

Step-by-step guide: Analyzing gene expression data using a web application.

Scribe

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

- 2 Click "Use Sample Data"

Limma Analysis

Select CSV File to Import

No file selected

Show head Use Sample Data

Transpose data Log2 Transform Log10 Transform

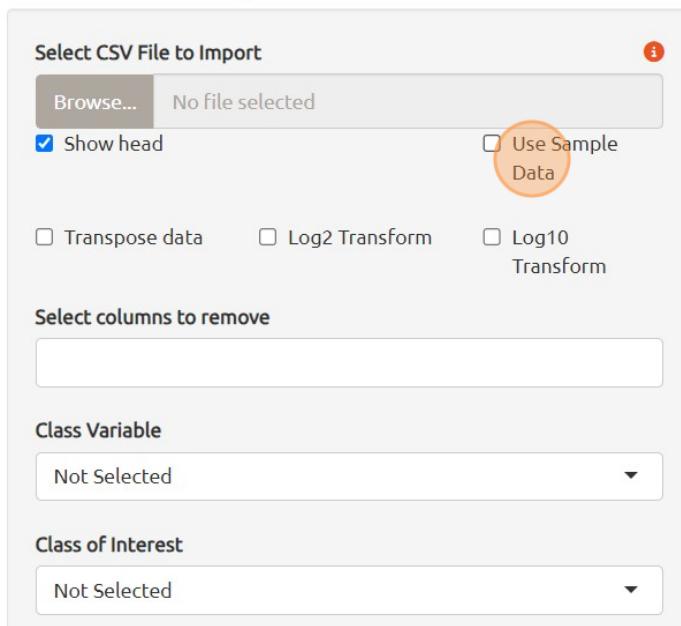
Select columns to remove

Class Variable

Not Selected

Class of Interest

Not Selected



Data Results Top 50 Volcano Plot

Sample Data

| Condition | Protein.1 | Protein.2 | Protein.3 |
|-----------|-----------|-----------|-----------|
| C1 | 3.09 | 2.03 | 2.22 |
| C1 | 1.11 | 2.11 | 2.21 |
| C2 | 1.17 | 2.00 | 1.83 |
| C1 | 1.10 | 2.02 | 2.70 |
| C3 | 2.69 | 2.34 | 2.49 |
| C3 | 2.71 | 2.12 | 2.20 |
| C4 | 2.64 | 2.26 | 1.99 |
| C4 | 2.65 | 1.98 | 1.97 |
| C1 | 2.74 | 2.04 | 1.97 |

3 Click here.

Show head Use Sample Data

Transpose data Log2 Transform Log10 Transform

Select columns to remove

Class Variable

Class of Interest

Contrast variable

Contrast other classes 

Sample Data

| Condition | Protein.1 | Protein.2 | Protein.3 |
|-----------|-----------|-----------|-----------|
| C1 | 3.09 | 2.03 | 2.22 |
| C1 | 1.11 | 2.11 | 2.21 |
| C2 | 1.17 | 2.00 | 1.83 |
| C1 | 1.10 | 2.02 | 2.70 |
| C3 | 2.69 | 2.34 | 2.49 |
| C3 | 2.71 | 2.12 | 2.20 |
| C4 | 2.64 | 2.26 | 1.99 |
| C4 | 2.65 | 1.98 | 1.97 |
| C1 | 2.74 | 2.04 | 1.97 |
| C3 | 2.68 | 2.02 | 1.96 |
| C3 | 2.26 | 1.94 | 2.57 |
| C3 | 2.24 | 1.94 | 2.17 |
| C2 | 2.30 | 2.31 | 2.24 |

4 Click "Condition"

Transpose data Log2 Transform Log10 Transform

Select columns to remove

Class Variable

Condition

Contrast variable

Contrast other classes 

Log fold-change variable

| | | | |
|----|------|------|------|
| C1 | 3.09 | 2.03 | 2.22 |
| C1 | 1.11 | 2.11 | 2.21 |
| C2 | 1.17 | 2.00 | 1.83 |
| C1 | 1.10 | 2.02 | 2.70 |
| C3 | 2.69 | 2.34 | 2.49 |
| C3 | 2.71 | 2.12 | 2.20 |
| C4 | 2.64 | 2.26 | 1.99 |
| C4 | 2.65 | 1.98 | 1.97 |
| C1 | 2.74 | 2.04 | 1.97 |
| C3 | 2.68 | 2.02 | 1.96 |
| C3 | 2.26 | 1.94 | 2.57 |
| C3 | 2.24 | 1.94 | 2.17 |
| C2 | 2.30 | 2.31 | 2.24 |
| C1 | 4.09 | 1.23 | 2.20 |
| C2 | 1.11 | 2.73 | 2.07 |

5 Click here.

Class Variable

Condition

Class of Interest

C1

Contrast variable

C1

Contrast other classes

Log fold-change variable

P-value variable

P-values for Multiple Comparisons

| | 1.10 | 2.02 | 2.70 |
|----|------|------|------|
| C3 | 2.69 | 2.34 | 2.49 |
| C3 | 2.71 | 2.12 | 2.20 |
| C4 | 2.64 | 2.26 | 1.99 |
| C4 | 2.65 | 1.98 | 1.97 |
| C1 | 2.74 | 2.04 | 1.97 |
| C3 | 2.68 | 2.02 | 1.96 |
| C3 | 2.26 | 1.94 | 2.57 |
| C3 | 2.24 | 1.94 | 2.17 |
| C2 | 2.30 | 2.31 | 2.24 |
| C1 | 4.09 | 1.23 | 2.20 |
| C2 | 1.11 | 2.73 | 2.07 |
| C1 | 1.16 | 1.91 | 2.21 |
| C1 | 2.64 | 2.97 | 2.28 |
| C4 | 2.68 | 1.95 | 2.35 |
| C4 | 2.61 | 2.68 | 2.07 |

6 Click "C2"

Condition

Class of Interest

C1

Contrast variable

C1

Condition

C1

C2

C3

C4

P-value variable

P-values for Multiple Comparisons

| | 2.71 | 2.12 | 2.20 |
|----|------|------|------|
| C3 | 2.71 | 2.12 | 2.20 |
| C4 | 2.64 | 2.26 | 1.99 |
| C4 | 2.65 | 1.98 | 1.97 |
| C1 | 2.74 | 2.04 | 1.97 |
| C3 | 2.68 | 2.02 | 1.96 |
| C3 | 2.26 | 1.94 | 2.57 |
| C3 | 2.24 | 1.94 | 2.17 |
| C2 | 2.30 | 2.31 | 2.24 |
| C1 | 4.09 | 1.23 | 2.20 |
| C2 | 1.11 | 2.73 | 2.07 |
| C1 | 1.16 | 1.91 | 2.21 |
| C1 | 2.64 | 2.97 | 2.28 |
| C4 | 2.68 | 1.95 | 2.35 |
| C4 | 2.61 | 2.68 | 2.07 |
| C1 | 3.78 | 2.15 | 2.49 |

7 Click "Perform Limma"

Contrast variable
C2

□ Contrast other classes

Log fold-change variable

P-value variable

Adjust P-values for Multiple Comparisons
BH

▶ Perform Limma

| | C1 | C1.T | C2.T | TOT |
|----|------|------|------|-----|
| C3 | 2.68 | 2.02 | 1.96 | |
| C3 | 2.26 | 1.94 | 2.57 | |
| C3 | 2.24 | 1.94 | 2.17 | |
| C2 | 2.30 | 2.31 | 2.24 | |
| C1 | 4.09 | 1.23 | 2.20 | |
| C2 | 1.11 | 2.73 | 2.07 | |
| C1 | 1.16 | 1.91 | 2.21 | |
| C1 | 2.64 | 2.97 | 2.28 | |
| C4 | 2.68 | 1.95 | 2.35 | |
| C4 | 2.61 | 2.68 | 2.07 | |
| C1 | 3.78 | 2.15 | 2.49 | |

8 Click "Results"

Differential Expression Automated Differential Expression Consensus Clustering About

ysis

ort

selected

Use Sample Data

Log2 Transform Log10 Transform

ove

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

Sample Data

| Condition | Protein.1 | Protein.2 | Protein.3 | Protein.4 | Protein.5 |
|-----------|-----------|-----------|-----------|-----------|-----------|
| C1 | 3.09 | 2.03 | 2.22 | 1.52 | 1.48 |
| C1 | 1.11 | 2.11 | 2.21 | 2.18 | 1.87 |
| C2 | 1.17 | 2.00 | 1.83 | 1.90 | 1.91 |
| C1 | 1.10 | 2.02 | 2.70 | 1.96 | 1.55 |
| C3 | 2.69 | 2.34 | 2.49 | 2.77 | 1.70 |
| C3 | 2.71 | 2.12 | 2.20 | 2.49 | 1.91 |
| C4 | 2.64 | 2.26 | 1.00 | 2.17 | 1.62 |

