

Package ‘seqplots’

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Title An interactive tool for visualizing NGS signals and sequence motif densities along genomic features using average plots and heatmaps

Description SeqPlots is a tool for plotting next generation sequencing (NGS) based experiments' signal tracks, e.g. reads coverage from ChIP-seq, RNA-seq and DNA accessibility assays like DNase-seq and MNase-seq, over user specified genomic features, e.g. promoters, gene bodies, etc. It can also calculate sequence motif density profiles from reference genome. The data are visualized as average signal profile plot, with error estimates (standard error and 95% confidence interval) shown as fields, or as series of heatmaps that can be sorted and clustered using hierarchical clustering, k-means algorithm and self organising maps. Plots can be prepared using R programming language or web browser based graphical user interface (GUI) implemented using Shiny framework. The dual-purpose implementation allows running the software locally on desktop or deploying it on server. SeqPlots is useful for both for exploratory data analyses and preparing replicable, publication quality plots. Other features of the software include collaboration and data sharing capabilities, as well as ability to store pre-calculated result matrixes, that combine many sequencing experiments and in-silico generated tracks with multiple different features. These binaries can be further used to generate new combination plots on fly, run automated batch operations or share with colleagues, who can adjust their plotting parameters without loading actual tracks and recalculating numeric values. SeqPlots relays on Bioconductor packages, mainly on rtracklayer for data input and BSgenome packages for reference genome sequence and annotations.

URL <http://github.com/przemol/seqplots>

BugReports <http://github.com/przemol/seqplots/issues>

biocViews ChIPSeq, RNASeq, Sequencing, Software, Visualization

Depends R (>= 3.2.0)

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getPlotSetArray *Process genomic signal*

Description

Function to process genomic signal from tracks and/or motif data, calculate statistics. This function should be used as the entry point to the SeqPlots pipeline and followed by plotting function(s).

Usage

```
getPlotSetArray(tracks, features, refgenome, bin = 10L, rm0 = FALSE,
  ignore_strand = FALSE, xmin = 2000L, xmax = 2000L, xanchored = 1000L,
  type = "pf", add_heatmap = TRUE, verbose = FALSE, stat = "mean",
  lvl1m = message, lvl2m = message)
```

Arguments

tracks	Character vector or list of BigWig track paths. For motif density plots MotifSetup class containing motifs setup and/or BigWig track paths (see details)
features	Character vector or list containing feature file paths (BED or GFF).
refgenome	The UCSC code of reference genome, e.g. 'hg19' for Homo sapiens (see details)
bin	Binning window size in base pairs, defaults to 1L
rm0	Remove zeros from mean/error estimate calculation, 0 in track file will be treated as missing data, defaults to FALSE
ignore_strand	If TRUE the directionality is ignored, that is all features' strands, regardless of annotation in GFF/BED file, are treated as undetermined ("*"), defaults to FALSE
xmin	Upstream calculation distance in base pairs, defaults to 200L
xmax	Downstream calculation distance in base pairs, defaults to 2000L
xanchored	Anchored, feature body pseudo length in base pairs. The features will be extended or shrunk using linear approximation. Used only if /codetype="af", defaults to 1000L
type	The type of the calculation, "pf" for point features (default), "mf" for midpoint features, "ef" for endpoint features and "af" for anchored features, see details
add_heatmap	Add the heatmap data to output, must be on to plot heatmap form output PlotSetArray class, defaults to TRUE
verbose	Print various messages and warnings, defaults to FALSE
stat	If set to "median" the median is used as summarizing statistic for linear plots instead of mean
lvl1m	function to handle lvl 1 messages, useful when invoked in Shiny GUI environment, defaults to message
lvl2m	function to handle lvl 2 messages, useful when invoked in Shiny GUI environment, defaults to message

Details

This function takes genomic coordinates in BED or GFF format, and extracts the signal from track files (BigWig) and/or calculates motif density in these regions. Then it computes the statistics required for average and heatmap plots. Returns the `PlotSetArray` class, which can be further subsisted, and used for plotting.

