

Labeled Volcano Plot DSP DA Script Vignette

Introduction

The Labeled Volcano Plot DSP DA script creates publication-ready labeled volcano plots based on the researchers' input and statistical study results. The script also creates a table of tagged genes.

Use of the “LabeledVolcanoPlot” DSP DA script

Intended use

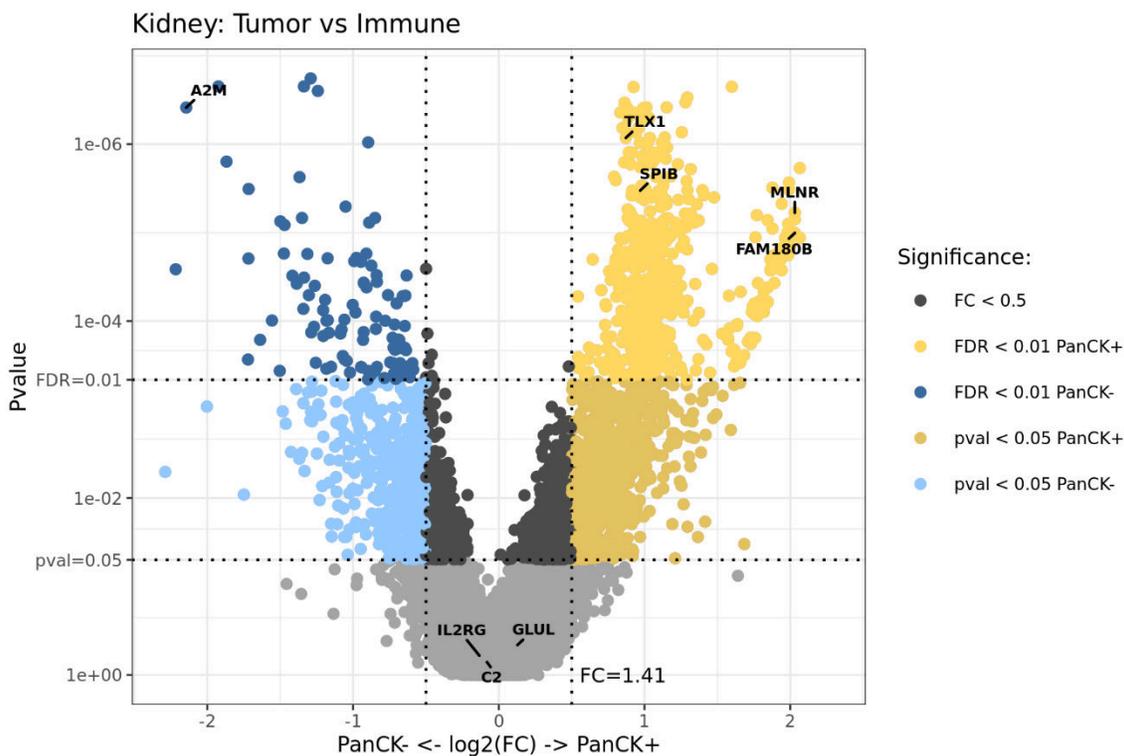
The **Labeled Volcano Plot DSP DA script** supports the **GeoMx nCounter** (*protein or RNA*) or **GeoMx NGS (CTA)** readout applications. The script creates publication-ready labeled volcano plots based on user input and statistical test results. The script also generates a table with the tagged genes.

