the heatmap colours in View/Plot Settings/More
11. Zoom in the variables by using the arrows in View/Plot Settings. Reset by pressing Auto.
12. Export the plot (File/Export/Image)
13. Open the exported file
14. Close the file and the dataset



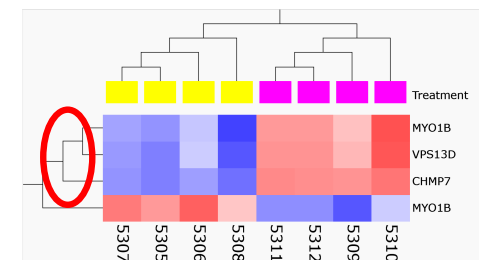
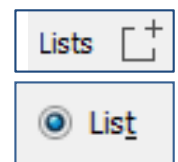
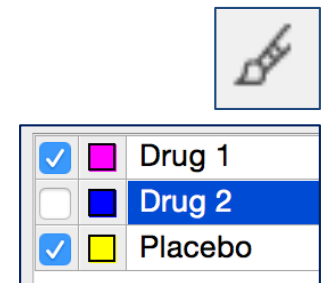
Exercise 4 ANOVA: Steps

1. Open the Data Import Example gedata-file and select Heat map in the Methods tab.
2. Colour according to Treatment and perform hierarchical clustering
3. Select Multi group comparison in the Statistics dialogue.
4. Select the annotation Treatment.
5. Drag the p-value slider in the Statistics dialog. Monitor the p-values and q-values of the remaining variables. Note that the heatmap is instantly updated
6. Create a list of the variables by making a copy of the active variable list (Data Import Example) in the Variables tab.
7. Double click on the list to rename it.
8. Add more information to the list by clicking on the column selector icon and select symbol. Add p-value and q-value.
9. Add a comment. (*Mark the list and select the comment tab in the bottom part of the Window*)
10. Export the list to your computer (select the Export icon) (include annotations)
11. Open the list and look at the content.



Exercise 5 t-test: Steps

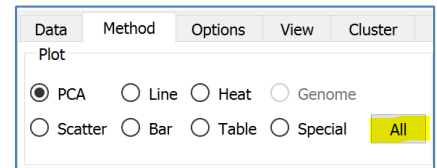
1. Select the Qlucore Test Data Set from the Help Menu/Example Files
2. Select Window/New synchronized plot and Tile the Plots using CTRL+T or Cmd+T
3. Colour the the Sample PCA plot according to Treatment
4. Make the other plot a heat map, hierarchically clustered and coloured according to Treatment
5. Deselect Drug 2 under Treatment annotation in the sample tab - uncheck the check box.
6. Select Two Group comparison in the Statistics dialogue and Treatment = Drug 1
7. Drag the statistical slider to find discriminating variables, stop at a q-value = 0.01
8. Note that the same clusters can be viewed in both the sample PCA and the heatmap
9. Create New Variable list by clicking on the icon in the variables tab.
10. Change the Mouse tool to List
11. Select the upregulated genes for the Drug 1 group with the list tool (select the node in the heatmap)
12. Rename the list and export it.
13. Go to the log tab and print a pdf report summary



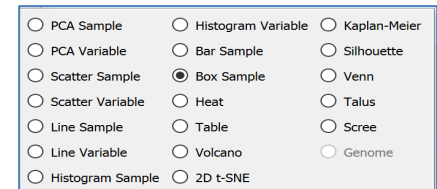
Extra Exercise – make a t-test of Placebo vs both the treated groups

Exercise 6 Box Plot: Steps

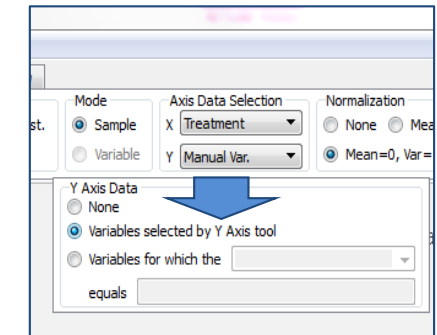
1. Select the Qlucore Test Data Set from the Help Menu/Example Files
2. Open a Box Plot by pressing the “All” button in the Method tab and select Box Sample
3. Select the Treatment annotation in the X-axis data selection (1st)
4. Select “Variables selected by y-axis tool” on y-axis data selection and select one or several variables from the active list in the Variable tab with the mouse
5. Double click on the y-axis to open the plot settings and change Variable title to Gene symbol.
6. Go to the Options tab and tick the box samples to show both samples and boxes.
7. Tick and untick the other boxes and see how it affects the plot.
8. Add different p-values
9. Go to the Method tab and add Gender as a 2nd x-axis
10. Export the image – File/Export/Image in your preferred file format.
11. Open the exported file.



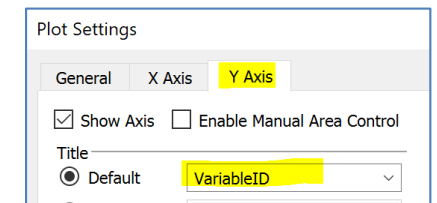
Method tab screenshot showing the 'All' button highlighted in the 'Method' section.