Modes of operation: The function operate in three modes, determined by type parameter:

- **Point Features** - anchor plot on the start of a feature. By default, plot will be directional if strand information is present (i.e, use start position and plot on positive strand for + strand features and use end position and plot on negative strand for minus strand features). If strand information is not present in the feature file (or if the "ignore strand" option is chosen), plot will use start position of feature and be plotted on the positive strand (see explanations). User chooses length of upstream and downstream sequence to plot.
- **Midpoint Features** - similar to point feature, but plot is centred on the midpoint of the feature.
- **Endpoint Features** - similar to point feature, but plot is centred on the end point (most downstream) of the feature.
- **Anchored Features** - features are anchored at start and stop positions and given pseudo-length chosen by the user. Additionally, the user chooses the length of sequence upstream of the start and downstream of the end to plot.

Binning the track: bin numeric parameter determines the resolution of data acquisition. The default value 10 means that 10bp intervals within the plotting range will be summarized by calculating the mean. Higher values increases the speed of calculation and produces smoother plots, but decreases resolution.

DNA motifs: The `MotifSetup` class allows to calculate and plot the density of any user-defined motif around the chosen genomic feature using the reference sequence package. Motif plots can be mixed with track files' signal plots. The `MotifSetup` can be initialized in following way:

```
ms <- MotifSetup()
ms$addMotif("TATA", window=200L, heatmap=TRUE, revcomp=TRUE, name=pattern)
ms$addMotif("GAGA", window=100L)$addBigWig("path/to/file.bw")
```

The `addMotif` methods accepts following parameters:

`motif` The DNA motif sequence.

`window` Sliding window size in base pairs [bp] - the size of the sliding window for motif calculation. The value (number of matching motifs within the window) is reported in the middle of the window, e.g. if window is set to 200bp, DNA motif is "GC" and there are 8 CpGs in first 200 bp of the chromosome the value 8 will be reported at 100th bp.

`name` Display name - The name of the motif that will be shown in key and heatmap labels. Leave blank to use DNA motif value.

`heatmap` Plot heatmap or error estimates - this checkbox determines if heatmap matrix and error estimates should be calculated. If unchecked much faster algorithm will be used for motif density calculation, but only the average plot without the error estimates will be available.

`revcomp` Match reverse complement as well - select if reverse complement motif should be reported as well. For example the TATA motif will report both TATA and ATAT with this option selected.

Reference genomes: The `refgenome` parameter determines the reference genome to be used chromosome naming convention (e.g. `chrX` vs. `X`) and chromosome lengths. Also for motif plots the genomic sequence is used to calculate motif density tracks. To check which genomic packages are installed in current R session use `installed.genomes` function. `available.genomes` gives the list of all reference genome packages currently supplied by BioConductor. Please refer to `BSgenome` package documentation for installing and forging new genome packages.

Value

The `PlotSetArray` object.

See Also

Other plotting functions: `plotAverage`, `plotHeatmap`, `plot`

Examples

```
# Get the paths of example files
bed1 <- system.file("extdata",
  "Transcripts_ce10_chrI_100Kb.bed", package="seqplots")
bed2 <- system.file("extdata",
  "GSM1208361_chrI_100Kb_PeakCalls.bed", package="seqplots")
bw1 <- system.file("extdata",
  "GSM1208360_chrI_100Kb_q5_sample.bw", package="seqplots")

#If required install C. elegans genomic package from Bioconductor
if(!"BSgenome.Celegans.UCSC.ce10" %in% BSgenome::installed.genomes()) {
  if(.Platform$OS.type != "windows" || .Machine$sizeof.pointer != 4) {
    source("http://bioconductor.org/biocLite.R")
    biocLite("BSgenome.Celegans.UCSC.ce10")
  }
}

#Get getPlotSetArray for track and feature files
#Does not work on Windows i386 (32 bit)
if(.Platform$OS.type != "windows" || .Machine$sizeof.pointer != 4) {
  plotset1 <- getPlotSetArray(bw1, c(bed1, bed2), 'ce10')
} else {
  load(system.file("extdata", "precalc_plotset.Rdata", package="seqplots"))
}
plot(plotset1) #Average plot
plot(plotset1[1,], what='h') #Heatmap

#Get getPlotSetArray for motifs, track and feature files
ms <- MotifSetup()
ms <- MotifSetup()
ms$addMotif('GAGA')
ms$addMotif('TATA')
ms$addBigWig(bw1)
if(.Platform$OS.type != "windows" || .Machine$sizeof.pointer != 4) {
  plotset2 <- getPlotSetArray(ms, c(bed1, bed2), 'ce10')
```

```

}
plot(plotset2) #Average plot
plot(plotset2[1,], what='h') #Heatmap

```

MotifSetup-class *MotifSetup Reference Class*

Description

This class is used to initialize motif plots and mix them with track plots.

