Technical Bulletin – BIN06
RBioPlot: Automated statistical analysis and data visualization
Hanane Hadj-Moussa and Jing Zhang PhD, 2016.

<u>**Overall:**</u>

RBioplot is an R pipeline designed for comprehensive statistical analysis and data visualization, it represents a fully automated and versatile data processing solution for molecular biology and biochemistry. We need to break free from the confinements of SigmaPlot 12 and Jing Zhang PhD has developed a new statistical and graphing R package for molecular biology that is here to help us do just that. RBioplot is a black box that will do all our graphing, plotting, and statistical analyses for us with minimal data reorganization, time, and energy. This techbull is the RBioplot for dummies user's manual.

*Note: This techbull will refer you to various websites and to the RBioplot webpage that is hosted on our lab website (http://kenstoreylab.com/?page_id=2448). There, you can find all the sample input and output files used to make this techbull.

Before using RBioplot you should read the following paper that explains all the functions, commands, and options. Also, ****when using this program remember to cite****:

Zhang J, Storey KB. (2016) RBioplot: an easy-to-use R pipeline for automated statistical analysis and data visualization in molecular biology and biochemistry. PeerJ 4:e2436.

I. Installing R and RStudio

To run RBioplot you must first install R on your computer, then install RStudio the user interface for R. Links to both of these programs are on the lab website (www.kensotreylab.com) → Research → Research Tools → RBioplot (http://kenstoreylab.com/?page_id=2448).

A. Installing "R"
1) To install '**R**' visit (https://www.r-project.org) and then select your CRAN preferred CRAN mirror, we will use the University of Toronto's (http://cran.utstat.utoronto.ca/).
2) Download the version of R that corresponds with your operating system. For example, if you are working on a Windows computer click **Download R for Windows** → **install R for the first time** → **Download R 3.3.1 for Windows**
3) Save the installation .exe file → open the file → follow the R for Windows 3.3.1 Setup Wizard's simple installation instructions.
    *Note: You should install all the installation components and make sure to accept the default start-up options.

B. Installing RStudio
1) To install **RStudio** visit (https://www.rstudio.com/). Click **RStudio** → **Desktop** → **Download RStudio Desktop**
2) Download the RStudio version that corresponds with your operating system. For example, if you are working on a Windows computer click **RStudio 0.99.903 - Windows Vista/7/8/10**
3) Save the installation .exe file → open the file → follow the RStudio Setup Wizard's simple installation instructions.
    *Note: Once the program has installed you should locate your RStudio shortcut and place it on your desktop.

II. Setting up RBioplot

Open the RStudio application, this will be the platform you will use to run RBIoplot. The picture below is a breakdown of the different RStudio quadrants and panels. **RStudio is case-sensitive.**
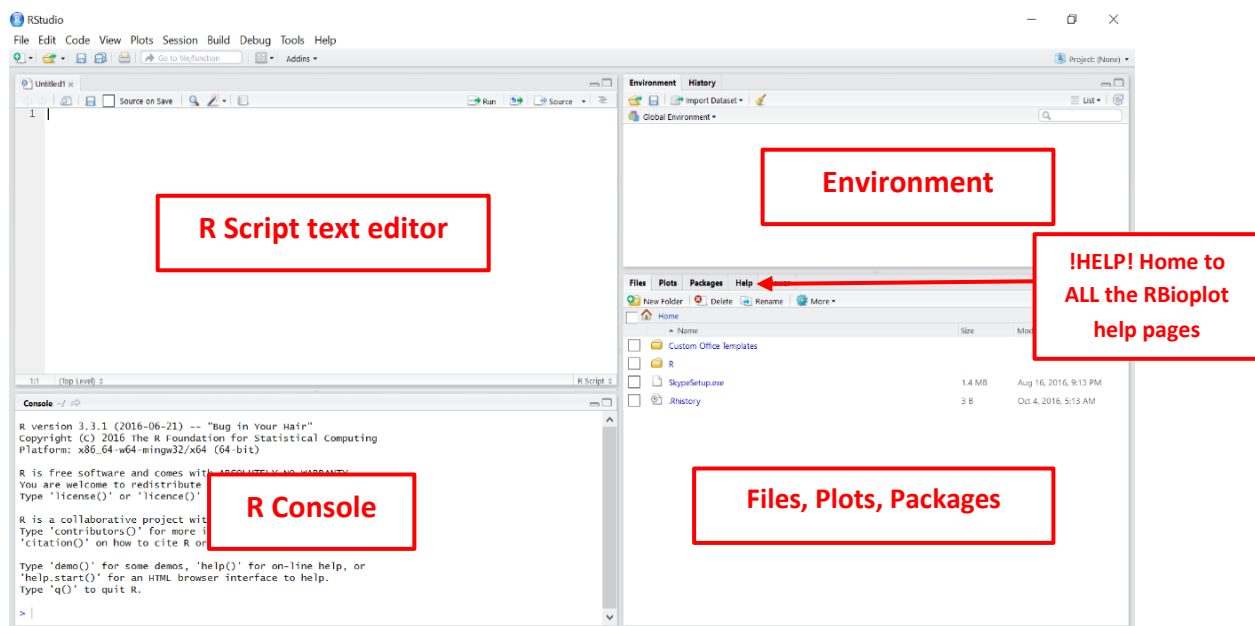
1) To setup the R Script text editor go to **File → New File → R Script**. The text editor is where you should prepare your R script before running your commands in the console. The text editor also allows you to run multiple commands at the same time (as long as you highlight all the commands you want to run).

