

# Creating gene lists from ANOVA results

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## Creating a gene list with the ANOVA Streamlined list manager

Now that you have obtained statistical results from the microarray experiment, you can create new spreadsheets containing just those genes that pass certain criteria. This will streamline data management by focusing on just those genes with the most significant differential expression or substantial fold change. The *List Manager* can be used to specify numerous conditions for selecting genes of interest. In this tutorial, we are going to create a gene list of gene with a fold change between -1.3 to 1.3 that has an unadjusted p-value of  $< 0.0005$ .


- Invoke the *List Manager* dialog by selecting **Create Gene List** in the *Analysis* section of the *Gene Expression* workflow
- Ensure that the *1/ANOVA-3way (ANOVAResults)* spreadsheet is selected as this is the spreadsheet we will be using to create our new gene list as shown (Figure 1)
- Select the **ANOVA Streamlined** tab.
- Set *Contrast: find genes that change between two categories* panel, to **Down Syndrome vs. Normal** and select **Have Any Change** from the *Setting* drop-down menu

This will find genes with different expression levels in the different types of samples.

- In the *Configuration for "Down Syndrome vs Normal"* panel, check that **Include size of the change** is selected and enter 1.3 into *Change >* and -1.3 in *OR Change <*
- Select **Include significance of the change**, choose **unadjusted p-value** from the dropdown menu, and  $< 0.001$  for the cutoff

The number of genes that pass your cutoff criteria will be shown next to the *# Pass* field. In this example, 30 genes pass the criteria.

- Set **Save the list** as *A*
- Select **Create** to generate the new list *A*
- Select **Close** to view the new gene list spreadsheet


List Manager

List

Spreadsheet Name

A

1 (Down\_Syndrome-GE)

B

1/ANOVA-3way (ANOVA)

List Info

Venn Diagram

ANOVA Streamlined

Advanced

Single factor: find genes that vary across all samples upon single factor

Name

Setting

# Pass

☐ Type

☐ Tissue

Interaction: find genes that vary across all samples upon interaction ?

Name

Setting

# Pass

☐ Type \* Tissue

Contrast: find genes that change between two categories

Name

Setting

# Pass

☒ Down Syndrome vs. Normal

Have Any Change

30

Configuration for "Down Syndrome vs. Normal"

# Pass: 30

☒ Include size of the change

Fold change > 1.3 OR Fold change < -1.3

☒ Include significance of the change

unadjusted p-value < 0.001 ?

30 genes passed the specified criteria.

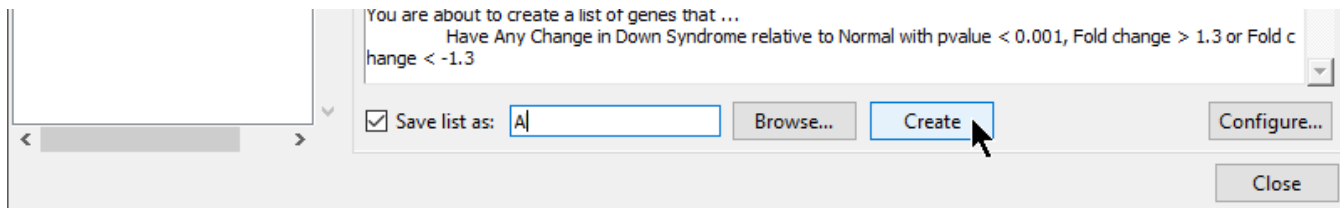


Figure 7. Creating a gene list from ANOVA results

The spreadsheet *Down\_Syndrome\_vs\_Normal (A)* will be created as a child spreadsheet under the *Down\_Syndrome-GE* spreadsheet.

This gene list spreadsheet can now be used for further analysis such as hierarchical clustering, gene ontology, integration of copy number data, or be exported into other data analysis tools such as pathway analysis.

You can practice creating new gene list criteria of your own to become familiar with the *List Manager* tool. For more information, you can always click on the (?) buttons.

## Creating a gene list from a volcano plot

Next, we will generate a list of genes that passed a p-value threshold of 0.05 and fold-changes greater than 1.3 using a volcano plot.

