

Step-by-step Guide to Perform Differential Expression Analysis using FlexStatv1 Pipeline - Using Sample Data



This feature facilitates pairwise differential expression analysis with integrated multiple-testing corrections. Users have the option to filter results by configuring cutoffs for log fold change and p-values.

This functionality includes visual representations of differential expression results, including boxplots, volcano plots, and heat maps.

Protein type-specific principal component analysis is a prominent aspect of this feature.

This tutorial is based on the sample data provided with the application.

1

Navigate to <https://jglab.shinyapps.io/flexstatv1-pipeline-only/>

2

Go to the "Differential Expression" tab.

3 Click the "Use Sample Data" field.

FlexStat 1.0

Differential Expression

Automated Differential Expression

Consensus Clustering

About

Limma Analysis

Select CSV File to Import

Browse...

No file selected

☒ Show head

☐ Use Sample Data

☐ Transpose data

☐ Log2 Transform

☐ Log10 Transform

Select columns to remove

Class Variable

Not Selected

Class of Interest

Data

Results

Top 50

Sample Data

Condition	O76070	P01344
A	28.41	27.36
A	28.46	27.40
A	28.41	27.47
B	24.28	24.63
B	24.28	24.73
B	24.20	24.66

4 Select Class variable as "Condition"

Browse...

No file selected

☒ Show head

☒ Use Sample Data

☐ Transpose data

☐ Log2 Transform

☐ Log10 Transform

Select columns to remove

Class Variable

Not Selected

Not Selected

Condition

Contrast variable

Not Selected

☐ Contrast other classes

Sample Data

Condition	O76070	P01344
A	28.41	27.3
A	28.46	27.4
A	28.41	27.4
B	24.28	24.6
B	24.28	24.7
B	24.20	24.6

5 Select class of interest from the dropdown

☐ Transpose data ☐ Log2 Transform ☐ Log10 Transform

Select columns to remove

Class Variable


Condition ▼


Class of Interest

Not Selected ▼

Contrast variable

Not Selected ▼

☐ Contrast other classes 

Log fold-change variable 

Condition	O76070	P0134
A	28.41	27.3
A	28.46	27.4
A	28.41	27.4
B	24.28	24.6
B	24.28	24.7
B	24.20	24.6

6 Select contrast variable from the dropdown

Class Variable


Condition ▼

Class of Interest

A ▼

Contrast variable

A ▼

☐ Contrast other classes 

Log fold-change variable 

P-value variable 

Adjust P-values for Multiple Comparisons

Condition	O76070	P0134
A	28.41	27.4
B	24.28	24.6
B	24.28	24.7
B	24.20	24.6

7


[Optional] Change log fold change cutoff and p-value cutoff

Condition

Class of Interest

Contrast variable

☐ Contrast other classes

Log fold-change variable 

P-value variable

Adjust P-values for Multiple Comparisons

B	24.28	24.7
B	24.20	24.6

8 Click "Perform Limma"

Contrast variable

B ▼

☐ Contrast other classes ⓘ

Log fold-change variable ⓘ

P-value variable ⓘ

Adjust P-values for Multiple Comparisons ⓘ

Benjamini-Hochberg ▼

▶ Perform Limma

Check the Result Tab

9 Click "Results" tab

Automated Differential Expression Consensus Clustering About

Data **Results** Top 50 Volcano Plot Annotated Volcano Plot Heatmap

☒ Use Sample Data

☐ Log10 Transform

Sample Data

Condition	O76070	P01344	P01579	P00709	P41159	P00918	P01344
A	28.41	27.36	27.40	27.14	28.23	28.04	28.41
A	28.46	27.40	27.37	27.05	28.14	28.07	28.46
A	28.41	27.47	27.37	27.08	28.23	28.03	28.41
B	24.28	24.63	23.63	22.84	24.47	24.17	24.28
B	24.28	24.73	23.44	23.03	24.72	24.52	24.28
B	24.20	24.66	23.68	22.76	24.66	24.47	24.20