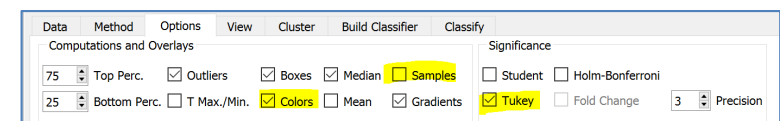
Screenshot showing the selection of 'Box Sample' under the 'Method' tab.



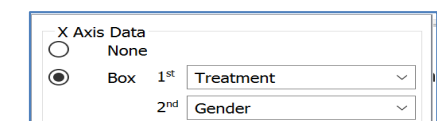
Screenshot showing the 'Y Axis Data' selection with 'Variables selected by Y Axis tool' chosen. A blue arrow points to this option.



Plot Settings dialog box showing the 'Y Axis' tab with 'VariableID' selected as the title.



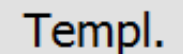
Options tab screenshot showing various checkboxes for computations and overlays, with 'Samples' and 'Colors' highlighted.



Screenshot showing the 'X Axis Data' selection with 'Box' selected and 'Treatment' and 'Gender' assigned to the 1st and 2nd axes respectively.

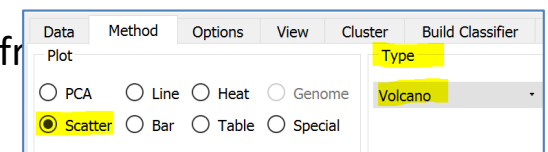
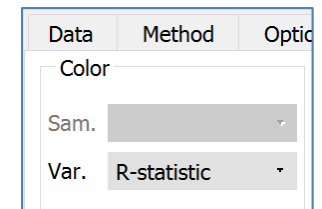
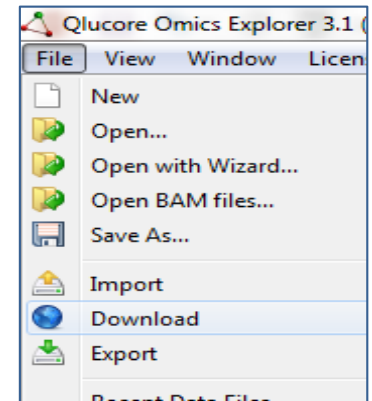
Exercise 7 Templates: Steps

1. Open the Qlucore Test Data set from the Help menu.
2. Click on the launch button for the Templates.
3. Template 1: Select the Heatmap sample clustering template, select the annotation Treatment, press Execute
4. Template 2: Select the Multigroup ANOVA template/annotation Treatment/q=0.1/Execute
5. Template 3: Select t-test & Fold change/annotation Treatment= Placebo/q=0,1/Fold change=2/Execute

A rectangular button with a thin blue border and a light gray background, containing the text "Templ." in a black sans-serif font.

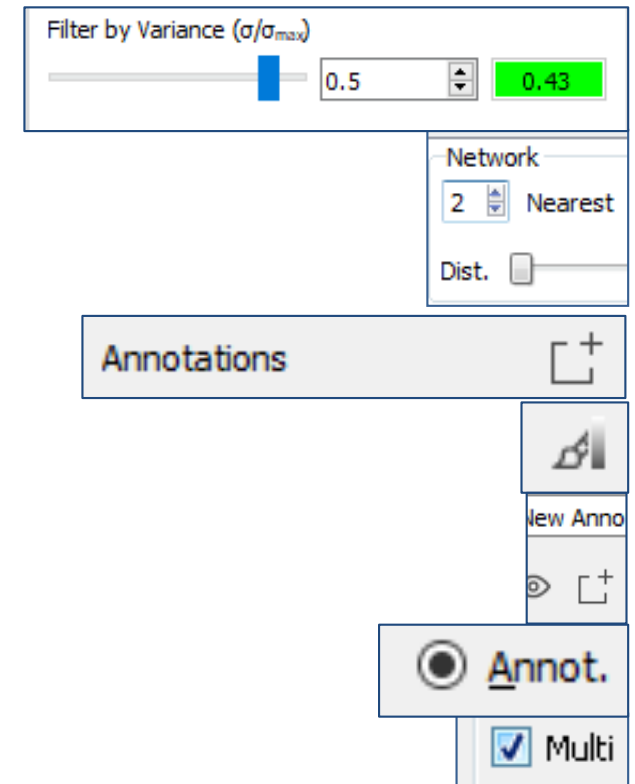
Exercise 8 GEO data: Steps

1. Import a data set from GEO. Search for GSE13425 (childhood ALLs). (If no internet connection - find the data set on a USB stick)
2. Colour the samples according to the annotation characteristics_ch1.
3. Select Multi group comparison in the Statistics dialog.
4. Select the annotation characteristics_ch1.
5. Drag the p-value slider in the Statistics dialog and filter to the 800 most significant variables that separates the disease subtypes.
6. Open up a new synchronized plot, and make it a heatmap
7. Select hierarchical clustering for both samples and variables.
8. Colour the samples according to characteristics_ch1.
9. Show all the sample annotations in the heatmap (View/Color/Sam.)
10. Colour the variables according to q-value(View/Colour/Var.)
11. Copy the active list, rename, add more information and export it.
12. Change the statistical test to a Two Group comparison (t-test) on T-ALL and p-value filtration down to 800 variables
13. Open up a new synchronized plot and make it a Volcano plot – select from
14. Colour it according to R-statistics (View/Colour/Var)
15. Tile the plots (Ctrl T/Cmd T)