9 Click here.

The screenshot shows a software interface for analyzing gene expression data. On the left, there are input fields for 'Use Sample Data' (checked) and 'Log10 Transform'. The main area displays a table of results with columns: Gene, logFC, AveExpr, t, and PValue. The first row, which has a circled value in the logFC column, is highlighted. Below the table, it says 'Showing 1 to 10 of 10 entries' and provides 'Previous' and 'Next' links.

| Gene | logFC | AveExpr | t | PValue |
|-------------|---|---|--|---------------------|
| Protein.1 | 0.9370492497499991 | 2.20862929345451.408874883424218 | 0.18457063198132720 | 0.16883407997478140 |
| Protein.8 | -0.5617557730416678 | 4.156447826545454-1.465417375018756 | -1.465417375018756 | 0.15078348221267910 |
| Protein.9 | -0.3883712163333342 | 1.325348759636364-1.536069417024783 | -1.536069417024783 | 0.33091337253038030 |
| Protein.2 | -0.2884856054166676 | 2.135455021636364-1.013843322507447 | -1.013843322507447 | 0.17530472450365920 |
| Protein.3 | 0.236516405541666 | 2.2196728796363641.441651775780344 | 1.441651775780344 | 0.7697139251779320 |
| Protein.7 | -0.1958905979583343 | 2.595223139909091-0.98321005333205820.34515501189272570 | -0.98321005333205820.34515501189272570 | 0.52400075282368140 |
| Protein.6 | -0.138486765833344 | 2.285561559454545-0.656575931446834 | -0.656575931446834 | 0.72865094204469760 |
| Protein.5 | 0.05647668787499871 | 1.7935192778181820.3552429230079786 | 0.3552429230079786 | 0.72865094204469760 |
| Protein.100 | 0.04373664579166636 | 2.164099909 | 0.2995447006876253 | 0.7697139251779320 |
| Protein.4 | 0.0033577425833328792.18368493527270.0131852280062833 | 0.98969942121705290 | | |

10 Click "-0.3883712163333342"

This screenshot shows the same software interface after clicking the circled value in the previous step. The table now highlights the entire row corresponding to Protein.9. The circled value '-0.3883712163333342' is now bolded in the logFC column.

| Gene | logFC | AveExpr | t | PValue |
|------------|----------------------------|---|--|---------------------|
| Protein.1 | 0.9370492497499991 | 2.20862929345451.408874883424218 | 0.18457063198132720 | 0.16883407997478140 |
| Protein.8 | -0.5617557730416678 | 4.156447826545454-1.465417375018756 | -1.465417375018756 | 0.15078348221267910 |
| Protein.9 | -0.3883712163333342 | 1.325348759636364 -1.536069417024783 | -1.536069417024783 | 0.33091337253038030 |
| Protein.2 | -0.2884856054166676 | 2.135455021636364-1.013843322507447 | -1.013843322507447 | 0.17530472450365920 |
| Protein.3 | 0.236516405541666 | 2.2196728796363641.441651775780344 | 1.441651775780344 | 0.17530472450365920 |
| Protein.7 | -0.1958905979583343 | 2.595223139909091-0.98321005333205820.34515501189272570 | -0.98321005333205820.34515501189272570 | 0.52400075282368140 |
| Protein.6 | -0.138486765833344 | 2.285561559454545-0.656575931446834 | -0.656575931446834 | 0.52400075282368140 |
| Protein.5 | 0.05647668787499871 | 1.7935192778181820.3552429230079786 | 0.3552429230079786 | 0.72865094204469760 |
| Protein.10 | 0.04373664579166636 | 2.164099909 | 0.2995447006876253 | 0.7697139251779320 |

11 Double-click "0.003357742583332879"

| | | | | |
|------------|----------------------|-------------------|---------------------|--------------|
| Protein.8 | -0.5617557730416678 | 4.156447826545454 | -1.465417375018756 | 0.1688340799 |
| Protein.9 | -0.3883712163333342 | 1.325348759636364 | -1.536069417024783 | 0.1507834822 |
| Protein.2 | -0.2884856054166676 | 2.135455021636364 | -1.013843322507447 | 0.3309133725 |
| Protein.3 | 0.236516405541666 | 2.219672879636364 | 1.441651775780344 | 0.1753047245 |
| Protein.7 | -0.1958905979583343 | 2.595223139909091 | -0.9832100533320582 | 0.3451550118 |
| Protein.6 | -0.1384867658333344 | 2.285561559454545 | -0.656575931446834 | 0.5240007528 |
| Protein.5 | 0.05647668787499871 | 1.793519277818182 | 0.3552429230079786 | 0.7286509420 |
| Protein.10 | 0.04373664579166636 | 2.164099909 | 0.2995447006876253 | 0.7697139251 |
| Protein.4 | 0.003357742583332879 | 2.183684935272727 | 0.0131852280062833 | 0.9896994212 |

Showing 1 to 10 of 10 entries

12 Double-click here.

| | | | | |
|------------|----------------------|-------------------|---------------------|--------------|
| Protein.8 | -0.5617557730416678 | 4.156447826545454 | -1.465417375018756 | 0.1688340799 |
| Protein.9 | -0.3883712163333342 | 1.325348759636364 | -1.536069417024783 | 0.1507834822 |
| Protein.2 | -0.2884856054166676 | 2.135455021636364 | -1.013843322507447 | 0.3309133725 |
| Protein.3 | 0.236516405541666 | 2.219672879636364 | 1.441651775780344 | 0.1753047245 |
| Protein.7 | -0.1958905979583343 | 2.595223139909091 | -0.9832100533320582 | 0.3451550118 |
| Protein.6 | -0.1384867658333344 | 2.285561559454545 | -0.656575931446834 | 0.5240007528 |
| Protein.5 | 0.05647668787499871 | 1.793519277818182 | 0.3552429230079786 | 0.7286509420 |
| Protein.10 | 0.04373664579166636 | 2.164099909 | 0.2995447006876253 | 0.7697139251 |
| Protein.4 | 0.003357742583332879 | 2.183684935272727 | 0.0131852280062833 | 0.9896994212 |

No definition found.
Search the web for "0.003357742583332879" »

Showing 1 to 10 of 10 entries

13 Click "Top 50"

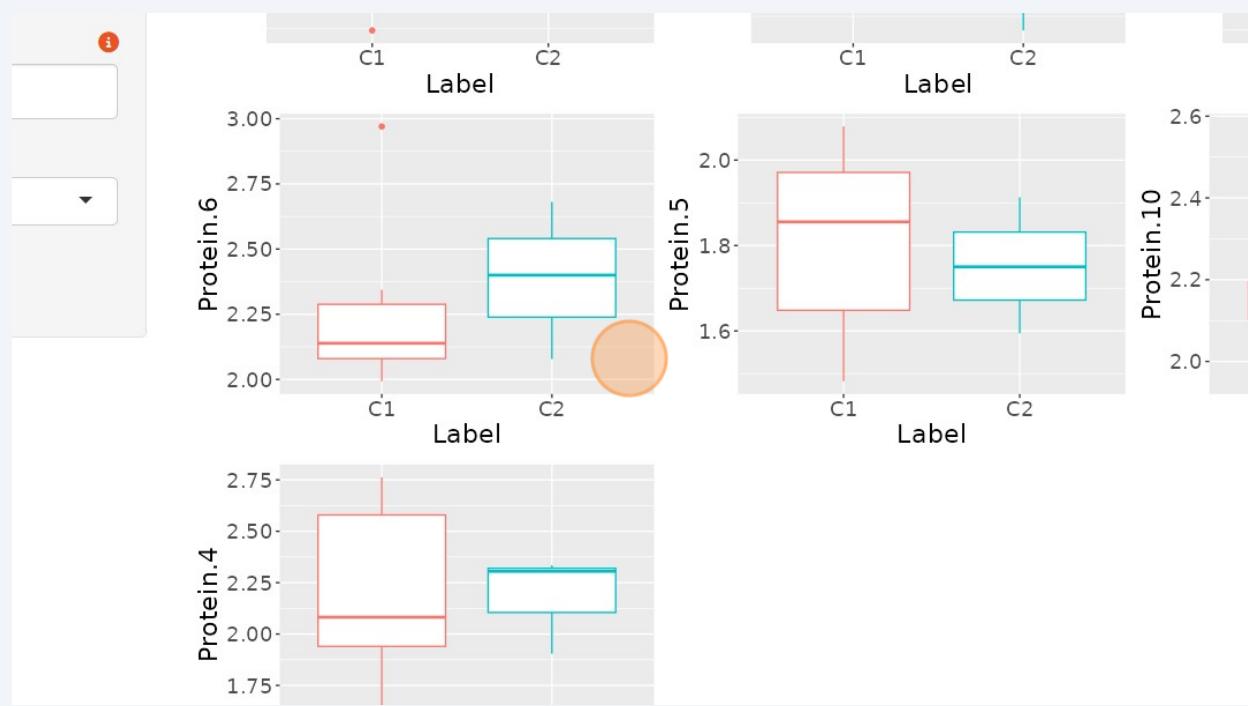
The screenshot shows a software interface for analyzing gene expression data. At the top, there are tabs: Data, Results, Top 50 (which is highlighted with an orange circle), Annotated Volcano Plot, and Heatmap. Below the tabs, there is a search bar with 'Show 10 entries' and buttons for 'Download Current Page' and 'Download Full Results'. A table follows, with columns: Gene, logFC, AveExpr, and t. The table lists several entries, with 'Protein.9' highlighted in blue.