10 Click "Download Current Page" to download the visible results on the current page as a csv file.

Automated Differential Expression Consensus Clustering About

Data Results Top 50 Volcano Plot Annotated Volcano Plot Heatmap PCA

Show 10 entries **Download Current Page** Download Full Results

Gene	logFC	AveExpr	negLog10P
P01008	5.1769	26.2195	7.3010
P08758	5.0920	26.3756	6.2980
P61626	4.4942	25.3483	7.3010
P63165	4.4371	25.6728	7.3010
P04040	4.4325	27.5286	27.5286
P62037	4.2760	24.9159	4.0000

11 Click "Download Full Results" to download all results as a csv file.

The screenshot shows the 'Clustering' interface with a navigation bar containing 'Clustering' and 'About'. Below the navigation bar, there are tabs for 'Data', 'Results', 'Top 50', 'Volcano Plot', 'Annotated Volcano Plot', 'Heatmap', and 'PCA'. The 'Results' tab is selected. Below the tabs, there is a 'Show' dropdown set to '10' and a label 'entries'. To the right of the dropdown are two buttons: 'Download Current Page' and 'Download Full Results'. The 'Download Full Results' button is highlighted with an orange circle. Below the buttons is a table with the following columns: 'Gene', 'logFC', 'AveExpr', 't', and 'P.Value'. The table contains six rows of data.

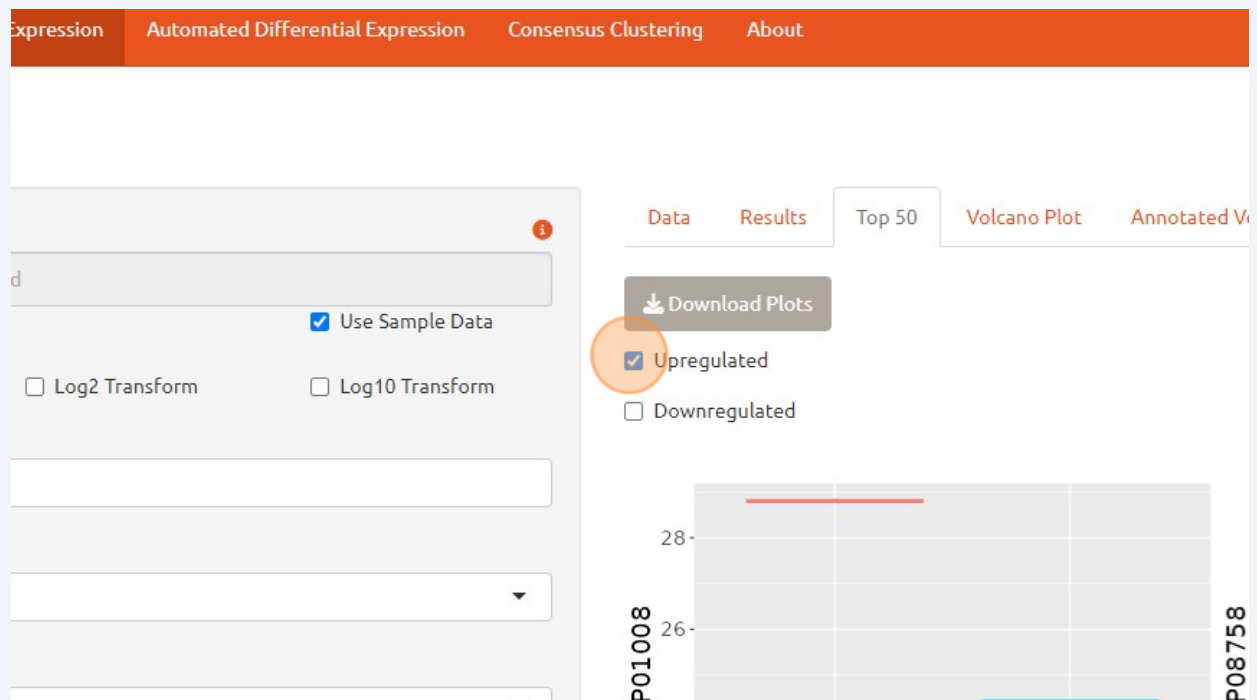
Gene	logFC	AveExpr	t	P.Value
P01008	5.1769	26.2195	7.4393	0.00
P08758	5.0920	26.3756	6.5047	0.00
P61626	4.4942	25.3483	7.3858	0.00
P63165	4.4371	25.6728	7.1539	0.00
P04040	4.4325	27.5286	27.4902	0.00
P62937	4.2760	24.9159	10.9160	0.00