- Select the *1/ANOVA-3way (ANOVAResults)* spreadsheet in the *Analysis* tab. This is the spreadsheet our gene list will be drawn from
- Select **View > Volcano Plot** from the Partek Genomics Suite main menu (Figure 2)

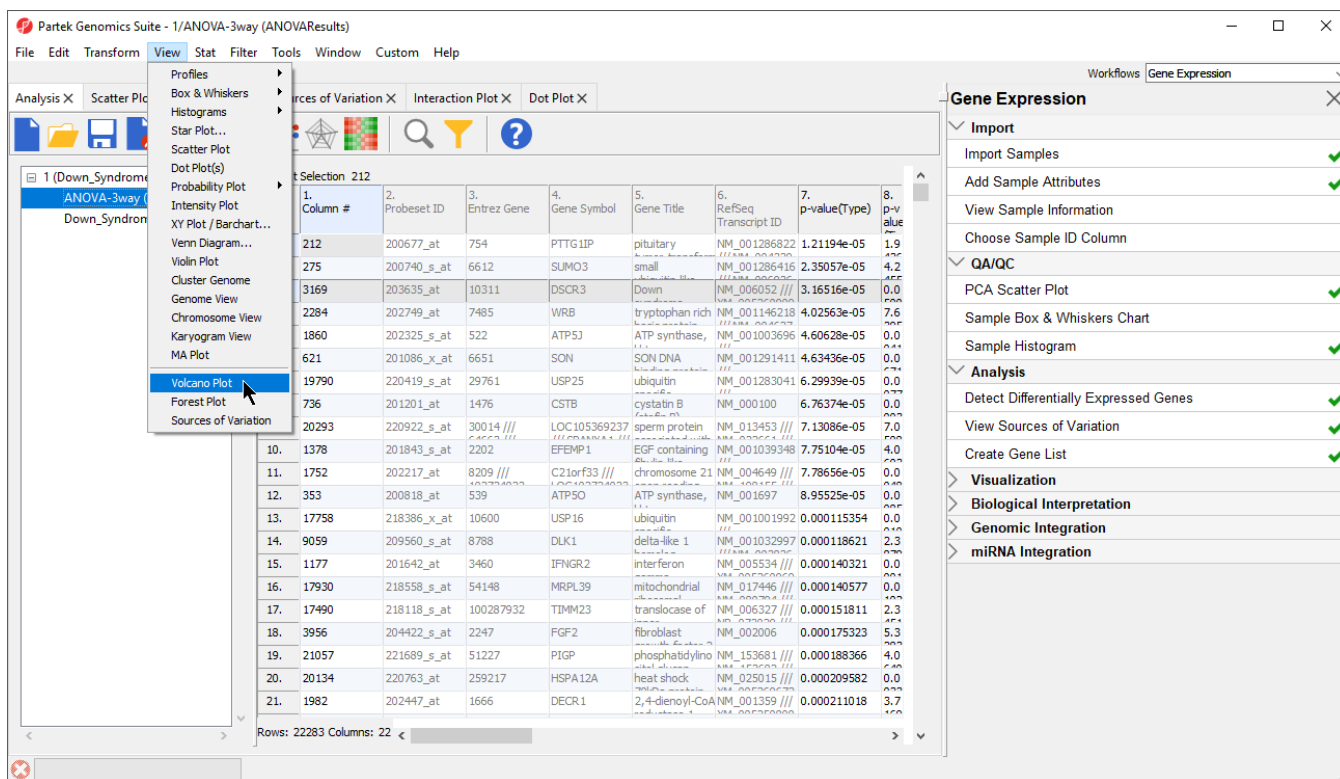


Figure 8. Generating a Volcano Plot from ANOVA results

- Set *X Axis (Fold-Change)* to **12. Fold-Change(Down Syndrome vs. Normal)**, and the *Y axis (p-value)* to be **10. p-value(Down Syndrome vs. Normal)**
- Select **OK** to generate a *Volcano Plot* tab for genes in the ANOVA spreadsheet (Figure 3)

