

“How To ViolinBox”

Introduction

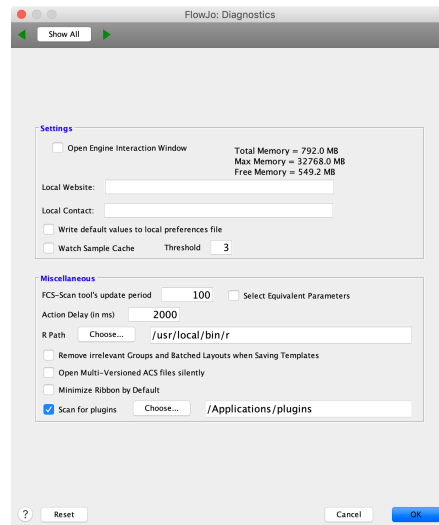
Violin, box and whisker plots, and heatmaps are all popular ways of illustrating expression patterns between populations genes or proteins of interest. The plots available in this plugin include a ‘half violin’, jittered data points, and a standard set of the boxplot and violins. The plugin also offers a heatmap function, Spearman correlation plots and some simple statistical tests. This plugin has some dependencies in R.

Setting Up the ViolinBox Plugin

There are detailed instructions on how to set up plugins in our [online documentation](#).

The ViolinBox plugin can be set up with the following steps:

1. Place the ViolinBox JAR file into a “plugins” folder.
2. Ensure that the [R program] (<https://cran.r-project.org/>) is installed on the computer.
3. Go to the Preferences (heart) icon in FlowJo or SeqGeq and select Diagnostics.
4. Select the plugins folder.
5. Set the correct R path in the R Path field.



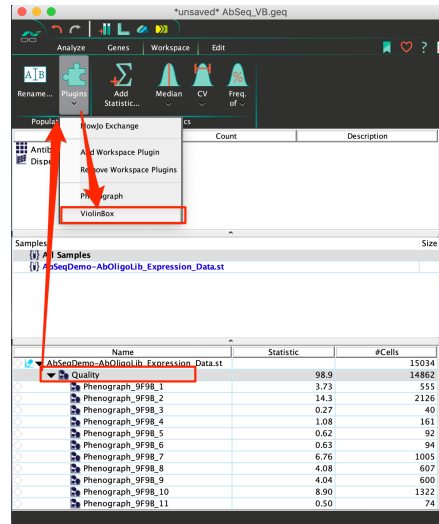
6. Restart the application.
7. When first running the ViolinBox plugin it should automatically install required R packages.

If you would like to manually install the R dependencies, the commands below can be copy/pasted into a R console:

```
install.packages(c("shiny", "shinydashboard", "shinyBS", "shinybusy", "readr", "dplyr",  
                  "viridisLite", "DT", "ggplot2", "cowplot", "RColorBrewer", "ggpubr",  
                  "shinyWidgets", "ggsignif", "broom", "gginnards", "tidyverse",  
                  "gtools", "plotly", "heatmaply"))
```

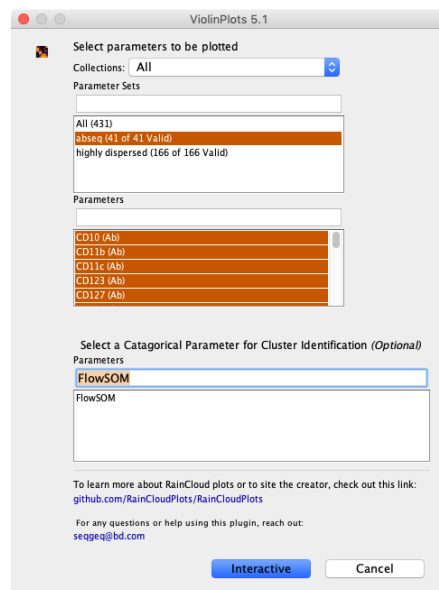
Using the ViolinBox Plugin

After performing analysis and isolating populations of interest the ViolinBox plugin can be used to take a further look at the populations identified there within categorical parameters. Select a population to analyze and choose ViolinBox from the plugin drop down menu.



A parameter selector window will appear. Choose the parameters to include in the violin plots. It is better to select all of your parameters here as you can choose to not plot them later once the interactive Shiny application opens in your web browser. There is also an option to select a “categorical parameter” such as a clustering parameter or one that you created during the concatenation of populations.

At the bottom of the parameter window are options to press “Interactive” or “Cancel”.