12 Click "Top 50" to see the highly varied 50 proteins/genes between the classes

The screenshot shows the 'Differential Expression' interface with a navigation bar containing 'Differential Expression', 'Consensus Clustering', and 'About'. Below the navigation bar, there are tabs for 'Data', 'Results', 'Top 50', 'Volcano Plot', 'Annotated Volcano Plot', and 'Heatmap'. The 'Top 50' tab is highlighted with an orange circle. To the left of the tabs is a sidebar with a red information icon at the top. The sidebar contains a checkbox labeled 'Use Sample Data' which is checked, and a checkbox labeled 'Log10 Transform' which is unchecked. Below these checkboxes are two input fields: a text field and a dropdown menu. To the right of the sidebar, there is a 'Download Plots' button with a download icon. Below this button are two checkboxes: 'Upregulated' and 'Downregulated', both of which are unchecked.

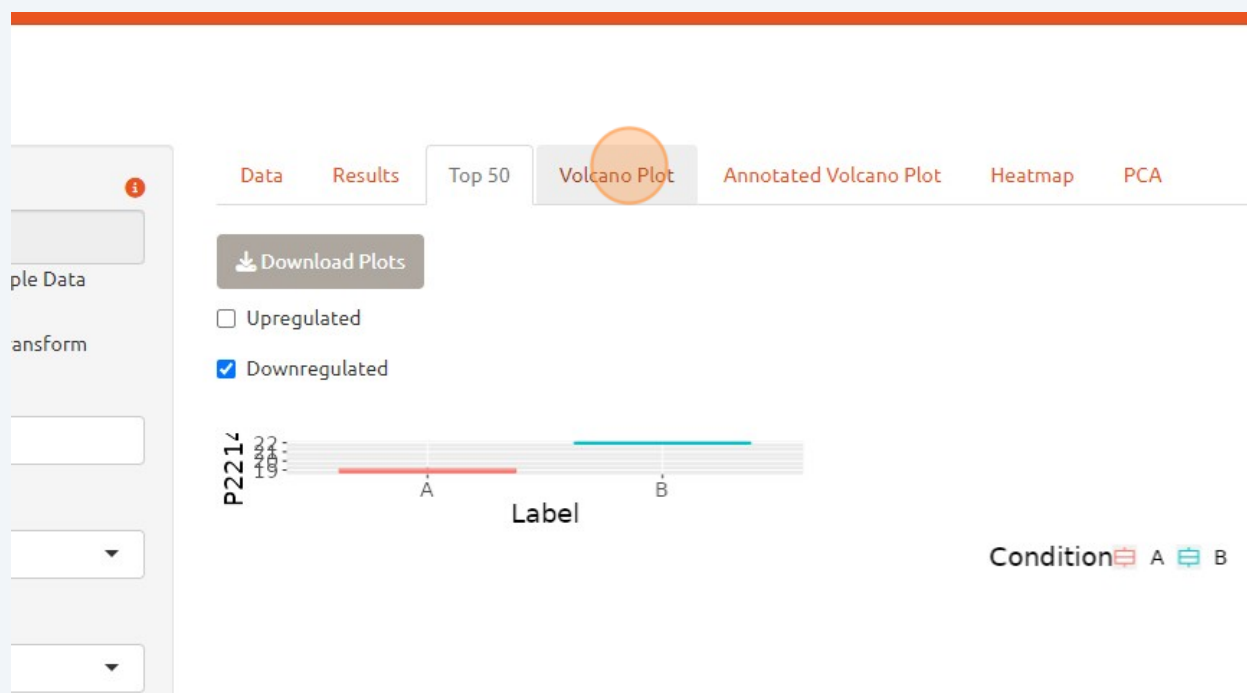
13

Click "Upregulated" to filter the boxplots of upregulated proteins/genes between the classes