| Gene | logFC | AveExpr | t |
|-----------|---------------------|-------------------|---------------------|
| Protein.1 | 0.9370492497499991 | 2.208629293454545 | 1.408874883424218 |
| Protein.8 | -0.5617557730416678 | 4.156447826545454 | -1.465417375018756 |
| Protein.9 | -0.3883712163333342 | 1.325348759636364 | -1.536069417024783 |
| Protein.2 | -0.2884856054166676 | 2.135455021636364 | -1.013843322507447 |
| Protein.3 | 0.236516405541666 | 2.219672879636364 | 1.441651775780344 |
| Protein.7 | -0.1958905979583343 | 2.595223139909091 | -0.9832100533320582 |
| Protein.6 | -0.1384867658333344 | 2.285561559454545 | -0.656575931446834 |
| Protein.5 | 0.05647668787499871 | 1.793519277818182 | 0.3552429230079786 |

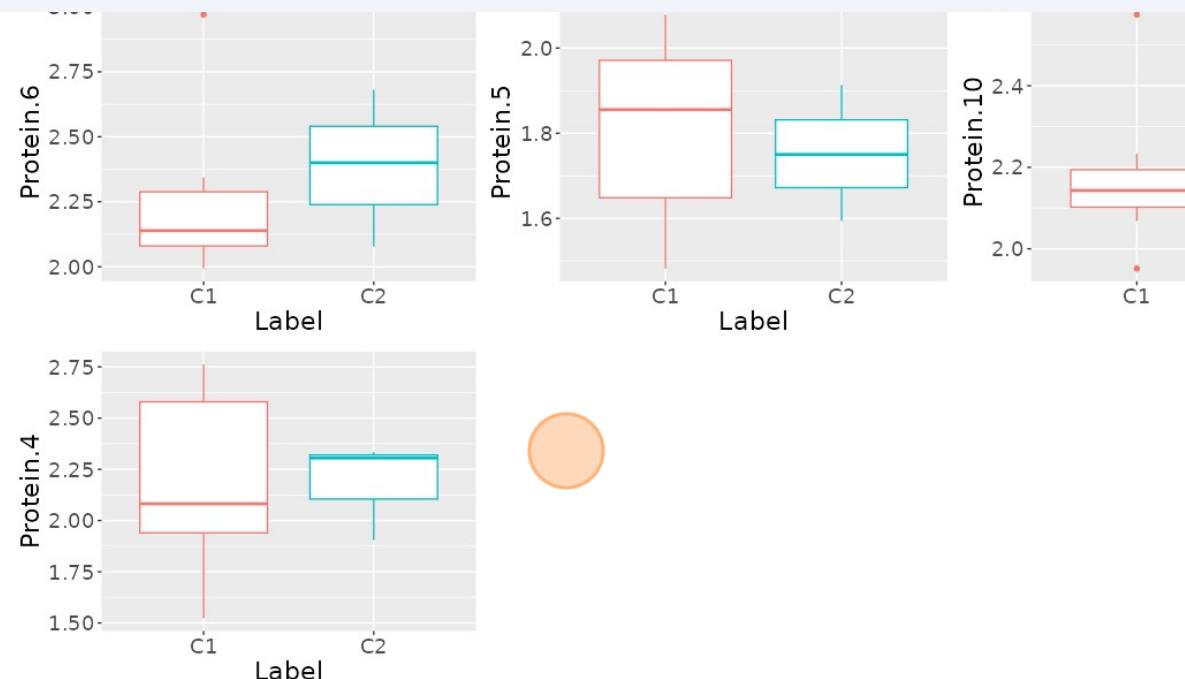
14 Click here.

The screenshot shows a heatmap visualization of gene expression data. On the left, there are dropdown menus and checkboxes for 'Upregulated' and 'Downregulated' genes. The main area displays a grid of colored bars representing expression levels, with a large orange circle highlighting a specific row or column. To the right of the heatmap, there are three vertical red bars.

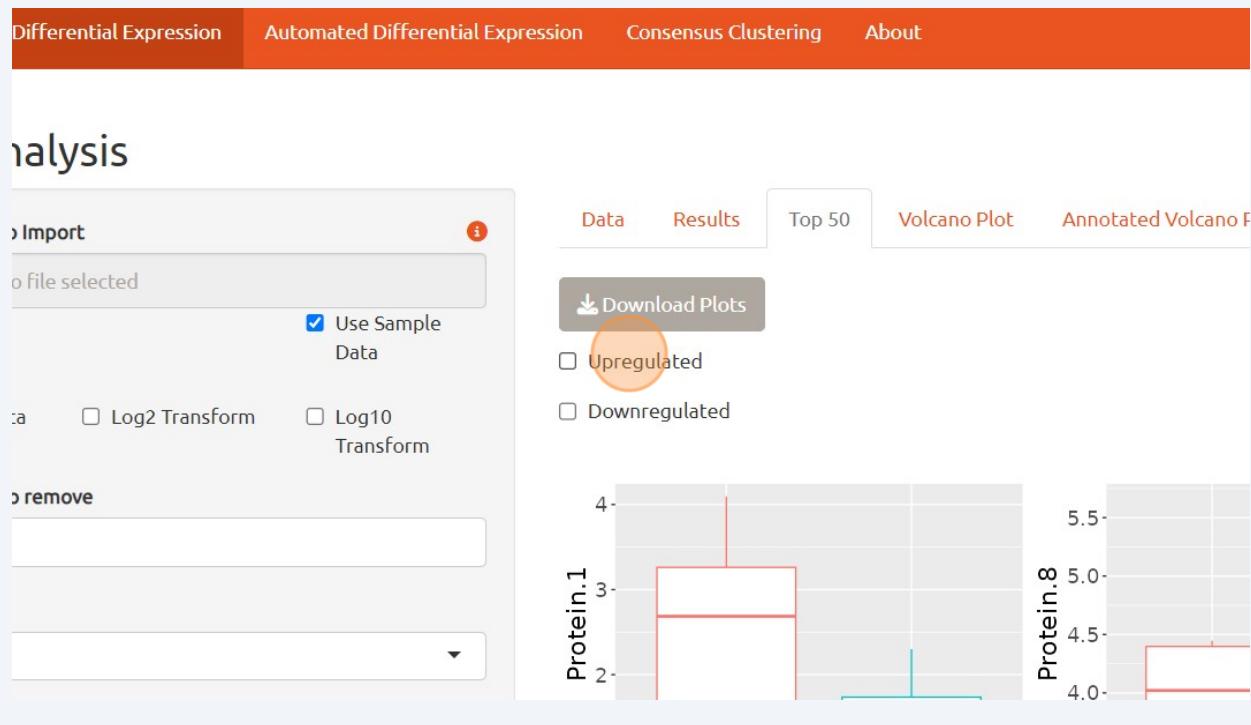
15 Right-click this image.