Interactive

The “Interactive” option will launch an interactive [Shiny](#) application within the computer’s web browser. This interactive window has further options for formatting the ViolinBox plots and displaying data. On the left-hand side of the webpage are tabs that navigate to the “Data”, “Heatmaps”, “Raincloud”, “Correlation Plots” plot types, as well as an “Options” section for additional customizations.

The “Data” tab of the web browser displays a table of the mean parameter intensities. Categorical parameter are shown as rows. If no categorical is chosen, the rows will represent the individual events. There are also options to copy this table so you can paste it into a spreadsheet or to download the table as a .csv file. The left-hand bar has options under the “Data” tab for selecting parameters and populations to include in figures.

ViolinBox

RECALCULATE

COPY

Data

Heatmaps

Rainclouds

Correlation_Plots

Options

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CD11b
(Ab)

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CD123
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|------------|-------|---------|---------|--------|-------|--------|--------|---------|-------|--------|-------|--------|------|-------|-------|--------|-------|-------|------|--------|--------|--------|
| FlowSOM_0 | 2.12 | 6.88 | 9.3 | 3.67 | 70.21 | 16.08 | 4.64 | 27.26 | 6.41 | 54.77 | 2.97 | 0.82 | 7.41 | 20.62 | 10.47 | 1.8 | 6.28 | 3.48 | 12.5 | 217.87 | 125.88 | 159.92 |
| FlowSOM_1 | 1.2 | 8.75 | 8.27 | 2.01 | 38.91 | 10.24 | 4.67 | 24.09 | 5.3 | 32.96 | 3.73 | 0.67 | 2.42 | 9.94 | 6.55 | 1.46 | 5.66 | 3.02 | 6.83 | 235.38 | 65.07 | 76.08 |
| FlowSOM_2 | 11.03 | 76.91 | 483.91 | 224.09 | 5.6 | 24.54 | 87.78 | 31.45 | 3.73 | 57.78 | 0.51 | 0.81 | 3.78 | 16.09 | 6.36 | 1.64 | 17.58 | 4.68 | 1.97 | 14.55 | 80.46 | 7.62 |
| FlowSOM_3 | 6.69 | 536.55 | 450.44 | 15.2 | 5.78 | 52.42 | 457 | 140.76 | 3.82 | 14.47 | 0.41 | 0.99 | 3.91 | 4.57 | 3.99 | 1.78 | 13.81 | 4.02 | 2.04 | 4.41 | 15.51 | 2.7 |
| FlowSOM_4 | 17.17 | 1321.36 | 796.88 | 14.81 | 8.33 | 128.36 | 981.89 | 61.58 | 5.64 | 21.74 | 0.61 | 1.41 | 6.86 | 9.11 | 6.99 | 2.76 | 17.16 | 6.72 | 3.7 | 6.87 | 32.56 | 4.46 |
| FlowSOM_5 | 19.98 | 24.31 | 59.65 | 11.22 | 36.1 | 31.27 | 22.96 | 69.69 | 10.78 | 103.22 | 95.04 | 172.04 | 12.1 | 87.9 | 13.45 | 115.04 | 31.29 | 67.92 | 8.88 | 321.31 | 338.88 | 68.78 |
| FlowSOM_6 | 5.05 | 164.87 | 1163.51 | 45.48 | 16.62 | 63.72 | 116.4 | 1590.18 | 4.76 | 32.54 | 0.55 | 1.14 | 5 | 4.69 | 6.03 | 2.98 | 17.71 | 5.46 | 1.85 | 8.17 | 10.45 | 3.51 |
| FlowSOM_7 | 1.85 | 20.4 | 14.59 | 2.24 | 32.1 | 13.18 | 7.03 | 111.78 | 7.75 | 40.18 | 2.37 | 1.24 | 3.09 | 10.39 | 6.38 | 2.28 | 11.32 | 5.35 | 3.97 | 166.55 | 51.94 | 49.98 |
| FlowSOM_8 | 1.53 | 15.49 | 10.43 | 1.8 | 45.56 | 14.73 | 5.77 | 68.77 | 12.64 | 44.78 | 2.88 | 0.8 | 2.95 | 11.76 | 9 | 2.52 | 15.92 | 3.88 | 5.68 | 273.2 | 73.03 | 81.78 |
| FlowSOM_9 | 6 | 35.7 | 64.3 | 3.4 | 21.35 | 22.6 | 13 | 266.35 | 15.35 | 30.2 | 2.25 | 1.35 | 4.35 | 9.15 | 6.6 | 3.05 | 24.4 | 13.1 | 1.85 | 74.25 | 33.8 | 29.45 |
| FlowSOM_10 | 6.5 | 11.71 | 26.21 | 1 | 25.1 | 8.7 | 2.61 | 55.36 | 21.09 | 45.17 | 0.62 | 0.83 | 0.93 | 9.6 | 2.35 | 1.57 | 4.48 | 2.94 | 1.29 | 146.07 | 27.82 | 34.96 |
| FlowSOM_11 | 2.17 | 53.34 | 54.36 | 1.3 | 7.24 | 6.51 | 5.3 | 887.21 | 10.04 | 17.72 | 0.28 | 0.6 | 1.12 | 1.57 | 1.63 | 1.45 | 6.34 | 2.23 | 0.99 | 7.75 | 4.61 | 1.45 |
| FlowSOM_12 | 5.19 | 95.95 | 107.99 | 6.31 | 41.14 | 29.1 | 66.22 | 150.57 | 14.68 | 71.82 | 1.38 | 1.9 | 6.71 | 17.83 | 14.47 | 3.91 | 33.65 | 10.05 | 3.1 | 312.01 | 80.34 | 60.34 |
| FlowSOM_13 | 0.96 | 9.23 | 8.27 | 1.34 | 28.57 | 9.83 | 3.18 | 36.22 | 15.42 | 38.17 | 0.8 | 0.8 | 1.37 | 8.34 | 7.38 | 2.67 | 18.11 | 2.87 | 1.6 | 201.88 | 41.17 | 41.4 |
| FlowSOM_14 | 2.27 | 52.81 | 44.65 | 1.66 | 11.91 | 7.52 | 3.48 | 924.57 | 16.01 | 19.76 | 0.41 | 0.54 | 0.99 | 5.8 | 1.73 | 1.56 | 8.79 | 2.1 | 1.05 | 27.41 | 7.75 | 11.56 |