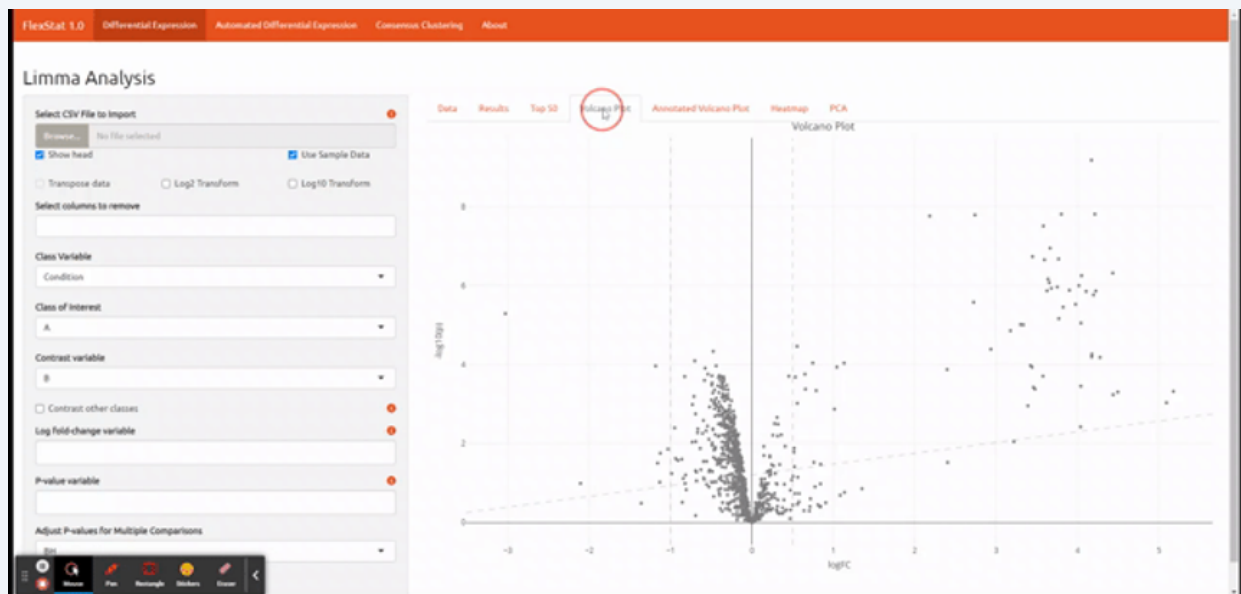


14

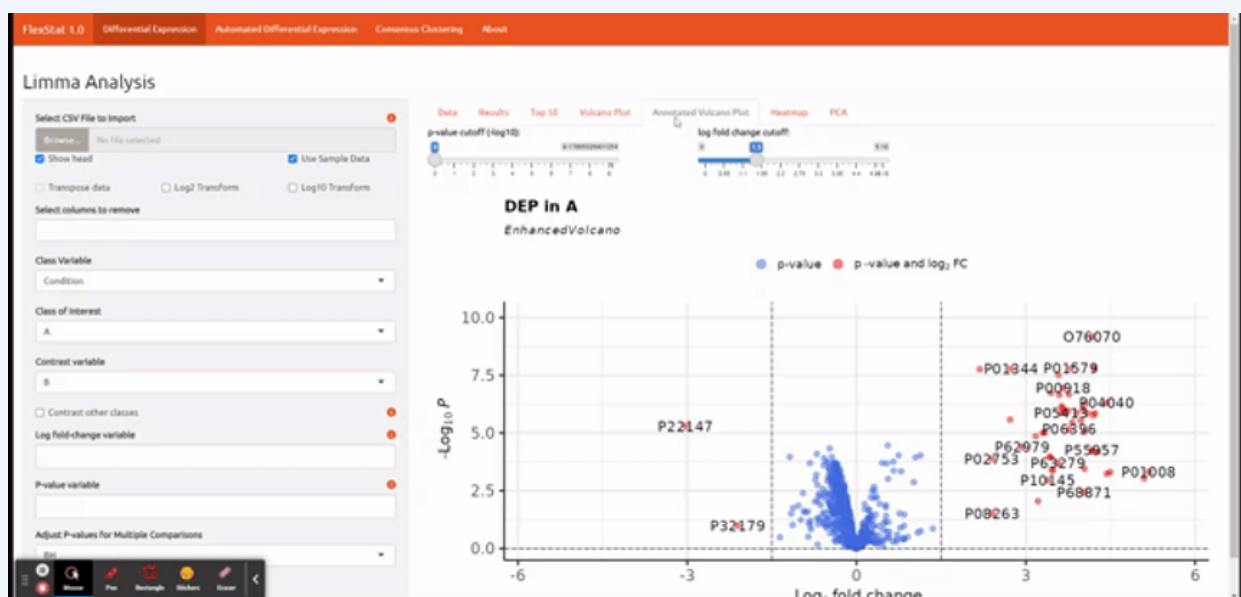
Click "Downregulated" to filter the boxplots of upregulated