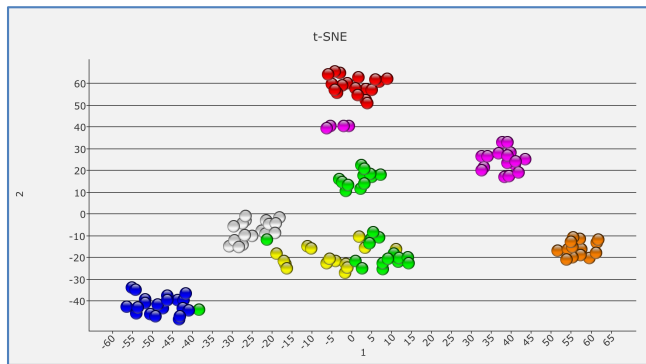
Exercise 9 Exploration: Steps

1. Load the Acute Lymphoblastic Leukemia.gedata data set from the Help menu.
2. Look at the data. Can you see any patterns?
3. Remove variables with low variance by moving the variance slider
4. Find the maximum Projection Score, 0.43.
5. Use networks to connect each sample to its closest neighbors.
6. Create a new sample annotation.
7. Color the data set according to the new annotation.
8. Create a new annotation value.
9. Select the Mouse tool Annotate and annotate the found group by circling the samples clockwise with the mouse (*Make sure that the Multi checkbox is marked*)
10. Deselect the found group.
11. Repeat steps 5-10.



Exercise 10 - t-SNE plot

- Keep the settings from previous exercise.
- Open a new synchronized plot and change it to a t-SNE plot. (Scatter/Type = 2D t-SNE)
- Press Calc (Method tab)
- Change Perplexity to 5 and press Calc again.
- Test other Perplexity values
- Use the annotate tool to re-annotate if needed



t-SNE

Perplexity
5

More
Calc

Steps to add annotations

1. Select File/Open with Wizard
2. Select the file Data Import Example Without Annotations
3. Check the sample annotations in the Sample tab and see that you only have one annotation – Sample id
4. Select File/Import/Sample annotations
5. Select the file Sample Annotations.txt
6. Import all the annotations
7. Check the sample annotations in the Sample tab and see that you now have all 6 annotations

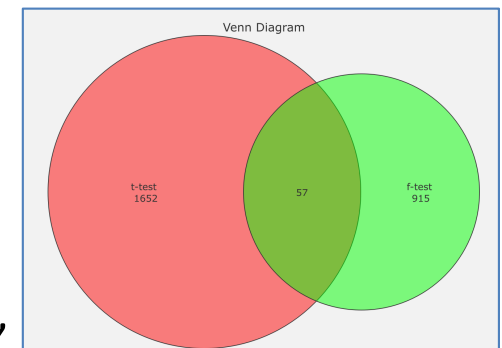
Another way of creating the sample annotation file:

1. Close the dataset and import it again (without annotations)
2. Perform File/Export/Sample annotations
3. Open the file in Excel and add one column per annotation.
4. Save the file (txt) and import as in steps 4-6.

EE3: Steps

1. Load the Leukemia dataset from the Help menu
2. Perform a Multigroup comparison on Leukemia Subtype – use the p-value slider and filter to approx 500 variables
3. Copy the list and call it "F-test"
4. Perform a two group comparison on Leukemia subtype T-ALL and filter to app. 500 variables
5. Copy the list and call it "t-test"
6. Use the Set Operations tool and make an intersection of the lists – rename the list "Intersection"
7. Use the Set Operations tool and make a difference between F-test and t-test. Rename the list Diff.

Variables			
Variable Lists			
<input type="checkbox"/>	Search	0	0
<input checked="" type="checkbox"/>	Qlucore Test Data Set.geodata 1	50	0
<input checked="" type="checkbox"/>	Acute Lymphoblastic Leukemia.g...	501	501
<input type="checkbox"/>	F-test	502	502
<input type="checkbox"/>	t-test	501	501
<input type="checkbox"/>	Intersection	211	211
<input type="checkbox"/>	Diff	291	291



EE4- Steps

- Start by saving your open data set as a gedata file. File/Save as
- Go to the log tab and create a new log file
- Then create a logpoint and rename it.
- Close the volcano plot and change the statistical filtering
- Save a new logpoint – rename it.
- Close the software
- Click on the logfile to open the software and load the logfile.
- Restore logpoint 1 by clicking on the Restore icon.
- Then restore logpoint 2.