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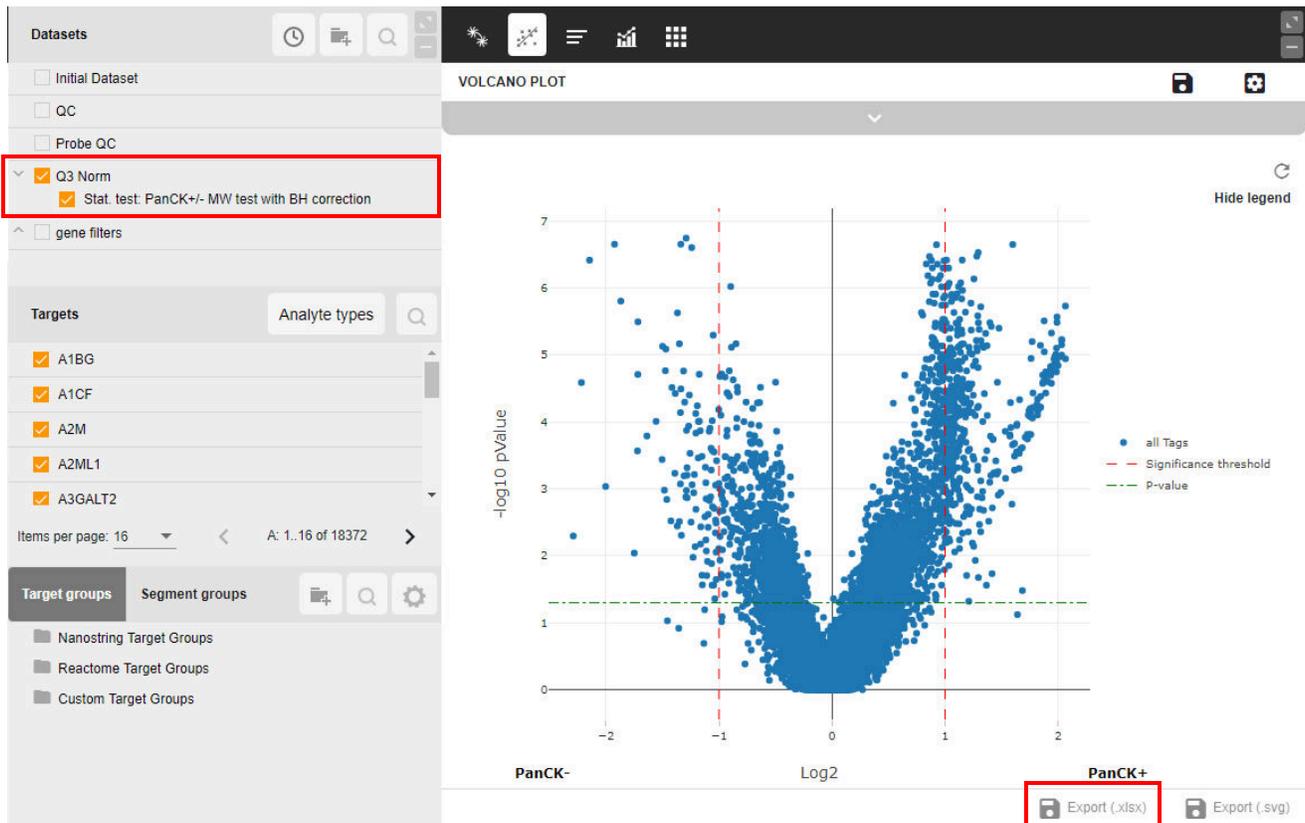
Page 6: Visual Example Parameter Set-up



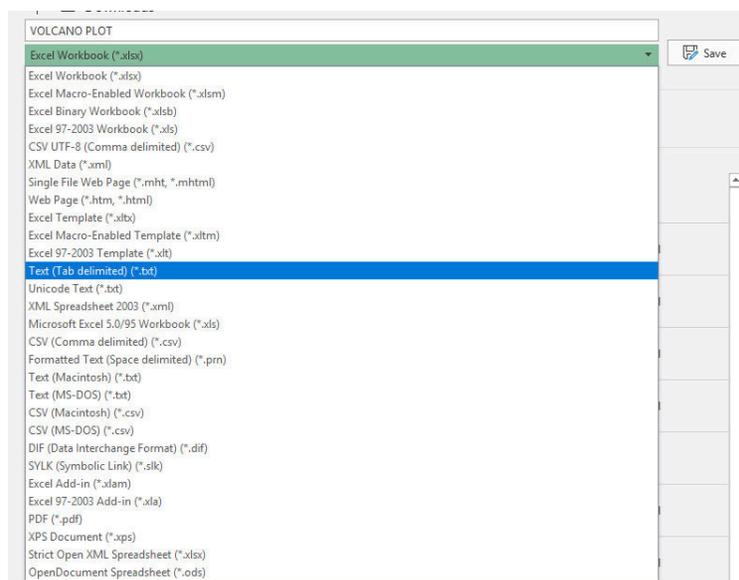
For more visual example graphs see page 6