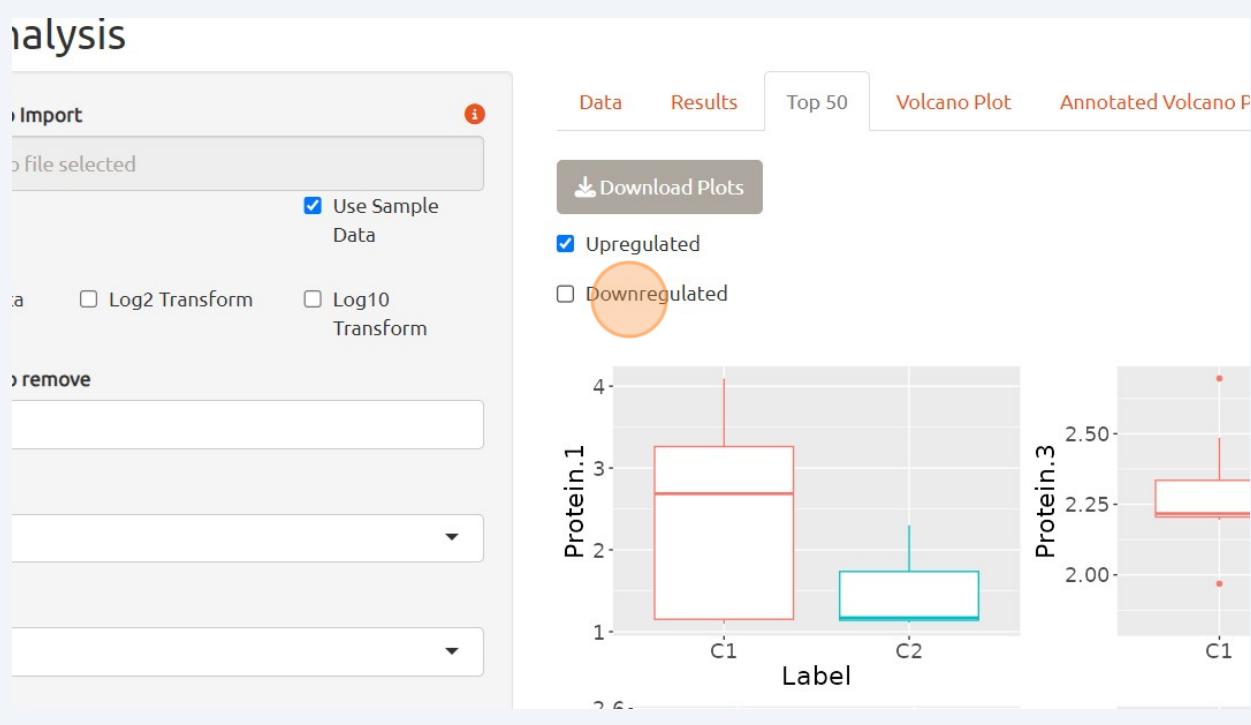
16 Click this image.



17 Click "Upregulated"



18 Click "Downregulated"



19 Click "Upregulated"

lysis

The screenshot shows the 'lysis' interface. On the left, there is a sidebar with sections for 'port' and 'move'. In the 'port' section, there is a dropdown menu with 'selected' and a checked checkbox labeled 'Use Sample Data'. Below it are two unchecked checkboxes: 'Log2 Transform' and 'Log10 Transform'. On the right, there is a navigation bar with tabs: 'Data', 'Results', 'Top 50', 'Volcano Plot' (which is highlighted in orange), and 'Annotated Volcano Plot'. Below the tabs is a button labeled 'Download Plots'. Underneath the button are two checkboxes: 'Upregulated' (which is checked and highlighted with an orange circle) and 'Downregulated' (which is also checked). A red vertical bar is visible on the far right.

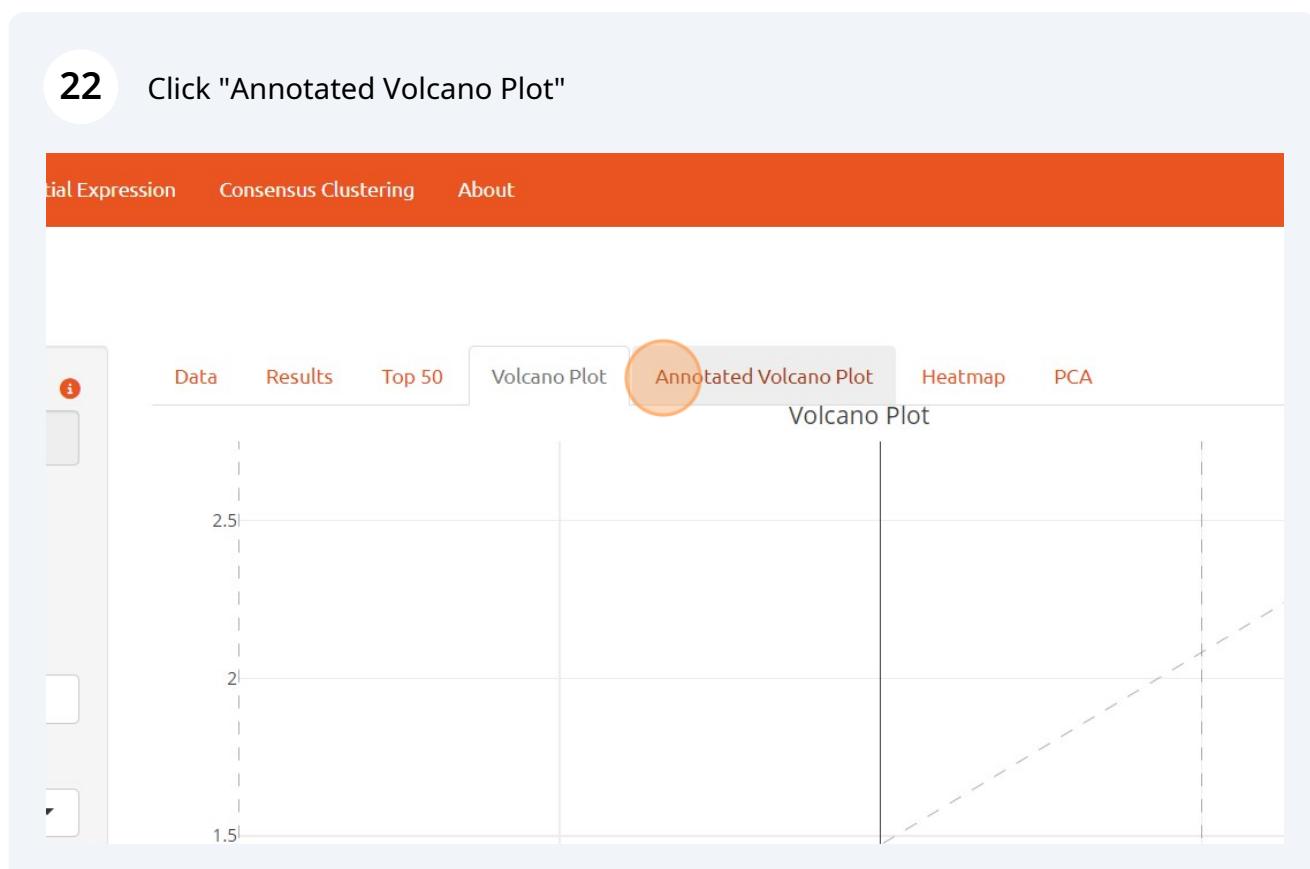
20 Click "Volcano Plot"

The screenshot shows the 'lysis' interface. At the top, there is a red header bar with tabs: 'Annotated Differential Expression', 'Consensus Clustering', and 'About'. Below the header, there is a sidebar with sections for 'port' and 'move'. In the 'port' section, there is a dropdown menu with 'selected' and a checked checkbox labeled 'Use Sample Data'. Below it are two unchecked checkboxes: 'Log10 Transform' and 'Log10 Transform'. On the right, there is a navigation bar with tabs: 'Data', 'Results', 'Top 50', 'Volcano Plot' (which is highlighted in orange), 'Annotated Volcano Plot', 'Heatmap', and 'PCA'. Below the tabs is a button labeled 'Download Plots'. Underneath the button are two checkboxes: 'Upregulated' (unchecked) and 'Downregulated' (checked). To the right of the checkboxes are two volcano plots. The left plot has a y-axis labeled 'Protein.8' ranging from 4.0 to 5.5 and an x-axis labeled 'Protein.2' ranging from 2 to 3. It shows a red box plot at approximately 4.2 and a cyan box plot at approximately 4.5. The right plot has a y-axis labeled 'Protein.9' ranging from 1.6 to 2.4 and an x-axis labeled 'Protein.2' ranging from 2 to 3. It shows a red box plot at approximately 1.6 and a cyan box plot at approximately 1.8. A red vertical bar is visible on the far right.