2) To use the latest version of RBioplot we need to first install **devtools,** this package contains some of the various dependencies that RBioplot needs to run. This is a one-time installation, to install 'devtools' rewrite the command below in the text editor.
```
install.packages("devtools")
```

   *Note: You must be connected to the internet. You should rewrite all the commands written in this techbull and not just copy them into the text editor. Microsoft word has altered the formatting and syntax of these codes and if you copy/paste this into RStudio, your command will not run as R Script has unique quotation marks. If you copy/paste then make sure you rewrite your quotation marks.

3) To run your R commands, you have two options:
   a. **R Script text editor**, (preferred): Highlight the command you want to run and press Ctrl+Enter. Or place your text cursor on the command you want to run and press Ctrl+Enter. If you separate your command into different lines then make sure all the different commands are highlighted and that they can
   b. **Console**: Copy your command from your text editor to the Console and press Enter.

4) Once your command has been successfully installed you should receive a message that reads something like this:
```
The downloaded binary packages are in
C:\Users\Hanane\AppData\Local\Temp\Rtmp4gUWDW\downloaded_packages
```

5) Now you are ready to install the RBioplot package. Run the following command:
```
devtools::install_github("jzhangc/git_R_STATS_KBS/package/RBioplot")
```

6) You may run into a few errors as the current version of **devtools** does not contain all the dependencies that RBioplot needs, which means you need to manually install the missing packages.

   Here are a few examples of errors and solutions, in this first one I was missing the **scales** package so I installed it manually:

```
Error in loadNamespace(i, c(lib.loc, .libPaths()), versionCheck = vI[[i]])
: there is no package called 'scales'
   install.packages("scales")
```

   After I installed the missing package (**scales)** I re-ran the RBioplot installation command in step 5.

```
Error in loadNamespace(j <- i[[1L]], c(lib.loc, .libPaths()), versionCheck
= vI[[j]]) : there is no package called 'sandwich'
```

   Now, I am missing the package (**sandwich)**, so I installed it with the command below and then re-ran the RBioplot installation command from step 5. Repeat this process until you have installed all the missing packages.
```
install.packages("sandwich")
```

   Once RBioplot has successfully been installed you will get this message:
```
* DONE (RBioplot)
```

7) The next step is to setup your working directory, this is where you will place all your input data files and where all your plots, stats, and graphs will be exported to. In this example my working

directory file is on my desktop and it's called "RBioplot stuffs". To setup your working directory run the following command and replace "C:\Users\Hanane\Desktop" with your folder address:

> For Windows: `setwd("C:\\Users\\Hanane\\Desktop\\RBioplot stuffs")`
> For Mac and Linux: `setwd("C:\Users\Hanane\Desktop\RBioplot stuffs")`

8) In the 'Files, Plots, and Packages' quadrant of the graph you should select 'Packages' and then find RBioplot in the list. Once you click on RBioplot you will be redirected to a page with the RBioplot documentation and all the help files for the different functions. If you get stuck using one of the commands use these help files to trouble shoot.

9) If your RBioplot stops working or there is an error that you have spent the last 3 weeks troubleshooting, then you should email the developer jzhangcad@gmail.com and beg for help.

III. RBiostats – "that p-value?"

Before starting you should visit the **rbiostats** help page (or the **rbiostats mini-HELP! box** on page 5), this will outline all the available functions and how they work. All sample files used and generated can be downloaded from the website. **rbiostats** is a simple to use function for comprehensive statistical analyses that generates a statistical report for various statistical test including: t-tests, ANOVA, Tukey, and Dunnett. Make sure to read up on the various tests and to ensure you are using the one most appropriate for your data set and experimental design.

1) Your data must be formatted in a very specific manner to ensure the values are analyzed properly by RBioplot. **All excel files MUST be saved as a Microsoft Excel Comma Separated Values File (.csv)**

2) The table on the right is my **Sample Proteins.csv** file, standardized western blot relative protein abundances. The values are normalized with coomassie blue loading dye. Experimental conditions are listed in the first column. The A1 cell should read 'Condition' – or something similar. Protein names are listed in the first row. The values I report are an average of the relative protein abundance for all my technical replicates that correspond to each of my biological replicates.

*Note: You can analyze and graph your data even with missing outliers in your table.
*Note: Even though I ran 4 different biological replicates for each experimental condition (ie. Control 1, 2, 3, and 4) I have to keep the exact same identifier for each condition or the program will separate them into different conditions.