Importing into the DSP DA

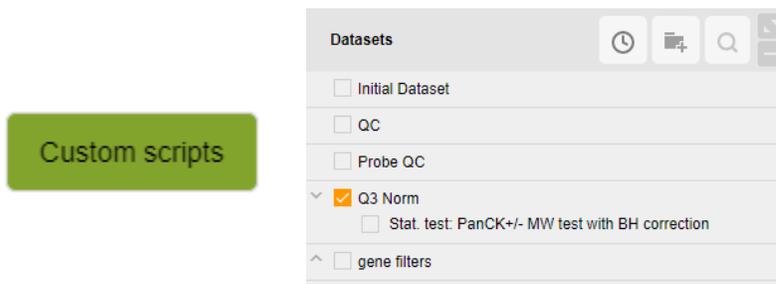
The **LabeledVolcanoPlot.R** script requires an extra file input from the DSP DA. After running a statistical test and creating a volcano plot in DSP DA, **export** the results as a **.xlsx** file. Statistical test results are under the dataset the test ran on.



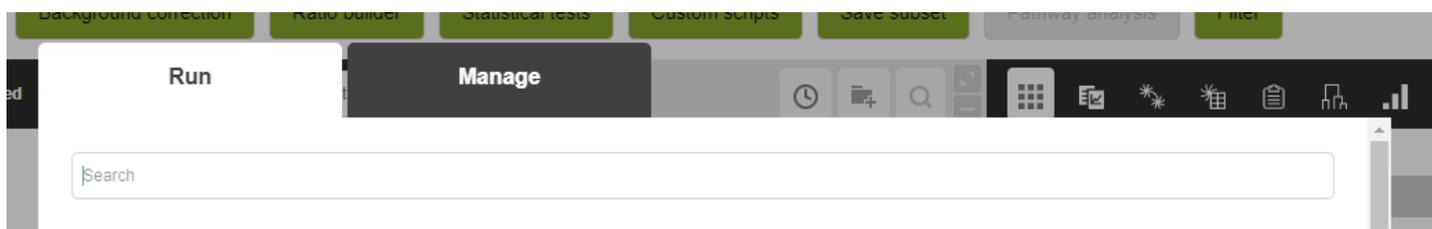
Change the file to a *tab-delimited file .txt* before running the script. The script will **NOT** run if file is in .xlsx format. **Open** the *Exported VOLCANO PLOT.xlsx* file in Excel and click **Save As**, to change the format to **Text (Tab delimited) (*.txt)**.



The **LabeledVolcanoPlot.R** loads into the custom scripts section of the DSP DA after you have a dataset processed and ready for analysis. To do so, **open** the custom script section by clicking on the unmarked statistical test dataset.



Select the **“Manage”** tab to open the area to load and edit scripts:



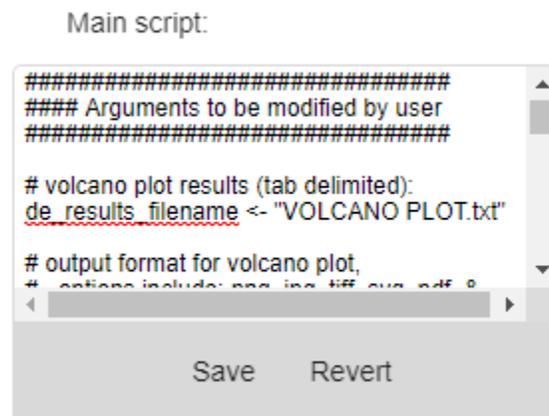
In the **Management** tab to add a new script and adjust parameters, fill out and then scroll to the bottom of the page. Use the **“+”** button to add the **LabeledVolcanoPlot.R** file to the script:



Use the **“+”** button to add the **VOLCANO PLOT.txt** file. Ensure the **LabeledVolcanoPlot.R** file is selected in the **dropdown menu**, indicated by a **star**.