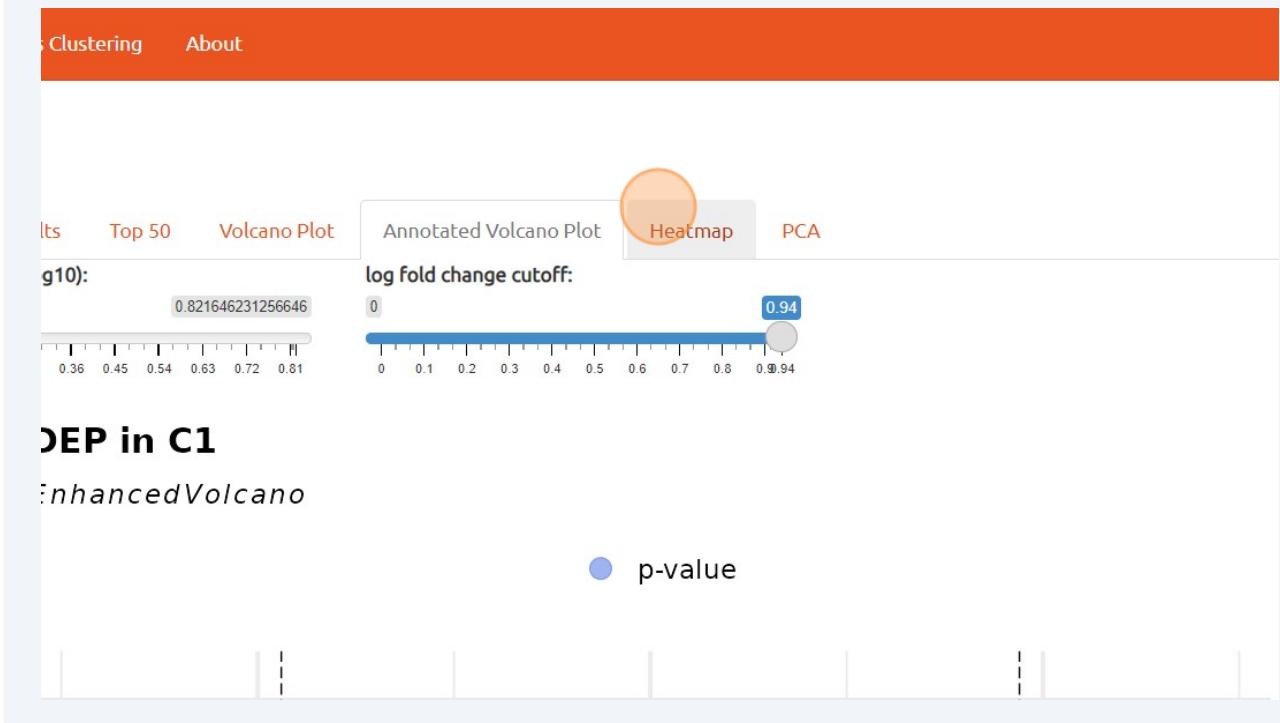
21 Click here.



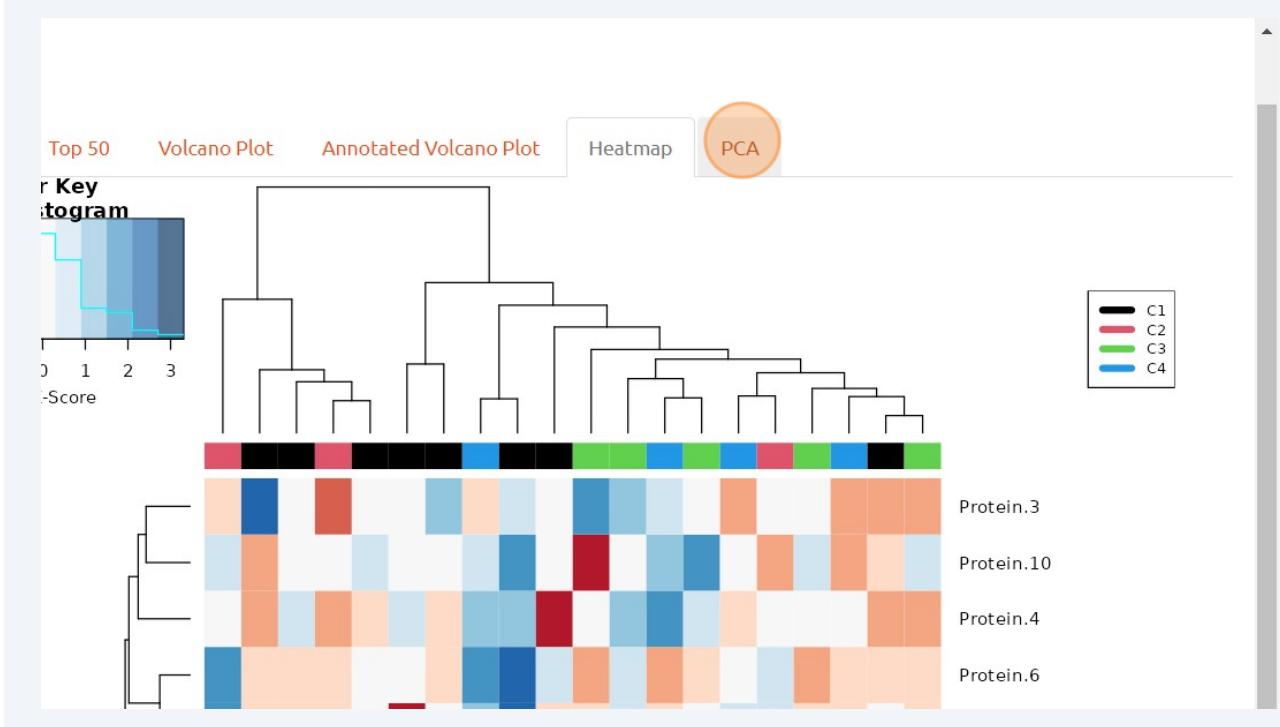
22 Click "Annotated Volcano Plot"



23 Click "Heatmap"



24 Click "PCA"



25 Click "Data"

The screenshot shows a software interface for data analysis. At the top, there is a navigation bar with tabs: Differential Expression, Automated Differential Expression, Consensus Clustering, and About. Below the navigation bar, the word "analysis" is partially visible. On the left side, there is a sidebar with sections for Import (a file is selected) and remove. In the main area, there is a header titled "Protein Functional Group" with a dropdown menu set to "Kinases". A checkbox labeled "Show label" is checked. Below this, a message states "Number of proteins identified as Kinases 536" and "No records to display". At the top of the main area, there are several tabs: Data (which is highlighted with an orange circle), Results, Top 50, Volcano Plot, and Annotated Volcano P.

26 Click "Contrast other classes"

The screenshot shows a configuration interface for contrasts. On the left, there are several dropdown menus and checkboxes: "Class Variable" (set to "Condition"), "Class of interest" (set to "C1"), "Contrast variable" (set to "C2"), and a checkbox "Contrast other classes" which is checked and highlighted with an orange circle. Below these are "Log fold-change variable" (empty), "P-value variable" (empty), and "Adjust P-values for Multiple Comparisons" (set to "BH"). On the right, there is a table with four columns and 16 rows of data. The columns are labeled C3, Z.09, Z.34, and Z.49. The data rows show values for C1 and C2 across these categories.

| | C3 | Z.09 | Z.34 | Z.49 |
|----|------|------|------|------|
| C3 | 2.71 | 2.12 | 2.20 | |
| C4 | 2.64 | 2.26 | 1.99 | |
| C4 | 2.65 | 1.98 | 1.97 | |
| C1 | 2.74 | 2.04 | 1.97 | |
| C3 | 2.68 | 2.02 | 1.96 | |
| C3 | 2.26 | 1.94 | 2.57 | |
| C3 | 2.24 | 1.94 | 2.17 | |
| C2 | 2.30 | 2.31 | 2.24 | |
| C1 | 4.09 | 1.23 | 2.20 | |
| C2 | 1.11 | 2.73 | 2.07 | |
| C1 | 1.16 | 1.91 | 2.21 | |
| C1 | 2.64 | 2.97 | 2.28 | |
| C4 | 2.68 | 1.95 | 2.35 | |
| C4 | 2.61 | 2.68 | 2.07 | |
| C1 | 3.78 | 2.15 | 2.49 | |