3) To statistically analyze your data using **rbiostats**, run the following command and replace "**Sample Proteins.csv**" with your "file name.csv" and select the stats test you need. (ANOVA is the default)
`RBioplot::rbiostats("Sample Proteins.csv", Tp = "ANOVA")`

4) As you are typing your command you will notice that RStudio has an auto-fill setting that you will find quite useful. If you hover your mouse over one of the options that appear, you will get a description of the function and the various arguments and customizations available.
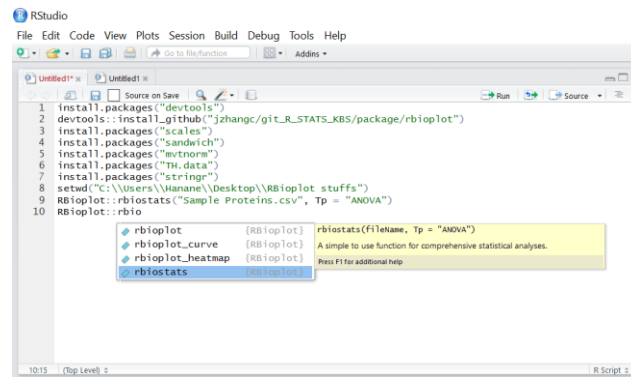
7) Outputs a **stats.txt** file with Shapiro-Wilk normality test results and the results of the statistical analysis of interest, this is automatically exported to your working directory. In this case, my file (on the left) was called **Sample Proteins.stats.txt**, the complete report can be downloaded from the website. For a detailed break-down of the statistical report refer to Zhang and Storey (2016). Value

---
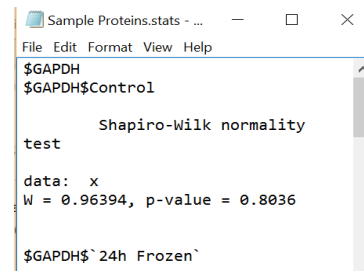
rbiostats   mini-HELP!

Usage
```
rbiostats(fileName, Tp = "ANOVA")
```

Arguments

`fileName` Input file name. Case sensitive and be sure to type with quotation marks. Currently only takes `.csv` files.

`Tp`   Type of the intended statistical test. Case sensitive and type with quotation marks. Options are: "t-test", "ANOVA", "Tukey" and "Dunnett". Default is "ANOVA".

IV. RBioplot – "that graph?"

After analyzing your statistical report, you can now graph your data. RBioplot allows you to generate Histograms and is excellent for applications such as western blotting, protein abundance levels, transcription factor-DNA binding activity, miRNA expression analyses, mRNA abundance, and many others. All sample files used and generated can be downloaded from the website.

*Note: You will find that the *Notes in this techbull will be useful for all the applications, not just the one it is listed in.*

A. Histogram: (rbioplot)

rbioplot is a simple to use function for plotting bas~~ed~~ing on the statistical analysis of choice. Before starting visit the **rbioplot** help page, this will outline the different functions and arguments and how to properly use them. You can also use the **rbioplot mini-HELP! box** on page 7.

1) For this example I used the western blotting data set from **Sample Proteins.csv** (check out rbiostats, step1). The protein abundance, PCR quantification, activity levels, etc. should all be in this format for **rbioplot.**

2) To generate a histogram run the following **rbioplot** command:

```
RBioplot::rbioplot("Sample Proteins.csv", Tp = "Tukey", Title = NULL,
errorbar = "SEM", errorbarWidth = 0.2, fontType = "sans", xLabel = NULL,
xTickLblSize = 10, xTickItalic = FALSE, xAngle = 0, xAlign = 0.5, yLabel =
NULL, yTickLblSize = 10, yTickItalic = FALSE, legendTtl = FALSE, plotWidth
= 170, plotHeight = 150, y_custom_tick_range = FALSE, y_lower_limit = 0,
y_upper_limit, y_major_tick_range, y_n_minor_ticks = 4)
```

3) The above command is very versatile and should be tailored to your specific dataset. You can use the customizable arguments to design what your graph looks like. Some of the customizable options include: Type of statistical test, Title, error bars, font, axes labels, Y-axis ticks, and plot height and width. For more information about the individual arguments go to the help pages described above.

4) Once you run the command, a preview of your graph will appear in the lower right quadrant of RStudio. A pdf version of your graph (600 dpi resolution.) will also be automatically exported into your working directory. In this example the pdf generated was titled **Sample Proteins.plot.pdf**.

   *Note: If you re-run the above command using the same input file name then the new generated graph will overwrite your old graph.
   *Note: Make sure the **.pdf** and **.plot.pdf** file that were generated are closed before re-running. You cannot generate a new file with the exact same name if the old version is active.
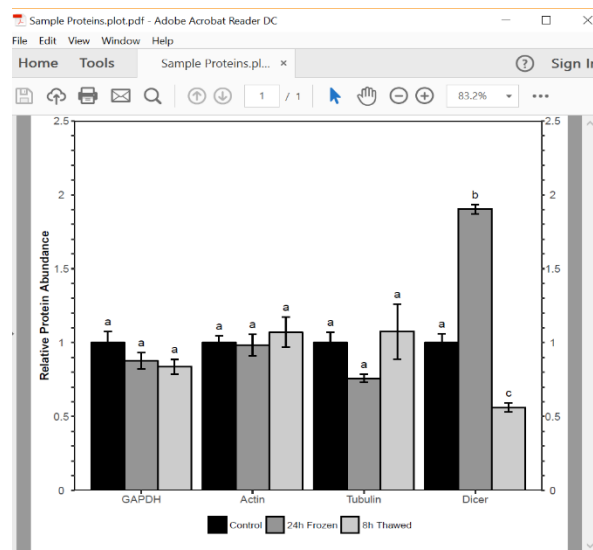
5) The above command also outputs a .csv file with detailed metrics for the plot, including Mean, SEM and significance labels. In this sample the exporeted file was named **Sample Proteins.plot.csv**:

rbioplot mini-HELP!