Download

Previous

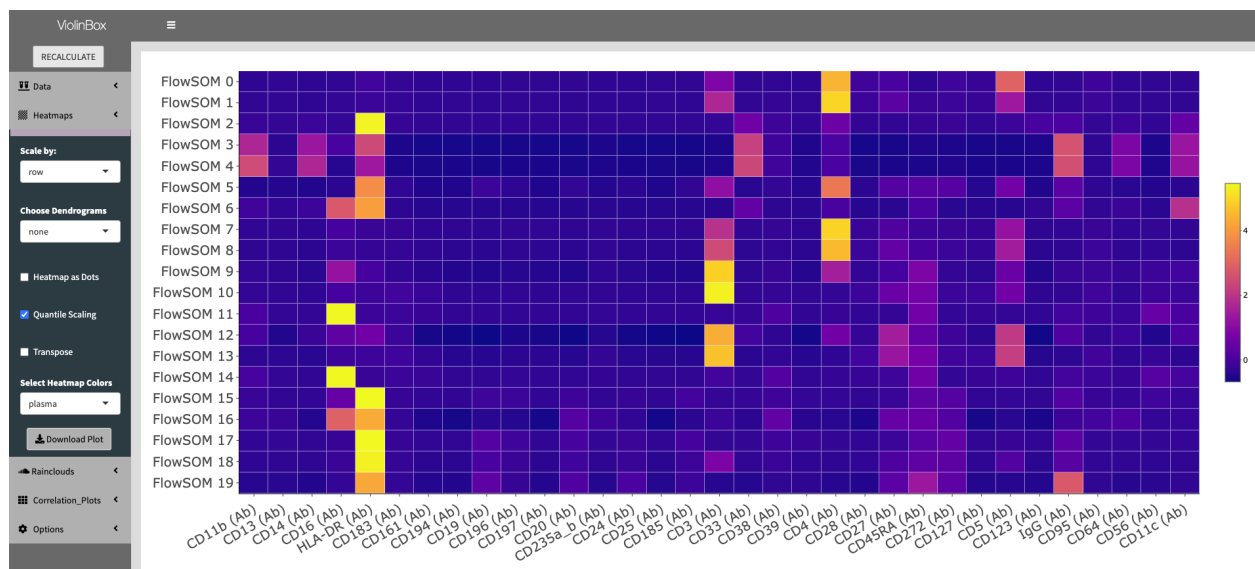
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Next