Details

Usage note - the addMotif and addBigWig methods can be chained in following way: MotifSetup()\$addMotif("GAGA", window=200L)\$addBigWig("path/to/file.bw")

Fields

data a nested list holding the data
 annotations list of annotations

Methods

addBigWig(file_path) Adds new BigWig file.
 addMotif(pattern, window = 200L, heatmap = TRUE, revcomp = TRUE, genome = NULL, name = pattern)
 Adds new motif.
 nmotifs() Prints number of motifs in class

See Also

Other classes: [PlotSetArray-class](#), [PlotSetList-class](#), [PlotSetPair-class](#)

Examples

```

#Motifs only
motif1 <- MotifSetup()
motif1$addMotif("GAGA", window=200L)
motif1$addMotif("TATA", window=100L, name="TATA box")

#Motifs and BigWigs
motif2 <- MotifSetup()
motif2$addMotif("GAGA", window=200L)
motif2$addBigWig("path/to/file.bw")

#Chaining commands
motif3 <- MotifSetup()$addMotif("GAGA")$addBigWig("path/to/file.bw")

```

plot *Generic plot function for SeqPlots package calsses*

Description

Generic plot function for SeqPlots package calsses

Usage

```
plot(x, y, ...)

## S4 method for signature 'PlotSetPair,ANY'
plot(x, what = "a", ...)

## S4 method for signature 'PlotSetList,ANY'
plot(x, what = "a", ...)

## S4 method for signature 'PlotSetArray,ANY'
plot(x, what = "a", ...)
```

Arguments

x	This argument should be one of SeqPlots classes: PlotSetArray , PlotSetList or PlotSetPair
y	For plotting SeqPlots classes this argument is ignored, used for default functionality of plot function.
...	Other parameters controlong the plot, see plotAverage for avareange plot and plotHeatmap for heatmaps.
what	This argument takes a character determining if avareange plot ("a", default) or heatmap ("h") will be plotted.

Value

Returns NULL for avareange plot and cluster report data . frame for hetamap - see [plotHeatmap](#) for details.

Methods (by class)

- x = PlotSetPair, y = ANY: Method plot for signature 'PlotSetPair'
- x = PlotSetList, y = ANY: Method plot for signature 'PlotSetList'
- x = PlotSetArray, y = ANY: Method plot for signature 'PlotSetPair'

See Also

[getPlotSetArray](#)

Other plotting.functions: [getPlotSetArray](#), [plotAverage](#), [plotHeatmap](#)

Examples

```
#load precalculated PlotSetArrays "plotset1" and "plotset2", usually these
#objects are the output of getPlotSetArray function
load(system.file("extdata", "precalc_plotset.Rdata", package="seqplots"))

#plot with default values
plot(plotset2) #Average plot
plot(plotset2[1,], what='h') #Heatmap

#setting plot options
plot(plotset2, main='Title', xlab='Relative position [bp]', ylab='Signal',
      colvec=rainbow(6, 0.7, 0.5), labels=LETTERS, legend_ext=TRUE,
      legend_ext_pos='topright', legend_pos='topleft', ln.h=9)

plot(plotset2[2,], what='h', main="The heatmap", labels=LETTERS,
      ord = c(3,1,2), sortrows = TRUE, clusters = 2, clsmethod = "hclust",
      cex.main = 20, cex.lab = 12, cex.legend = 12, xlab = "Rel. pos. [bp]",
      ylab = "Signal", autoscale = FALSE, zmin = 0, zmax = 20,
      clspace = rev(rainbow(4, 0.7, 0.5)) )
```

plotAverage

Create the average plot

Description

Draw an average plot from [PlotSetArray](#), [PlotSetList](#), [PlotSetPair](#) or properly formatted [list](#) in active graphics window. Axes and titles, keys and other plot elements are controlled by function parameters.