Usage

```
rbioplot(fileName, Tp = "Tukey", Title = NULL, errorbar = "SEM",
  errorbarWidth = 0.2, fontType = "sans", xLabel = NULL,
  xTickLblSize = 10, xTickItalic = FALSE, xAngle = 0, xAlign = 0.5,
  yLabel = NULL, yTickLblSize = 10, yTickItalic = FALSE,
  legendTtl = FALSE, plotWidth = 170, plotHeight = 150,
  y_custom_tick_range = FALSE, y_lower_limit = 0, y_upper_limit,
  y_major_tick_range, y_n_minor_ticks = 4)
```

Arguments

fileName	Input file name. Case sensitive and be sure to type with quotation marks. Currently only takes .csv files.
Tp	Type of the intended statistical test. Case sensitive and be sure to type with quotation marks. Options are: "t-test", "Tukey" and "Dunnett". Default is "Tukey".
Title	The displayed title on top of the plot. Be sure to type with quotation marks. Default is NULL.
errorbar	Set the type of errorbar. Options are standard error of mean ("SEM"), or standard deviation ("SD"). Default is "SEM".
errorbarWidth	Set the width for errorbar. Default is 0.2.
fontType	The type of font in the figure. Default is "sans". For all options please refer to R font table, which is avaiable on the website: http://kenstoreylab.com/?page_id=2448.
xLabel	x axis label. Type with quotation marks. Default is NULL.
xTickLblSize	Font size of x axis ticks. Default is 10.
xTickItalic	Set x axis tick font to italic. Default is FALSE.
xAngle	The rotation angle (degrees) of the x axis marks. Default is 0 - horizontal.
xAlign	The alignment type of the x axis marks. Options are 0, 0.5 and 1. The default value at 0 is especially useful when xAngle = 90.
yLabel	y axis label. Type with quotation marks. Default is NULL.
yTickLblSize	Font size of y axis ticks. Default is 10.
yTickItalic	Set y axis tick font to italic. Default is FALSE.
legendTtl	Hide/Display legend title. If TRUE or T, the name of the first column of the raw data file will display as the legend title. Default is FALSE.
plotWidth	The width of the plot (unit: mm). Default is 170. Default will fit most of the cases.
plotHeight	The height of the plot (unit: mm). Default is 150. Default will fit most of the cases.
y_custom_tick_range	To initiate setting the custom y_upper_limit, y_lower_limit, y_major_tick_range, y_n_minor_ticks. Default is FALSE.
y_lower_limit	Can only be set when y_custom_tick_range = TRUE. Set custom lower limt for y axis. Default is 0. Value can be obtained from autorange_bar_y.
y_upper_limit	Can only be set when y_custom_tick_range = TRUE. Set custom upper limt for y axis. Value can be obtained from autorange_bar_y.
y_major_tick_range	Can only be set when y_custom_tick_range = TRUE. Set custom major tick range for y axis. Value can be obtained from autorange_bar_y.
y_n_minor_ticks	Can only be set when y_custom_tick_range = TRUE. Set custom numbers of minor ticks. Default is 4. Value can be obtained from autorange_bar_y.

B. Heatmap: (rbioplot_heatmap)

The **rbioplot_heatmap** function can be used to analyze and graph largescale high-throughput studies such as large explorations of microRNA differential expression. **rbioplot_heatmap** is a function for plotting simple heatmaps based on the statistical analysis of your choice. Before starting visit the **rbioplot_heatmap** help page, this will outline the different functions and arguments and how to properly use them. You can also use the **rbioplot_heatmap mini-HELP! box** on page 10, for a quick reference.

1) The table below is my **Sample miRNAs.csv** file, the values are standardized miRNA relative abundance levels (obtained from qRT-PCR and normalized to the reference gene). The A1 cell should contain the word **microRNA** (or whatever you are measuring). The values I report are an average of the relative miRNA abundance for all my technical replicates that correspond to each of my biological replicates. This is just a small portion of the file- the full file contains 152 microRNAs.
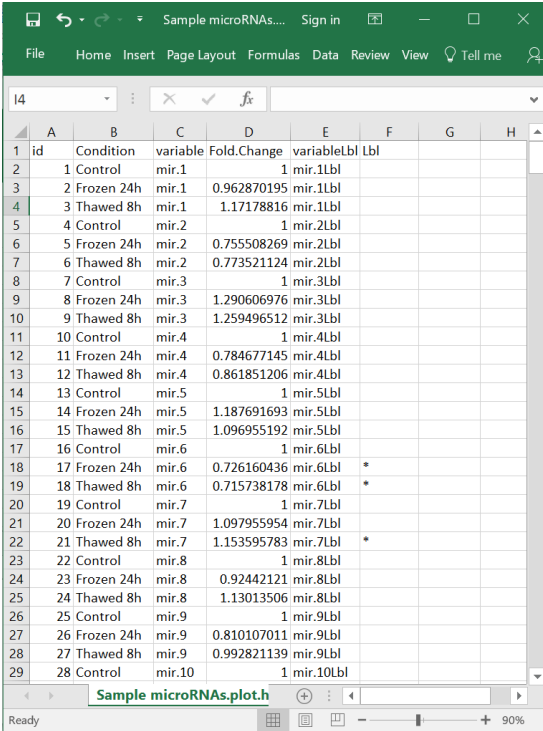