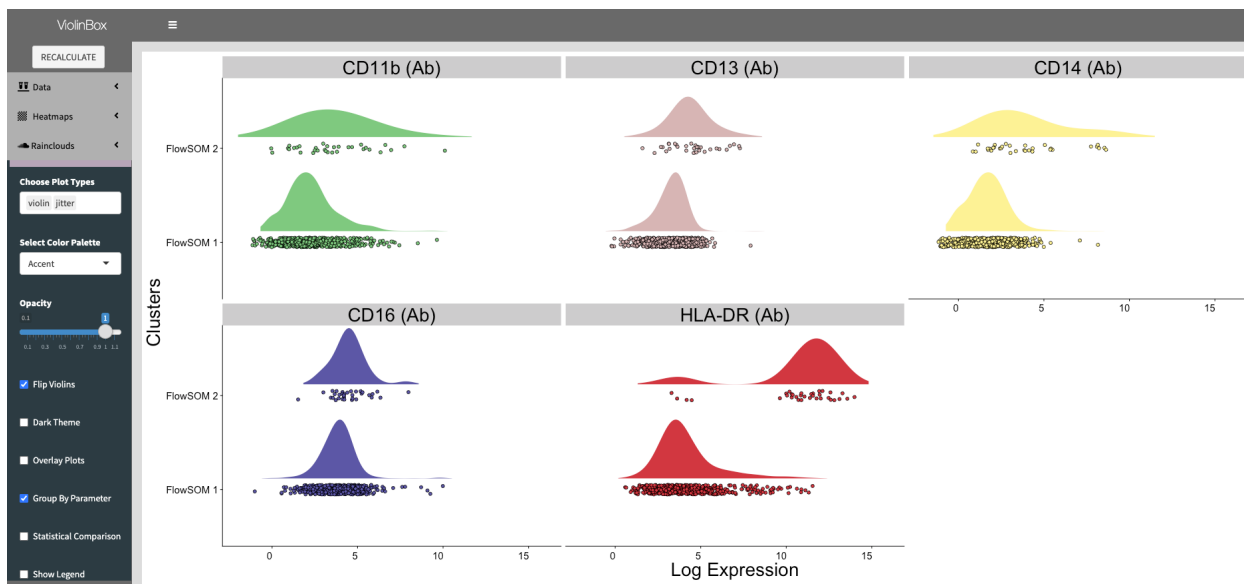
Heatmap

The “Heatmap” tab can be used to format and generate heatmaps. Color, scaling, fonts, and dendrogram options are available on the left. The “Recalculate” button generates the heatmap with the selected options using the data selected in the “Data” tab. The image can be saved to the computer by clicking the camera icon in the upper right side of the heatmap. This will download a high resolution .svg image. You can also click the ‘Download Plot’ button on the left side-bar to download an interactive .html file of your heatmap.