27 Click "Perform Limma"

The screenshot shows the 'Perform Limma' dialog box. It includes fields for 'Contrast variable' (set to C2), 'Log fold-change variable' (empty), 'P-value variable' (empty), and 'Adjust P-values for Multiple Comparisons' (set to BH). A checkbox for 'Contrast other classes' is checked. At the bottom is a 'Perform Limma' button, which is circled in orange.

| | C1 | 2.74 | 2.04 | 1.97 |
|----|------|------|------|------|
| C3 | 2.68 | 2.02 | 1.96 | |
| C3 | 2.26 | 1.94 | 2.57 | |
| C3 | 2.24 | 1.94 | 2.17 | |
| C2 | 2.30 | 2.31 | 2.24 | |
| C1 | 4.09 | 1.23 | 2.20 | |
| C2 | 1.11 | 2.73 | 2.07 | |
| C1 | 1.16 | 1.91 | 2.21 | |
| C1 | 2.64 | 2.97 | 2.28 | |
| C4 | 2.68 | 1.95 | 2.35 | |
| C4 | 2.61 | 2.68 | 2.07 | |
| C1 | 3.78 | 2.15 | 2.49 | |

28 Click "Results"

The screenshot shows the 'Results' tab selected in the software interface. On the left, there are transformation options: 'Log2 Transform' (checked) and 'Log10 Transform'. Below these are two dropdown menus. In the center, there are tabs for 'Data', 'Results' (which is highlighted with an orange circle), 'Top 50', 'Volcano Plot', and 'Annotated Volcano Plot'. To the right, there is a 'Sample Data' table.

| Condition | Protein.1 | Protein.2 | Protein.3 | Protein.4 | Protein.5 | Prot |
|-----------|-----------|-----------|-----------|-----------|-----------|------|
| C1 | 3.09 | 2.03 | 2.22 | 1.52 | 1.48 | |
| C1 | 1.11 | 2.11 | 2.21 | 2.18 | 1.87 | |
| C2 | 1.17 | 2.00 | 1.83 | 1.90 | 1.91 | |
| C1 | 1.10 | 2.02 | 2.70 | 1.96 | 1.55 | |
| C3 | 2.69 | 2.34 | 2.49 | 2.77 | 1.70 | |
| C3 | 2.71 | 2.12 | 2.20 | 2.49 | 1.91 | |
| C4 | 2.64 | 2.26 | 1.99 | 2.17 | 1.62 | |
| C4 | 2.65 | 1.98 | 1.97 | 2.40 | 1.83 | |
| C1 | 2.74 | 2.04 | 1.07 | 1.00 | 1.04 | |

29 Click here.

| | | | | | |
|------------|----------------------|---------------|---------------------|--------------------|--------------------|
| Protein.4 | -0.1829883555000009 | 2.29439369655 | -1.152930873344314 | 0.2614643996815371 | 0.8863240 |
| Protein.1 | 0.1516281485833324 | 2.3732112906 | 0.4104419178466993 | 0.6855007626012329 | 0.8863240 |
| Protein.2 | -0.1313824537500015 | 2.1356066015 | -0.8090844396784186 | 0.4272373879667872 | 0.8863240 |
| Protein.8 | 0.131257073458332 | 3.92448746255 | 0.5530134002632651 | 0.585899319678842 | 0.8863240 |
| Protein.3 | 0.1234566694583323 | 2.2101033522 | 1.142880061464126 | 0.265524819827718 | 0.8863240 |
| Protein.7 | -0.05091526545833425 | 2.57234759065 | -0.4169375590204463 | 0.6808142883884303 | 0.8863240 |
| Protein.6 | 0.04525452708333244 | 2.22063972525 | 0.3326233967869173 | 0.7426062118966059 | 0.8863240 |
| Protein.9 | -0.04303892258333386 | 1.24525269055 | -0.2708140624354476 | 0.7890898740405611 | 0.8863240 |
| Protein.10 | 0.02711150987499966 | 2.1597611792 | 0.2595007936198963 | 0.797691627125584 | 0.8863240 |
| Protein.5 | -0.01338923437500106 | 1.8169555515 | -0.1261468353212442 | 0.9007756338538664 | 0.9007756338538664 |

Showing 1 to 10 of 10 entries

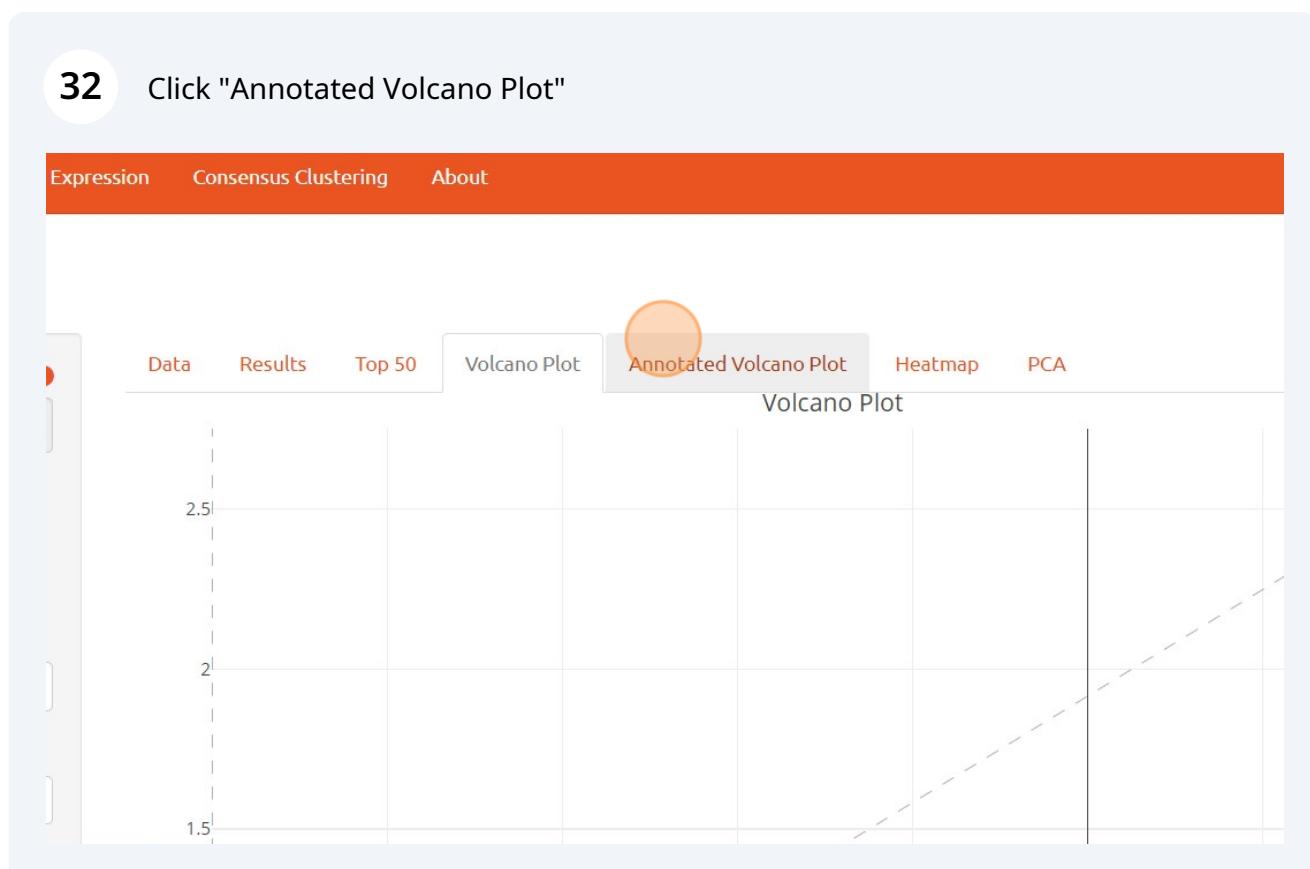
30 Click "Top 50"

| Gene | logFC | AveExpr | t |
|-----------|----------------------|---------------|---------------------|
| Protein.4 | -0.1829883555000009 | 2.29439369655 | -1.152930873344314 |
| Protein.1 | 0.1516281485833324 | 2.3732112906 | 0.4104419178466993 |
| Protein.2 | -0.1313824537500015 | 2.1356066015 | -0.8090844396784186 |
| Protein.8 | 0.131257073458332 | 3.92448746255 | 0.5530134002632651 |
| Protein.3 | 0.1234566694583323 | 2.2101033522 | 1.142880061464126 |
| Protein.7 | -0.05091526545833425 | 2.57234759065 | -0.4169375590204463 |
| Protein.6 | 0.04525452708333244 | 2.22063972525 | 0.3326233967869173 |