2) To generate a heatmap run the following **rbioplot** command:

RBioplot::rbioplot_heatmap("Sample microRNAs.csv", Tp = "Dunnett", rmCntl = FALSE, Title = NULL, fontType = "sans", tileLow = "firebrick1", tileHigh = "green2", tileLbl = TRUE, tileLblSize = 5, tileTxtColour = "white", tileLblPos = 0.5, xLabel = NULL, xTickLblSize = 10, xTickItalic = FALSE, xAngle = 0, xAlign = 0.5, yLabel = NULL, yTickLblSize = 10, yTickItalic = FALSE, legendTtl = FALSE, legendPos = "right", plotWidth = 170, plotHeight = 600)

3) The above command is very versatile and should be tailored to your specific dataset. You can also easily change the colour scheme of your heatmap, refer to **V. RBioplot Resources** for the full colour palette available on with R. For more information on how to customize the arguments go to the help page or the mini-help box.

4) The above **rbioplot_heatmap** command outputs a **.plot.csv** file with detailed metrics for the plot, including normalized mean and significance labels, as well as a plot image file (**.pdf**), with 600 dpi resolution.

id	Condition	variable	Fold.Change	variableLbl	Lbl
1	Control	mir.1	1	mir.1Lbl	
2	Frozen 24h	mir.1	0.962870195	mir.1Lbl	
3	Thawed 8h	mir.1	1.17178816	mir.1Lbl	
4	Control	mir.2	1	mir.2Lbl	
5	Frozen 24h	mir.2	0.755508269	mir.2Lbl	
6	Thawed 8h	mir.2	0.773521124	mir.2Lbl	
7	Control	mir.3	1	mir.3Lbl	
8	Frozen 24h	mir.3	1.290606976	mir.3Lbl	
9	Thawed 8h	mir.3	1.259496512	mir.3Lbl	
10	Control	mir.4	1	mir.4Lbl	
11	Frozen 24h	mir.4	0.784677145	mir.4Lbl	
12	Thawed 8h	mir.4	0.861851206	mir.4Lbl	
13	Control	mir.5	1	mir.5Lbl	
14	Frozen 24h	mir.5	1.187691693	mir.5Lbl	
15	Thawed 8h	mir.5	1.096955192	mir.5Lbl	
16	Control	mir.6	1	mir.6Lbl	
17	Frozen 24h	mir.6	0.726160436	mir.6Lbl	*
18	Thawed 8h	mir.6	0.715738178	mir.6Lbl	*
19	Control	mir.7	1	mir.7Lbl	
20	Frozen 24h	mir.7	1.097955954	mir.7Lbl	
21	Thawed 8h	mir.7	1.153595783	mir.7Lbl	*
22	Control	mir.8	1	mir.8Lbl	
23	Frozen 24h	mir.8	0.92442121	mir.8Lbl	
24	Thawed 8h	mir.8	1.13013506	mir.8Lbl	
25	Control	mir.9	1	mir.9Lbl	
26	Frozen 24h	mir.9	0.810107011	mir.9Lbl	
27	Thawed 8h	mir.9	0.992821139	mir.9Lbl	
28	Control	mir.10	1	mir.10Lbl	

Sample microRNAs.plot.h

rbioplot_heatmap mini-HELP!

Usage

```
rbioplot_heatmap(fileName, Tp = "Dunnett", rmCntl = FALSE, Title = NULL,
  fontType = "sans", tileLow = "skyblue", tileHigh = "midnightblue",
  tileLbl = TRUE, tileLblSize = 10, tileTxtColour = "white",
  tileLblPos = 0.5, xLabel = NULL, xTickLblSize = 10,
  xTickItalic = FALSE, xAngle = 0, xAlign = 0.5, yLabel = NULL,
  yTickLblSize = 10, yTickItalic = FALSE, legendTtl = FALSE,
  legendPos = "bottom", plotWidth = 170, plotHeight = 150)
```