proteins/genes between the classes



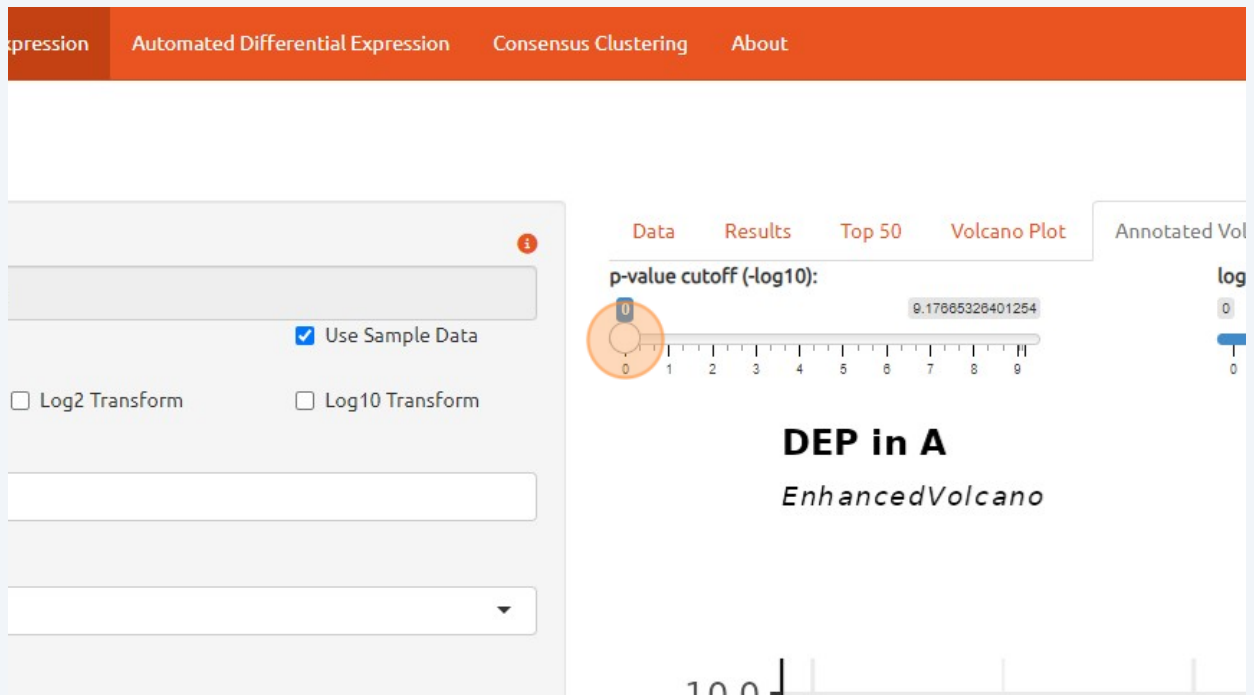
15 Click "Volcano Plot" to check the draft volcano plot