Once the desired parameters of the script are selected, the user can start making modifications, editing the top lines in the script by selecting the “**Save**” button. No need to check the *Create new dataset* button.



Setting and Modifying the User Parameters:

There are twenty-one settings the user can modify at the top of the plug-in script. These settings include:

Files

1. *de_results_filename*: (String) Name of the tab-delimited file you have uploaded to the DSP DA.
2. *output_format*: (String) Desired output format for the volcano plot figure.
 - Options: *PNG, JPG, TIFF, SVG, PDF, and BMP*

Labeling

The user must add labels from the DSP DA volcano plot.

3. *plot_title*: (String) Title for figure
4. *negative_label*: (String) Matching negative (left) x-axis label to the volcano plot in the DSP DA
5. *positive_label*: (String) Matching positive (right) x-axis label to the volcano plot in the DSP DA
6. *show_legend*: (Boolean) A color legend will appear
7. *n_genes*: (Numeric) Number of top genes by pvalue/fdr to label on figure. *gene_list* overrides this variable if set.
8. *gene_list*: (String) Labeled list of specified genes are on figure. Default labeling method over *n_genes*.

Thresholds

A line will appear on the figure to show the thresholds. Remove the line by setting the thresholds to **NULL**.

9. *pval_thresh*: (Numeric) p-value threshold on the y-axis
10. *fdr_thresh*: (Numeric) false discovery rate threshold on the y-axis
11. *fc_thresh*: (Numeric) log2 fold change cutoff on the x-axis.
12. *label_fc*: (Boolean) Should genes below the FC threshold be labeled if they are also above the significance threshold

Fonts

13. *font_size*: (Numeric) Font size on the figure
14. *label_size*: (Numeric) Size of the font for the gene labels

15. *font_family*: (String) Font family for all text on figure

- Options: *serif, sans, mono*

Plot Size

16. *plot_width*: (Numeric) Width of the saved figure in inches

17. *plot_height*: (Numeric) Height of saved figure in inches

Coloring

Colors that R can recognize should be either named colors (e.g., “orange2”) or hexadecimal colors (“#ABABAB”). See below for a chart of all named R colors.

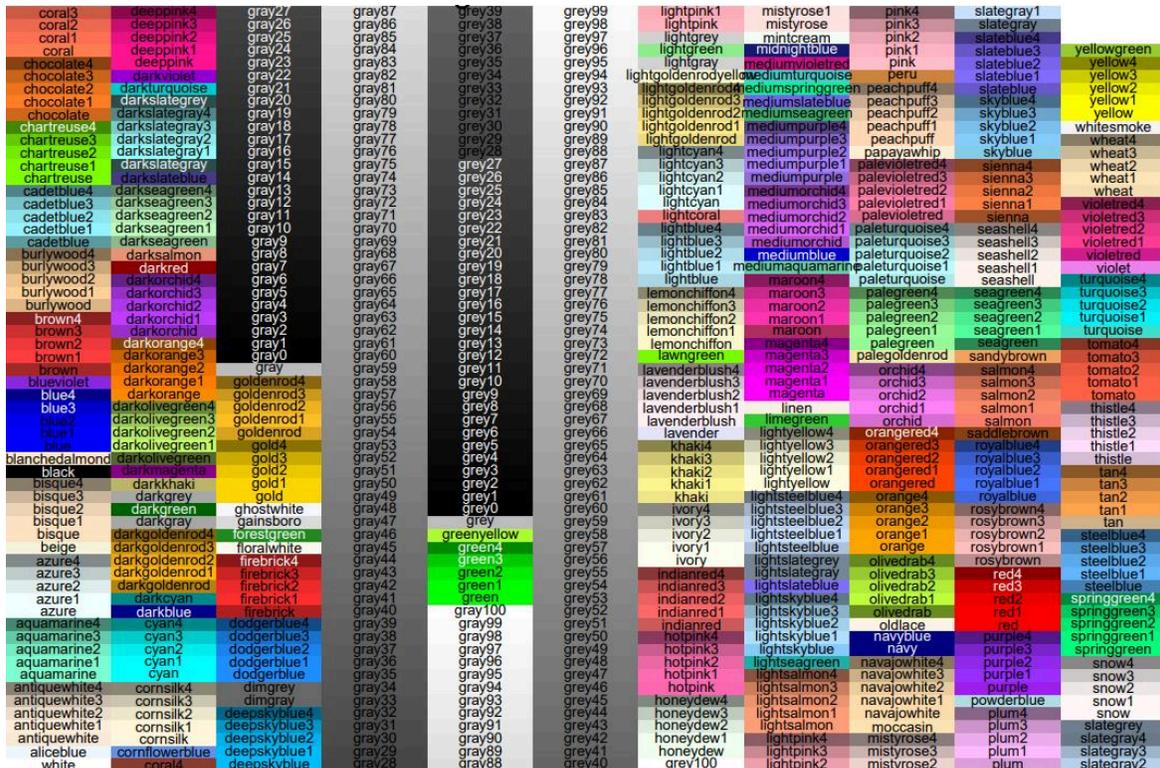
18. *default_color*: (String) Color of points not in target group or above significance threshold