Arguments

fileName	Input file name. Case sensitive and be sure to type with quotation marks. Currently only takes `.csv` files.
Tp	Type of the intended statistical test. Case sensitive and be sure to type with quotation marks. Options are: "t-test", "Tukey" and "Dunnett". Default is "Dunnett".
rmCntl	Remove the first column (i.e., control). Default is FALSE.
Title	The displayed title on top of the plot. Be sure to type with quotation marks. Default is NULL.
fontType	The type of font in the figure. Default is "sans". For all options please refer to R font table, which is avaiable on the website:http://kenstoreylab.com/?page_id=2448.
tileLow	Set the colour for the lower limit of the heatmap. Default is skyblue. For full colour options and names, refer to the websitehttp://kenstoreylab.com/?page_id=2448.
tileHigh	Set the colour for the upper limit of the heatmap. Default ismidnightblue. For full colour options and names, refer to the website http://kenstoreylab.com/?page_id=2448.
tileLbl	Enable or disable significant notation on the tiles. Default is TRUE.
tileLblSize	Set the font size of the tile label. Default is 10.
tileTxtColour	Set the colour of the on tile label. Default is "white". For full colour options and names, refer to the website http://kenstoreylab.com/?page_id=2448.
tileLblPos	Set the position of the tile lables. Options are 0, 0.5 and 1. Default is 0.5.
xLabel	x axis label. Type with quotation marks. Default is NULL.
xTickLblSize	Font size of x axis ticks. Default is 10.
xTickItalic	Set x axis tick font to italic. Default is FALSE.
xAngle	The rotation angle (degrees) of the x axis marks. Default is 0 - horizontal.
xAlign	The alignment type of the x axis marks. Options are 0, 0.5 and 1. The default value at 0 is especially useful when xAngle = 90.
yLabel	y axis label. Type with quotation marks. Default is NULL.
yTickLblSize	Font size of y axis ticks. Default is 10.
yTickItalic	Set y axis tick font to italic. Default is FALSE.
legendTtl	Hide/Display legend title. Default is FALSE.
legendPos	Set the legend position. Options are "top", "bottom", "left"and "right". Default is "bottom".
plotWidth	The width of the plot (unit: mm). Default is 170. Default will fit most of the cases.
plotHeight	The height of the plot (unit: mm). Default is 150. Default will fit most of the cases.
y_custom_tick_range	To initiate setting the custom y_upper_limit, y_lower_limit,y_major_tick_range, y_n_minor_ticks. Default is FALSE.
y_upper_limit	Can only be set when y_custom_tick_range = TRUE. Set custom upper limt for y axis. Value can be obtained fromautorange_bar_y.
y_lower_limit	Can only be set when y_custom_tick_range = TRUE. Set custom lower limt for y axis. Default is 0. Value can be obtained fromautorange_bar_y.
y_major_tick_range	Can only be set when y_custom_tick_range = TRUE. Set custom major tick range for y axis. Value can be obtained fromautorange_bar_y.
y_n_minor_ticks	Can only be set when y_custom_tick_range = TRUE. Set custom numbers of minor ticks. Default is 4. Value can be obtained from autorange_bar_y.

C. Joint-point curve: (rbioplot_curve)

rbioplot_curve is a simple to use function for plotting joining-point curve figures with continuous X-axis and Y-axis values. Before starting visit the **rbioplot_curve** help page, this will outline the different functions and arguments and how to properly use them. You can also use the **rbioplot_curve mini-HELP! box** on page 12.

1) In this example below I plotted my imaginary enzyme's activity for 18 eluted fractions from both control and 24 h frozen. I used the **Sample Elution Profile.csv** (below). The independent variables are listed in column A.

2) To generate a histogram run the following **rbioplot** command:

```
RBioplot::rbioplot_curve("Sample Elution Profile.csv", Title = NULL,
errorbar = "SEM", errorbarWidth = 0.2, fontType = "sans", symbolSize = 2,
xLabel = "Fraction", xTickLblSize = 10, xTickItalic = FALSE, xAngle = 0,
xAlign = 0.5, yLabel = "Relative Activity", yTickLblSize = 10, yTickItalic
= FALSE, legendTtl = FALSE, plotWidth = 170, plotHeight = 150,
x_custom_tick_range = TRUE, x_lower_limit = 5, x_upper_limit = 23,
x_major_tick_range = 1, x_n_minor_ticks = 0, y_custom_tick_range = TRUE,
y_lower_limit = -0.2, y_upper_limit = 1.2, y_major_tick_range = 0.2,
y_n_minor_ticks = 4)
```

3) The above **rbioplot_curve** command outputs a **.plot.csv** file with detailed metrics for the plot, including Mean and SEM, as well as a plot image file (**.pdf**), with 600 dpi resolution.

rbioplot_curve   mini-HELP!

Usage

```
rbioplot_curve(fileName, Title = NULL, errorbar = "SEM",
  errorbarWidth = 0.2, fontType = "sans", symbolSize = 2, xLabel = NULL,
  xTickLblSize = 10, xTickItalic = FALSE, xAngle = 0, xAlign = 0.5,
  yLabel = NULL, yTickLblSize = 10, yTickItalic = FALSE,
  legendTtl = FALSE, plotWidth = 170, plotHeight = 150,
  x_custom_tick_range = FALSE, x_lower_limit = 0, x_upper_limit,
  x_major_tick_range, x_n_minor_ticks = 0, y_custom_tick_range = FALSE,
  y_lower_limit = 0, y_upper_limit, y_major_tick_range, y_n_minor_ticks = 4)
```