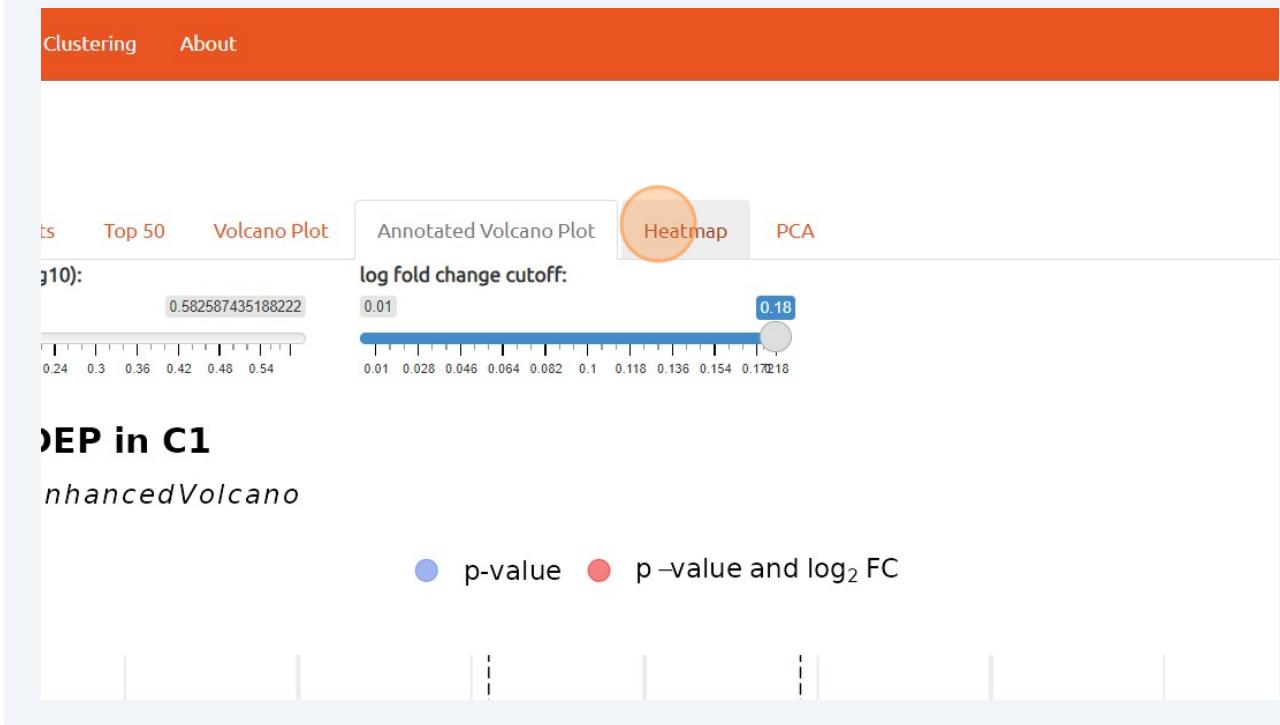
31 Click "Volcano Plot"



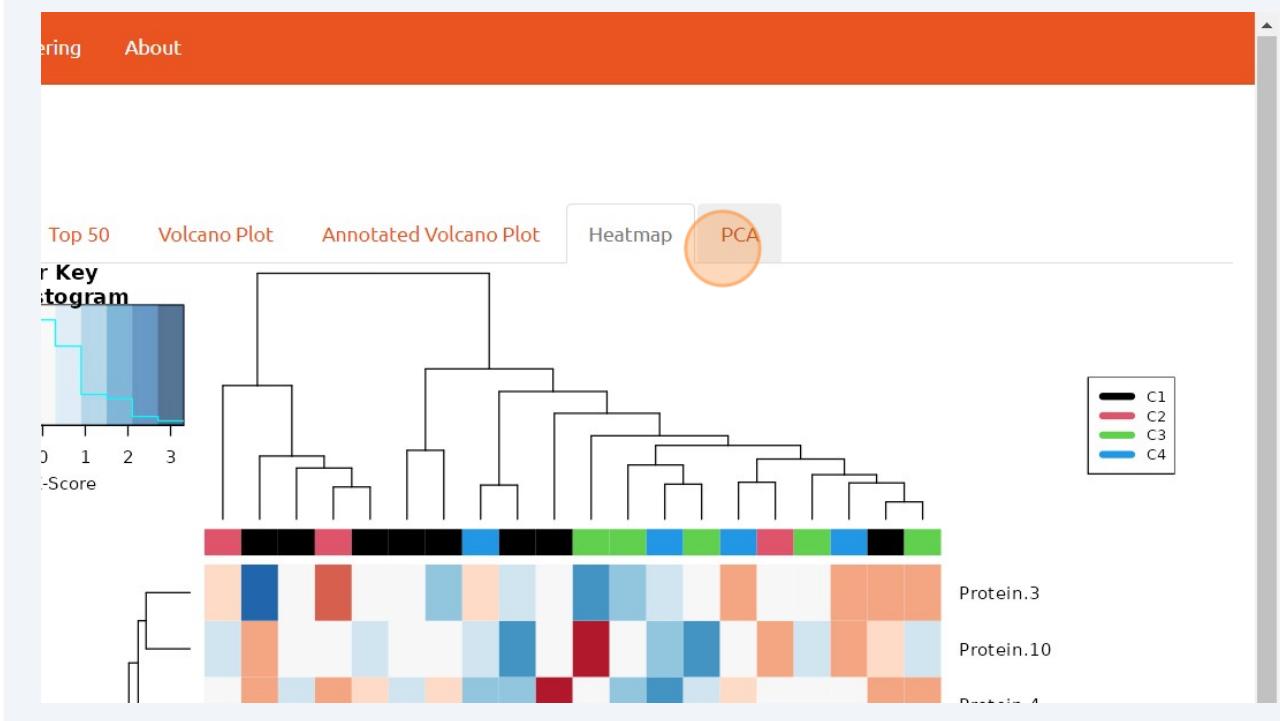
32 Click "Annotated Volcano Plot"



33 Click "Heatmap"



34 Click "PCA"



35 Click "Automated Differential Expression"

The screenshot shows the FlexStat 1.0 web application. At the top, there is a navigation bar with tabs: FlexStat 1.0, Differential Expression, Automated Differential Expression (which is highlighted with a red circle), Consensus Clustering, and About. Below the navigation bar, the title "Limma Analysis" is displayed. On the left side, there is a form titled "Select CSV File to Import" with a "Browse..." button and a message "No file selected". There are also checkboxes for "Show head", "Transpose data", "Log2 Transform", and "Log10 Transform". A section for "Select columns to remove" and a "Class Variable" dropdown set to "Condition" are also present. On the right side, there are tabs for Data, Results, Top 50, and Volcano Plot. Under "Protein Functional Group", a dropdown is set to "Kinases". Below it, a message states "Number of proteins identified as Kinases 536" and "No records to display".

36 Click "Use Sample Data"

The screenshot shows the FlexStat 1.0 web application. At the top, there is a navigation bar with tabs: FlexStat 1.0, Differential Expression, Automated Differential Expression (highlighted with a red circle), Consensus Clustering, and About. Below the navigation bar, the title "Combinatory Limma Analysis" is displayed. On the left side, there is a form titled "Select CSV File to Import" with a "Browse..." button and a message "No file selected". There is a checkbox for "Use Sample Data" which is highlighted with a red circle. A section for "Class Variable" with a dropdown set to "Not Selected" is also present. On the right side, there is a table titled "Sample Data" with four columns: Condition, Protein.1, Protein.2, and Protein.3. The data is as follows:

| Condition | Protein.1 | Protein.2 | Protein.3 |
|-----------|-----------|-----------|-----------|
| C1 | 3.09 | 2.03 | 2.22 |
| C1 | 1.11 | 2.11 | 2.21 |
| C2 | 1.17 | 2.00 | 1.83 |
| C1 | 1.10 | 2.02 | 2.70 |
| C3 | 2.69 | 2.34 | 2.49 |
| C3 | 2.71 | 2.12 | 2.20 |
| C4 | 2.64 | 2.26 | 1.99 |
| C4 | 2.65 | 1.98 | 1.97 |