19. *fc_color*: (String) Color of points below *fc_thresh* but above the significance threshold(s); change to same as default to not call out these targets

20. *target_groups*: (String) Color-specific gene target groups to be colored in plot. Labeled target groups are labeled in the *VOLCANO PLOT.xlsx* file. All genes in the given *target_group* are colored no matter where they are in the figure. If no group (*NULL*) will appear, locate the colored targets above the *pval/fdr* threshold.

21. *color_options*: (String) List of colors to use in the figure. Must have at least the number of *target_groups*.

Named R Colors Chart:



Example Parameter Set-up

The **LabeledVolcanoPlot.r** script outputs a typical volcano plot figure with log2 fold change on the x-axis and the pvalue or FDR on the y-axis for each target. A table of labeled genes in the figure is also output.

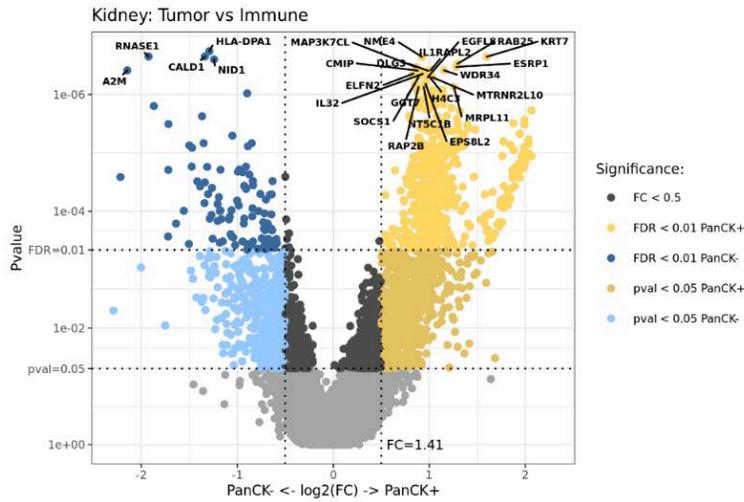
Example figures with different input arguments.

Example 1:

n_genes = 25
fdr_thresh = 0.01

pval_thresh = 0.05
fc_thresh = 0.5

label_fc = FALSE
target_groups = NULL



Example 2:

**gene_list = c("IL2RG", "GLUL", "SPIB", "C2",
"A2M", "MLNR", "TLX1", "FAM180B")**
target_groups = c("Hemostasis", "DNA Repair")

pval_thresh = NULL
fc_thresh = 0.5
fdr_thresh = 0.01