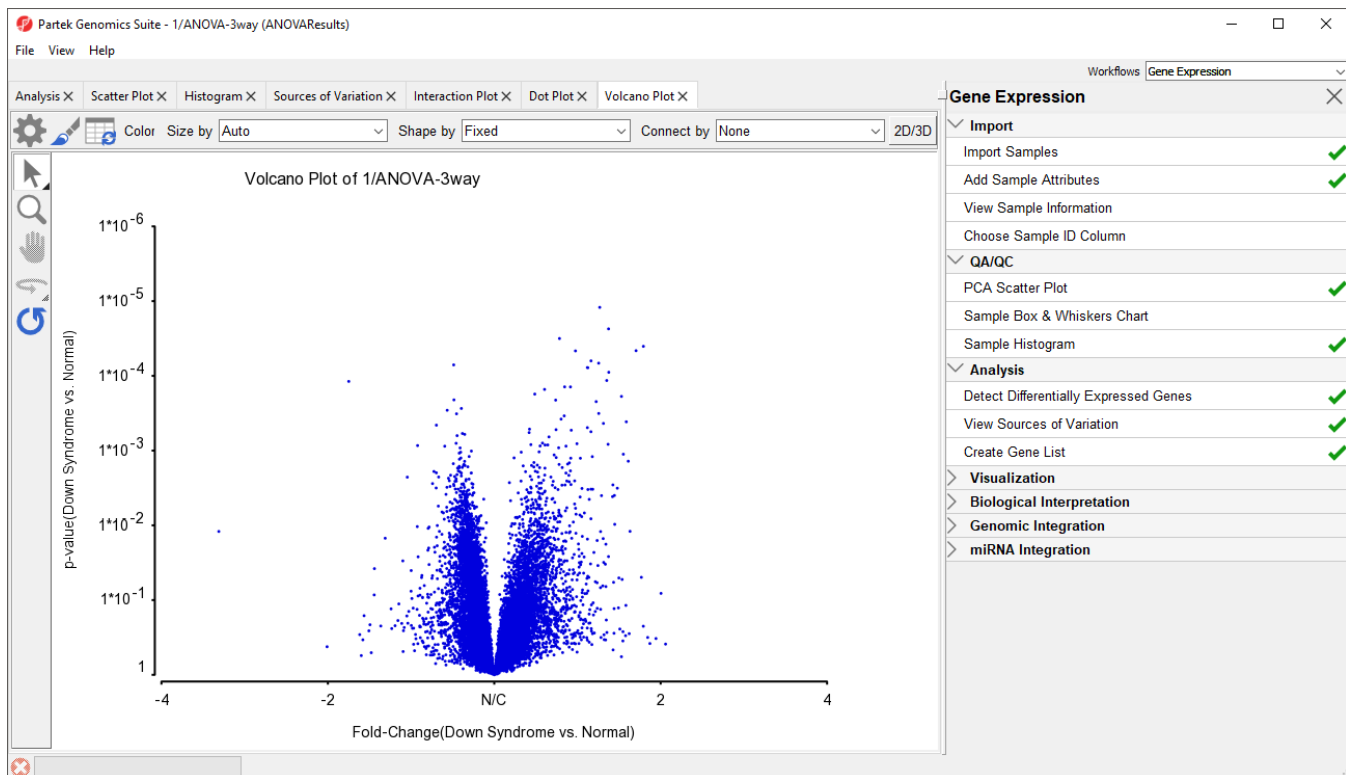


Figure 9. Volcano plot generated from ANOVA spreadsheet

In the plot, each dot represents a gene. The X-axis represents the fold change of the contrast (*Down syndrome vs. Normal*), and the Y-axis represents the range of p-values. The genes with increased expression in Down syndrome samples are on the right side of the N/C (no change) line; genes with reduced expression in Down syndrome samples are on the left. The genes become more statistically significant with increasing Y-axis position. The genes that have larger and more significant changes between the Down syndrome and normal groups are on the upper right and upper left corner.

In order to select the genes by fold-change and p-value, we will draw a horizontal line to represent the p-value 0.05 and two vertical lines indicating the -1.3 and 1.3-fold changes (cutoff lines).

- Select **Rendering Properties** ( )
- Choose the **Axes** tab
- Check **Select all points in a section** to allow Partek Genomics Suite to automatically select all the points in any given section
- Select the **Set Cutoff Lines** button and configure the *Set Cutoff Lines* dialog as shown (Figure 4)

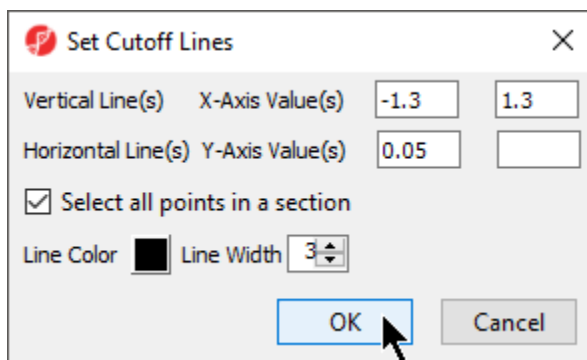


Figure 10. Setting cutoff lines for -1.3 to 1.3 fold changes and a p-value of 0.05

- Select **OK** to draw the cutoff lines

- Select **OK** in the *Plot Rendering Properties* dialog to close the dialog and view the plot

The plot will be divided into six sections. By clicking on the upper-right section, all genes in that section will be selected.