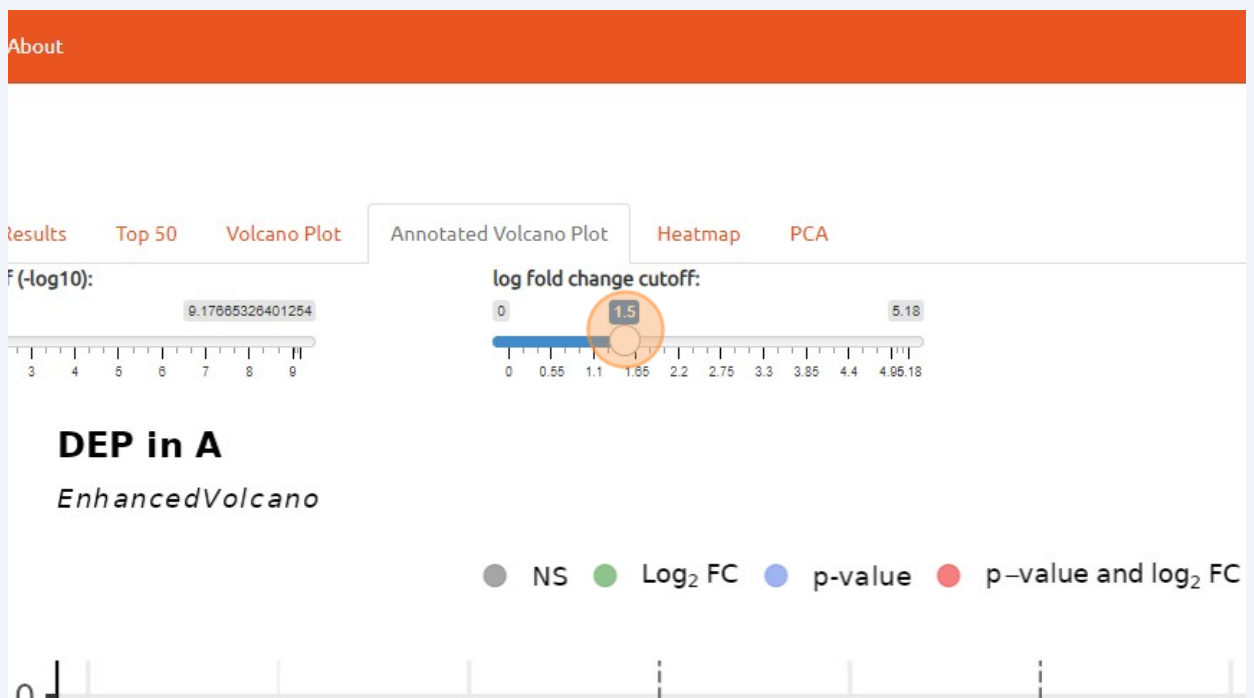
16 Click "Annotated Volcano Plot" to obtain the well-annotated points showing significant proteins/genes between the conditions.



17 [Optional] Change p-value cutoff based on your research requirements.



18 [Optional] Change log fold-change cutoff based on your research requirements.



19 Click "Heatmap" to obtain the heatmap