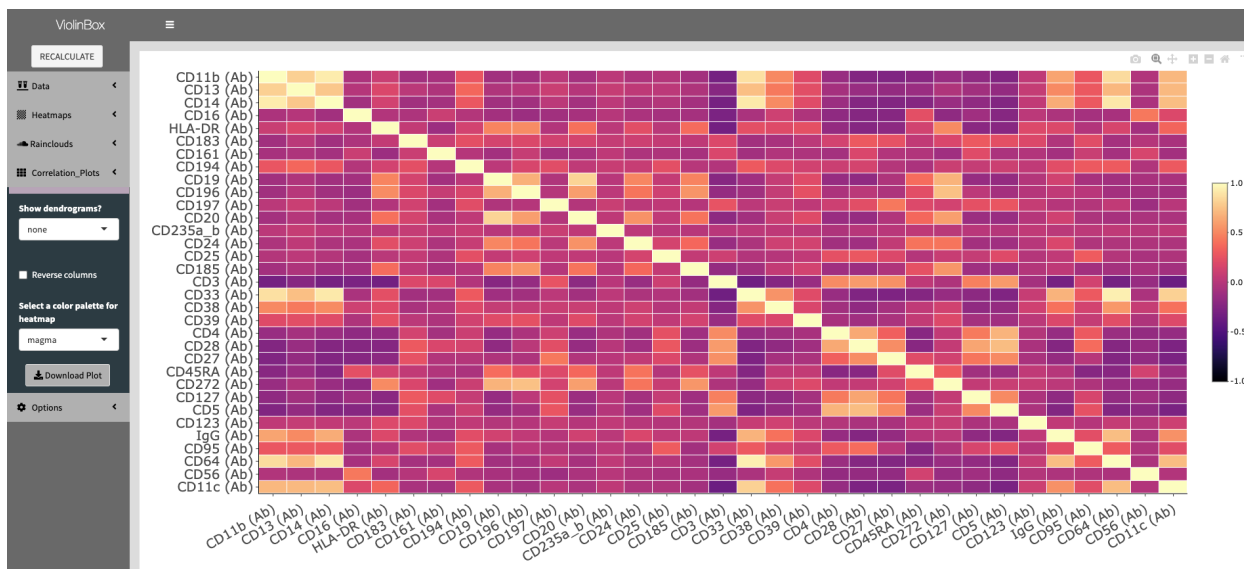
Rainclouds

The “Rainclouds” tab can be used to format and generate Violin plots, Raincloud plots, and Box-n-Whisker plots. On the left-hand side there are options for selecting the plot types and formatting. The “Choose plot type” box can be used to add and remove plot displays. The “Recalculate” button generates the plots with the selected options using the data selected in the “Data” tab. Images generated in the browser can be saved to the computer by right clicking on the graphic or clicking the Download Plot button.



Correlations

The “Correlation Plots” tab can be used to format and generate correlation plots of the selected parameters. The default method to calculate the correlation between the columns and rows is the Spearman’s rank correlation coefficient. Dendrogram, column order and color options are available on the left. The “Recalculate” button generates the correlation heatmap with the selected options using the data selected in the “Data” tab. The image can be saved to the computer by clicking the camera icon in the upper right side of the correlation heatmap. This will download a high resolution .svg image. You can also click the ‘Download Plot’ button on the left side-bar to download an interactive .html file of your correlation heatmap.



Closing the interactive webpage will delete the ViolinBox plot node in the workspace. Be sure to save any images before closing the webpage.

Note

- * The parameters or genes selected for plotting will be log scaled.
- * Selecting both the uncompensated & compensated options for the same parameter will cause an error.
- * Selecting a non-categorical parameter for the categorical parameter will cause an error. Appropriate categorical parameters are cluster parameters like XShift, Phenograph, FlowSOM, or parameterized keywords created during export & concatenation steps.
- * A keyword from the FlowJo workspace can be turned into a parameter to use as a categorical parameter by exporting populations of interest, adding a keyword to identify the samples, then concatenating the populations together including the keyword, while in the concatenate dialog window.
- * TIP When concatenating your samples together, uncheck the “spread distribution of keyword data” checkbox.

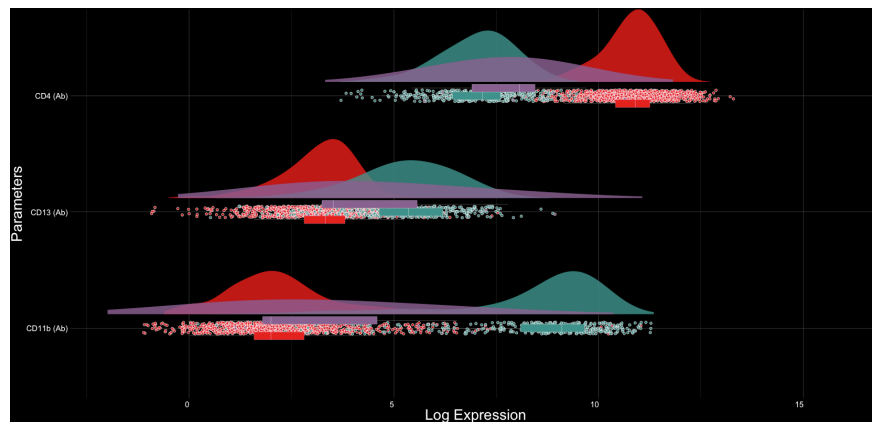


Figure 1: Here is a plot with all options selected. Showing the Half-Violin plot with Boxes and Jitter Points (Rain) below.

Leave us your feedback

Please write to flowjo@bd.com with any questions!

References

1. Allen M, Poggiali D, Whitaker K, Marshall TR, Kievit R. 2018. Raincloud plots: a multi-platform tool for robust data visualization. PeerJ Preprints 6:e27137v1 <https://doi.org/10.7287/peerj.preprints.27137v1>
2. Galili, Tal, O’Callaghan, Alan, Sidi, Jonathan, Sievert, Carson (2017). “heatmaply: an R package for creating interactive cluster heatmaps for online publishing.” Bioinformatics. doi: 10.1093/bioinformatics/btx657, <https://academic.oup.com/bioinformatics/article-pdf/doi/10.1093/bioinformatics/btx657/21358327/btx657.pdf>, <http://dx.doi.org/10.1093/bioinformatics/btx657>.
3. Sievert C (2020). Interactive Web-Based Data Visualization with R, plotly, and shiny. Chapman and Hall/CRC. ISBN 9781138331457, <https://plotly-r.com>.