- Right-click on the selected region in the plot and choose **Create List** to create a list including the genes from the section selected (Figure 5). Note that these p-values are uncorrected

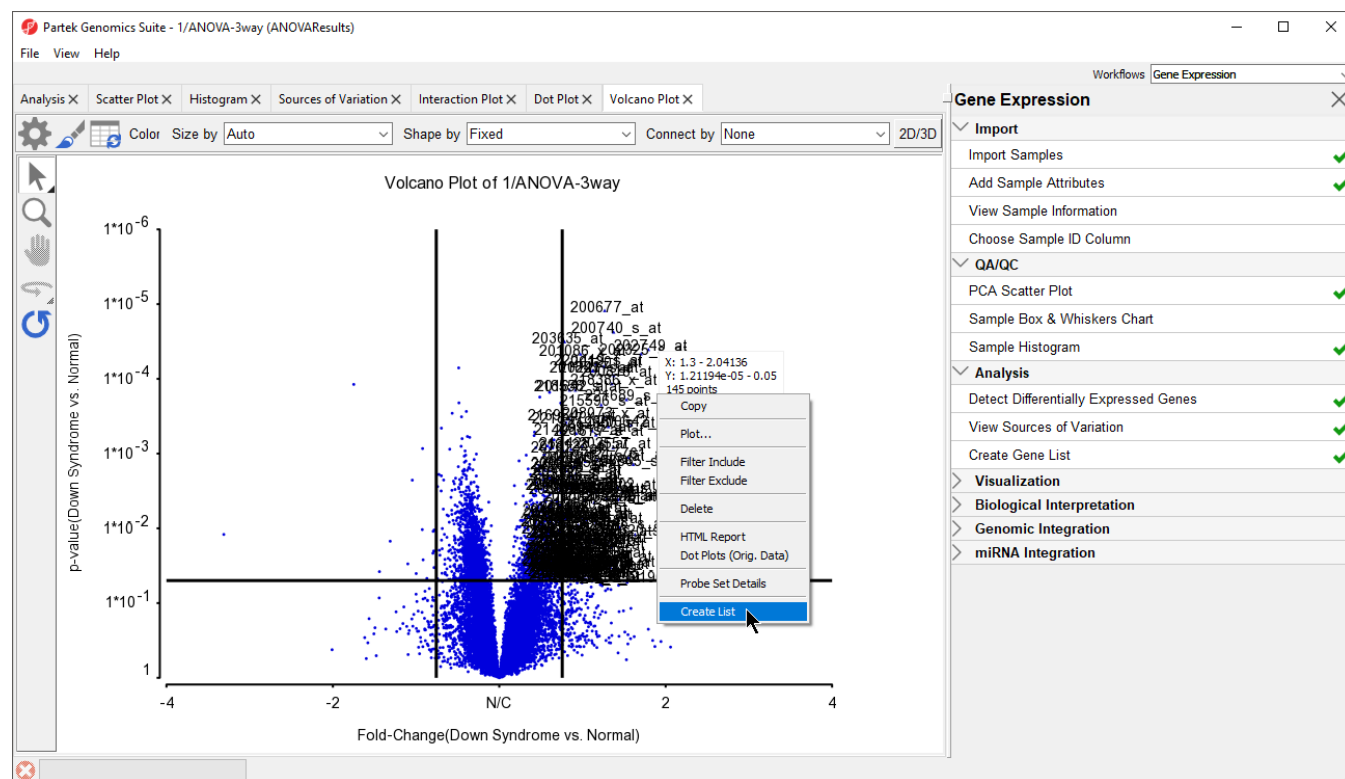


Figure 11. Creating a gene list from a volcano plot

Note: If no column is selected in the parent (ANOVA) spreadsheet, all of the columns will be included in the gene list; if some columns are selected, only the selected columns will be included in the list.

- Specify a name for the gene list (example: volcano plot list) and write a brief description about the list.

The description is shown when you right-click on the spreadsheet > Info > Comments. Here, I have named the list "volcano plot list" and described it as "Genes with >1.3 fold change and p-value <0.05" (Figure 6). The list can be saved as a text file (*File > Save As Text File*) for use in reports or by downstream analysis software.

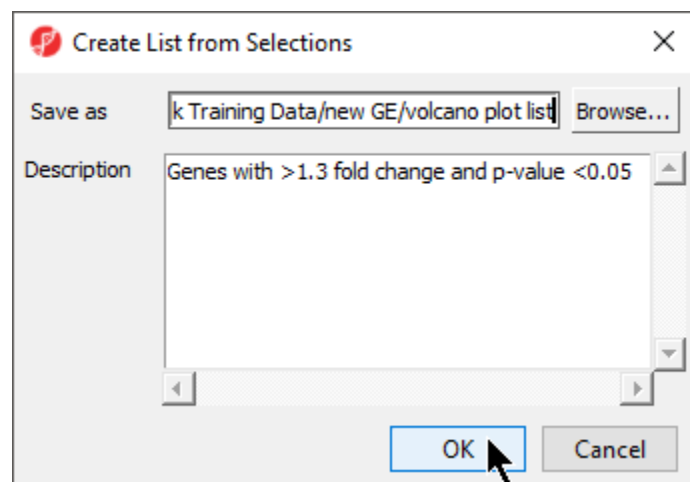


Figure 12. Saving a list created from a volcano plot

## Additional Assistance

If you need additional assistance, please visit [our support page](#) to submit a help ticket or find phone numbers for regional support.



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