Arguments

fileName	Input file name. Case sensitive and be sure to type with quotation marks. Currently only takes `.csv` files. Note that the column names (excluding the first column) need to be numeric.
Title	The displayed title on top of the plot. Be sure to type with quotation marks. Default is NULL.
errorbar	Set the type of errorbar. Options are standard error of mean ("SEM"), or standard deviation ("SD"). Default is "SEM".
errorbarWidth	Set the width for errorbar. Default is 0.2.
fontType	The type of font in the figure. Default is "sans". For all options please refer to R font table, which is avaiable on the website:http://kenstoreylab.com/?page_id=2448.
symbolSize	Set the size of symbols. Default is 2.
xLabel	x axis label. Type with quotation marks. Default is NULL.
xTickLblSize	Font size of x axis ticks. Default is 10.
xTickItalic	Set x axis tick font to italic. Default is FALSE.
xAngle	The rotation angle (degrees) of the x axis marks. Default is 0 - horizontal.
xAlign	The alignment type of the x axis marks. Options are 0, 0.5 and 1. The default value at 0 is especially useful when xAngle = 90.
yLabel	y axis label. Type with quotation marks. Default is NULL.
yTickLblSize	Font size of y axis ticks. Default is 10.
yTickItalic	Set y axis tick font to italic. Default is FALSE.
legendTtl	Hide/Display legend title. If TRUE or T, the name of the first column of the raw data file will display as the legend title. Default is FALSE.
plotWidth	The width of the plot (unit: mm). Default is 170. Default will fit most of the cases.
plotHeight	The height of the plot (unit: mm). Default is 150. Default will fit most of the cases.
x_custom_tick_range	To initiate setting the custom x_upper_limit, x_lower_limit,x_major_tick_range, x_n_minor_ticks. Default is FALSE.
x_lower_limit	Can only be set when x_custom_tick_range = TRUE. Set custom lower limt for x axis. Default is 0. Value can be obtained from autorange_curve.
x_upper_limit	Can only be set when x_custom_tick_range = TRUE. Set custom upper limt for x axis. Value can be obtained from autorange_curve.
x_major_tick_range	Can only be set when x_custom_tick_range = TRUE. Set custom major tick range for x axis. Value can be obtained from autorange_curve.
x_n_minor_ticks	Can only be set when x_custom_tick_range = TRUE. Set custom numbers of minor ticks. Default is 4. Value can be obtained from autorange_curve.
y_custom_tick_range	To initiate setting the custom y_upper_limit, y_lower_limit,y_major_tick_range, y_n_minor_ticks. Default is FALSE.
y_lower_limit	Can only be set when y_custom_tick_range = TRUE. Set custom lower limt for y axis. Default is 0. Value can be obtained from autorange_curve.
y_upper_limit	Can only be set when y_custom_tick_range = TRUE. Set custom upper limt for y axis. Value can be obtained from autorange_curve.
y_major_tick_range	Can only be set when y_custom_tick_range = TRUE. Set custom major tick range for y axis. Value can be obtained from autorange_curve.
y_n_minor_ticks	Can only be set when y_custom_tick_range = TRUE. Set custom numbers of minor ticks. Default is 4. Value can be obtained from autorange_curve.

V. RBioplot Resources

To customize your graphs and make them more visually appealing (or usually you have to make them more boring for journals), you can refer to the R colour palette and R font table below.