37 Click here.

Community Edition / Analysis

Select CSV File to Import

Browse... No file selected

Use Sample Data

Class Variable

Not Selected

Log fold-change variable

P-value variable

Adjust P-values for Multiple Comparisons

BH

Sample Data

| Condition | Protein.1 | Protein.2 | Protein.3 |
|-----------|-----------|-----------|-----------|
| C1 | 3.09 | 2.03 | 2.22 |
| C1 | 1.11 | 2.11 | 2.21 |
| C2 | 1.17 | 2.00 | 1.83 |
| C1 | 1.10 | 2.02 | 2.70 |
| C3 | 2.69 | 2.34 | 2.49 |
| C3 | 2.71 | 2.12 | 2.20 |
| C4 | 2.64 | 2.26 | 1.99 |
| C4 | 2.65 | 1.98 | 1.97 |
| C1 | 2.74 | 2.04 | 1.97 |
| C3 | 2.68 | 2.02 | 1.96 |
| C3 | 2.26 | 1.94 | 2.57 |
| C3 | 2.24 | 1.94 | 2.17 |

38 Click "Condition"

Select CSV File to Import

Browse... No file selected

Use Sample Data

Class Variable

Not Selected

Not Selected

Condition

P-value variable

Adjust P-values for Multiple Comparisons

BH

Sample Data

| Condition | Protein.1 | Protein.2 | Protein.3 |
|-----------|-----------|-----------|-----------|
| C1 | 3.09 | 2.03 | 2.22 |
| C1 | 1.11 | 2.11 | 2.21 |
| C2 | 1.17 | 2.00 | 1.83 |
| C1 | 1.10 | 2.02 | 2.70 |
| C3 | 2.69 | 2.34 | 2.49 |
| C3 | 2.71 | 2.12 | 2.20 |
| C4 | 2.64 | 2.26 | 1.99 |
| C4 | 2.65 | 1.98 | 1.97 |
| C1 | 2.74 | 2.04 | 1.97 |
| C3 | 2.68 | 2.02 | 1.96 |
| C3 | 2.26 | 1.94 | 2.57 |
| C3 | 2.24 | 1.94 | 2.17 |
| C2 | 2.30 | 2.31 | 2.24 |

39 Click "Perform Auto Limma"

Log fold-change variable i

P-value variable i

Adjust P-values for Multiple Comparisons

BH

▶ Perform Auto Limma



| | | | |
|----|------|------|------|
| C3 | 2.69 | 2.34 | 2.49 |
| C3 | 2.71 | 2.12 | 2.20 |
| C4 | 2.64 | 2.26 | 1.99 |
| C4 | 2.65 | 1.98 | 1.97 |
| C1 | 2.74 | 2.04 | 1.97 |
| C3 | 2.68 | 2.02 | 1.96 |
| C3 | 2.26 | 1.94 | 2.57 |
| C3 | 2.24 | 1.94 | 2.17 |
| C2 | 2.30 | 2.31 | 2.24 |
| C1 | 4.09 | 1.23 | 2.20 |
| C2 | 1.11 | 2.73 | 2.07 |
| C1 | 1.16 | 1.91 | 2.21 |
| C1 | 2.64 | 2.97 | 2.28 |
| C4 | 2.68 | 1.95 | 2.35 |
| C4 | 2.61 | 2.68 | 2.07 |

40 Click the "P-value variable" field.

USE SAMPLE Data

Class Variable

Condition

Log fold-change variable i

P-value variable i

▶ Perform Auto Limma



C1 vs C2
C1 vs C3
C1 vs C4
C2 vs C3
C2 vs C4
C3 vs C4
C1+C2+C4 vs C3
C1+C2+C3 vs C4
C1+C3+C4 vs C2
C2+C3+C4 vs C1
C1+C4 vs C2+C3
C1+C2 vs C3+C4
C1+C3 vs C2+C4

Show 10 entries

| Combination | Gene |
|-------------|-----------|
| | Protein.4 |
| | Protein.3 |
| | Protein.2 |

41 Type "0.1"

42 Click "Perform Auto Limma"

Use sample Data

Class Variable
Condition

Log fold-change variable

P-value variable
0.1

Adjust P-values for Multiple Comparisons
BH

 Perform Auto Limma

C1 vs C2
C1 vs C3
C1 vs C4
C2 vs C3
C2 vs C4
C3 vs C4
C1+C2+C4 vs C3
C1+C2+C3 vs C4
C1+C3+C4 vs C2
C2+C3+C4 vs C1
C1+C4 vs C2+C3
C1+C2 vs C3+C4
C1+C3 vs C2+C4

Show 10 entries

| Combination | Gene |
|-------------|-----------|
| | Protein.4 |
| | Protein.3 |

43 Click "Consensus Clustering"

The screenshot shows the "Auto limma Results" section of a bioinformatics tool. At the top, there are two download buttons: "Download Top 50" and "Download All". Below these buttons is a list of 12 comparison pairs:

- C1 vs C2
- C1 vs C3
- C1 vs C4
- C2 vs C3
- C2 vs C4
- C3 vs C4
- C1+C2+C4 vs C3
- C1+C2+C3 vs C4
- C1+C3+C4 vs C2
- C2+C3+C4 vs C1
- C1+C4 vs C2+C3

44 Click "Use Sample Data"

The screenshot shows the "Perform Consensus Clustering" page. On the left, there is a configuration panel with the following settings:

- Select CSV File:** A "Browse..." button and a text input field showing "No file selected".
- Use Sample Data:** A checkbox labeled "Use Sample Data" is checked.
- Maximum No. of Clusters:** A slider set to 6, with a range from 2 to 15.
- Clust Algorithm:** A dropdown menu set to "hc".
- Distance Measurement:** A dropdown menu showing an empty list.

On the right, there is a "Sample Data" table with columns X, S1, S2, S3, S4, and S5. The data rows are:

| X | S1 | S2 | S3 | S4 | S5 |
|-----|-------|-------|-------|-------|-------|
| P1 | -2.35 | -1.22 | -0.55 | 0.28 | 0.78 |
| P2 | -0.52 | -0.42 | 1.20 | 1.64 | 2.43 |
| P3 | 1.31 | 0.25 | 0.93 | 2.17 | 1.16 |
| P4 | -2.01 | -1.95 | -0.75 | -2.17 | -1.83 |
| P5 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| P6 | -0.33 | 0.07 | 0.63 | 2.06 | 2.71 |
| P7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| P8 | -0.74 | -0.46 | 0.88 | 0.19 | 1.26 |
| ... | ... | ... | ... | ... | ... |

45 Click "Perform Clustering"

The screenshot shows the 'Consensus Clustering' interface. On the left, there are three dropdown menus: 'Distance Measurement' set to 'pearson', 'Inner Linkage' set to 'ward.D2', and 'Final Linkage' set to 'ward.D2'. Below these is a large orange button labeled '► Perform Clustering'. To the right is a table with 20 rows, each representing a sample labeled P7 through P20, with numerical values for six columns. At the bottom right is a 'Download Results' button.

| | P7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|-----|-------|-------|-------|-------|------|------|
| P8 | -0.74 | -0.46 | 0.88 | 0.19 | 1.26 | |
| P9 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| P10 | -1.00 | 0.37 | 0.72 | 2.76 | 2.89 | |
| P11 | -0.71 | -1.28 | -0.23 | 0.45 | 1.02 | |
| P12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| P13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| P14 | -0.77 | -0.89 | 0.06 | 0.16 | 1.04 | |
| P15 | -2.34 | 0.52 | 0.49 | -1.13 | 1.74 | |
| P16 | -0.56 | -0.70 | 0.59 | 1.99 | 2.71 | |
| P17 | -0.15 | -0.02 | 0.41 | 0.50 | 1.18 | |
| P18 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| P19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| P20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |

Download Results

46 Click "About"

The screenshot shows the 'About' page of the application. At the top, there is a navigation bar with three tabs: 'Automated Differential Expression', 'Consensus Clustering', and 'About', where 'About' is highlighted with a yellow circle. Below the navigation bar, the word 'stering' is partially visible. On the left, there is a sidebar with a dropdown menu and a slider set to 15. In the center, there is a 'Download Results' button and a message stating 'Output files are in /home/shiny/consensus_plots_2024-01-03_hc_pearson_11232'. To the right is a heatmap titled 'consensus matrix legend' with a color scale from 0.6 (dark blue) to 1.0 (light blue).

Output files are in /home/shiny/consensus_plots_2024-01-03_hc_pearson_11232

consensus matrix legend

| | 1 | 0.9 | 0.8 | 0.7 | 0.6 |
|--|---|-----|-----|-----|-----|
|--|---|-----|-----|-----|-----|