R colour palette

brown4	darkorange4	gray	gray57	hotpink3	lightsalmon4	navajowhite1	plum3	slategray3	antiquewhite
brown3	darkorange3	goldenrod4	gray56	hotpink2	lightsalmon3	navajowhite	plum2	slategray2	aliceblue
brown2	darkorange2	goldenrod3	gray55	hotpink1	lightsalmon2	moccasin	plum1	slategray1	white
brown1	darkorange1	goldenrod2	gray54	hotpink	lightsalmon1	mistyrose4	plum	slategray	yellowgreen
brown	darkorange	goldenrod1	gray53	honeydew4	lightsalmon	mistyrose3	pink4	slateblue4	yellow4
blueviolet	darkolivegreen4	goldenrod	gray52	honeydew3	lightpink4	mistyrose2	pink3	slateblue3	yellow3
blue4	darkolivegreen3	gold4	gray51	honeydew2	lightpink3	mistyrose1	pink2	slateblue2	yellow2
blue3	darkolivegreen2	gold3	gray50	honeydew1	lightpink2	mistyrose	pink1	slateblue1	yellow1
blue2	darkolivegreen1	gold2	gray49	honeydew	lightpink1	mintcream	pink	slateblue	yellow
blue1	darkolivegreen	gold1	gray48	greenyellow	lightpink	midnightblue	peru	skyblue4	whitesmoke
blue	darkmagenta	gold	gray47	green4	lightgrey	mediumvioletred	peachpuff4	skyblue3	wheat4
blanchedalmond	darkkhaki	ghostwhite	gray46	green3	lightgreen	mediumturquoise	peachpuff3	skyblue2	wheat3
black	darkgrey	gainsboro	gray45	green2	lightgray	mediumspringgreen	peachpuff2	skyblue1	wheat2
bisque4	darkgreen	forestgreen	gray44	green1	lightgoldenrodyellow	mediumslateblue	peachpuff1	skyblue	wheat1
bisque3	darkgray	floralwhite	gray43	green	lightgoldenrod4	mediumseagreen	peachpuff	sienna4	wheat
bisque2	darkgoldenrod4	firebrick4	gray42	gray100	lightgoldenrod3	mediumpurple4	papayawhip	sienna3	violetred4
bisque1	darkgoldenrod3	firebrick3	gray41	gray99	lightgoldenrod2	mediumpurple3	palevioletred4	sienna2	violetred3
bisque	darkgoldenrod2	firebrick2	gray40	gray98	lightgoldenrod1	mediumpurple2	palevioletred3	sienna1	violetred2
beige	darkgoldenrod1	firebrick1	gray39	gray97	lightgoldenrod	mediumpurple1	palevioletred2	sienna	violetred1
azure4	darkgoldenrod	firebrick	gray38	gray96	lightcyan4	mediumpurple	palevioletred1	seashell4	violetred
azure3	darkcyan	dodgerblue4	gray37	gray95	lightcyan3	mediumorchid4	palevioletred	seashell3	violet
azure2	darkblue	dodgerblue3	gray36	gray94	lightcyan2	mediumorchid3	paleturquoise4	seashell2	turquoise4
azure1	cyan4	dodgerblue2	gray35	gray93	lightcyan1	mediumorchid2	paleturquoise3	seashell1	turquoise3
azure	cyan3	dodgerblue1	gray34	gray92	lightcyan	mediumorchid1	paleturquoise2	seashell	turquoise2
aquamarine4	cyan2	dodgerblue	gray33	gray91	lightcoral	mediumorchid	paleturquoise1	seagreen4	turquoise1
aquamarine3	cyan1	dimgrey	gray32	gray90	lightblue4	mediumorchid	paleturquoise	seagreen3	turquoise
aquamarine2	cyan	dimgray	gray31	gray89	lightblue3	mediumblue	palegreen4	seagreen2	tomato4
aquamarine1	cornsilk4	deepskyblue4	gray30	gray88	lightblue2	maroon4	palegreen3	seagreen1	tomato3
aquamarine	cornsilk3	deepskyblue3	gray29	gray87	lightblue1	maroon3	palegreen2	seagreen	tomato2
antiquewhite4	cornsilk2	deepskyblue2	gray28	gray86	lightblue	maroon2	palegreen1	sandybrown	tomato1
antiquewhite3	cornsilk1	deepskyblue1	gray27	gray85	lemonchiffon4	maroon1	palegreen	salmon4	tomato
antiquewhite2	cornsilk	deepskyblue	gray26	gray84	lemonchiffon3	maroon	palegoldenrod	salmon3	thistle4
antiquewhite1	cornflowerblue	deeppink4	gray25	gray83	lemonchiffon2	magenta4	orchid4	salmon2	thistle3
antiquewhite	coral4	deeppink3	gray24	gray82	lemonchiffon1	magenta3	orchid3	salmon1	thistle2
aliceblue	coral3	deeppink2	gray23	gray81	lemonchiffon	magenta2	orchid2	salmon	thistle1
white	coral2	deeppink1	gray22	gray80	lawngreen	magenta1	orchid1	saddlebrown	thistle
bisque3	coral1	deeppink	gray21	gray79	lavenderblush4	magenta	orchid	royalblue4	tan4
bisque2	coral	darkviolet	gray20	gray78	lavenderblush3	linen	orangered4	royalblue3	tan3
bisque1	chocolate4	darkturquoise	gray19	gray77	lavenderblush2	limegreen	orangered3	royalblue2	tan2
bisque	chocolate3	darkslategrey	gray18	gray76	lavenderblush1	lightyellow4	orangered2	royalblue1	tan1
beige	chocolate2	darkslategray4	gray17	gray75	lavenderblush	lightyellow3	orangered1	royalblue	tan
azure4	chocolate1	darkslategray3	gray16	gray74	lavender	lightyellow2	orangered	rosybrown4	steelblue4
azure3	chocolate	darkslategray2	gray15	gray73	khaki4	lightyellow1	orange4	rosybrown3	steelblue3
azure2	chartreuse4	darkslategray1	gray14	gray72	khaki3	lightyellow	orange3	rosybrown2	steelblue2
azure1	chartreuse3	darkslategray	gray13	gray71	khaki2	lightsteelblue4	orange2	rosybrown1	steelblue1
azure	chartreuse2	darkslateblue	gray12	gray70	khaki1	lightsteelblue3	orange1	rosybrown	steelblue
aquamarine4	chartreuse1	darkseagreen4	gray11	gray69	khaki	lightsteelblue2	orange	red4	springgreen4
aquamarine3	chartreuse	darkseagreen3	gray10	gray68	ivory4	lightsteelblue1	olivedrab4	red3	springgreen3
aquamarine2	cadetblue4	darkseagreen2	gray9	gray67	ivory3	lightsteelblue	olivedrab3	red2	springgreen2
aquamarine1	cadetblue3	darkseagreen1	gray8	gray66	ivory2	lightslategrey	olivedrab2	red1	springgreen1
aquamarine	cadetblue2	darkseagreen	gray7	gray65	ivory1	lightslategray	olivedrab1	red	springgreen
antiquewhite4	cadetblue1	darksalmon	gray6	gray64	ivory	lightslateblue	olivedrab	purple4	snow4
antiquewhite3	cadetblue	darkred	gray5	gray63	indianred4	lightskyblue4	oldlace	purple3	snow3
antiquewhite2	burlywood4	darkorchid4	gray4	gray62	indianred3	lightskyblue3	navyblue	purple2	snow2
antiquewhite1	burlywood3	darkorchid3	gray3	gray61	indianred2	lightskyblue2	navy	purple1	snow1
antiquewhite	burlywood2	darkorchid2	gray2	gray60	indianred1	lightskyblue1	navajowhite4	purple	snow
aliceblue	burlywood1	darkorchid1	gray1	gray59	indianred	lightskyblue	navajowhite3	powderblue	slategrey
white	burlywood	darkorchid	gray0	gray58	hotpink4	lightseagreen	navajowhite2	plum4	slategray4

R font table

plain	
URWTimes	NimbusRom
	URWPalladio
	CenturySch
	NimbusSanCond
URWHelvetica	NimbusSan
	NimbusMon
	URWBookman
	URWGothic
	Palatino
	NewCenturySchoolbook
	Helvetica-Narrow
	Bookman
	AvantGarde
serif	Times
sans	Helvetica
mono	Courier