Usage

```
plotAverage(plotset, keepratio = FALSE, labels = NULL, xlim = NULL,
            ylim = NULL, main = NULL, xlab = "", ylab = "",
            plotScale = "linear", type = "full", error.estimates = TRUE,
            legend = TRUE, legend_ext = FALSE, legend_pos = "topright",
            legend_ext_pos = "topleft", cex.axis = 14, cex.lab = 16,
            cex.main = 20, cex.legend = 10, ln.v = TRUE, ln.h = NULL,
            colvec = NULL, pointsize = 12, ...)

## S4 method for signature 'list'
plotAverage(plotset, keepratio = FALSE, labels = NULL,
            xlim = NULL, ylim = NULL, main = NULL, xlab = "", ylab = "",
            plotScale = "linear", type = "full", error.estimates = TRUE,
            legend = TRUE, legend_ext = FALSE, legend_pos = "topright",
            legend_ext_pos = "topleft", cex.axis = 14, cex.lab = 16,
            cex.main = 20, cex.legend = 10, ln.v = TRUE, ln.h = NULL,
            colvec = NULL, pointsize = 12, ...)
```



```
## S4 method for signature 'PlotSetPair'
plotAverage(plotset, keepratio = FALSE,
  labels = NULL, xlim = NULL, ylim = NULL, main = NULL, xlab = "",
  ylab = "", plotScale = "linear", type = "full",
  error.estimates = TRUE, legend = TRUE, legend_ext = FALSE,
  legend_pos = "topright", legend_ext_pos = "topleft", cex.axis = 14,
  cex.lab = 16, cex.main = 20, cex.legend = 10, ln.v = TRUE,
  ln.h = NULL, colvec = NULL, pointsize = 12, ...)

## S4 method for signature 'PlotSetList'
plotAverage(plotset, keepratio = FALSE,
  labels = NULL, xlim = NULL, ylim = NULL, main = NULL, xlab = "",
  ylab = "", plotScale = "linear", type = "full",
  error.estimates = TRUE, legend = TRUE, legend_ext = FALSE,
  legend_pos = "topright", legend_ext_pos = "topleft", cex.axis = 14,
  cex.lab = 16, cex.main = 20, cex.legend = 10, ln.v = TRUE,
  ln.h = NULL, colvec = NULL, pointsize = 12, ...)

## S4 method for signature 'PlotSetArray'
plotAverage(plotset, keepratio = FALSE,
  labels = NULL, xlim = NULL, ylim = NULL, main = NULL, xlab = "",
  ylab = "", plotScale = "linear", type = "full",
  error.estimates = TRUE, legend = TRUE, legend_ext = FALSE,
  legend_pos = "topright", legend_ext_pos = "topleft", cex.axis = 14,
  cex.lab = 16, cex.main = 20, cex.legend = 10, ln.v = TRUE,
  ln.h = NULL, colvec = NULL, pointsize = 12, ...)
```

Arguments

plotset	The dataset to plot - can be PlotSetArray , PlotSetList , PlotSetPair or properly formatted list
keepratio	If TRUE keep 1:1 aspect ratio of the figure; defaults to FALSE
labels	The character vector giving labels used in experiment key. The defaults NULL value indicates that feature/track file names will be used to generate the labels.
xlim	the x limits (x1, x2) of the plot. Note that x1 > x2 is allowed and leads to a "reversed axis". The default value, NULL, indicates that the whole range present in plotset will be plotted.
ylim	the y limits (y1, y2) of the plot. Note that x1 > x2 is allowed and leads to a "reversed axis". The default value, NULL, indicates that the range will be auto calculated including space for error estimates.
main	The main title of the plot, shown in top-centred part of the figure; defaults to NULL (not visible)
xlab	Label shown below horizontal axis; default to "" (empty)
ylab	Label shown below vertical axis; default to "" (empty)
plotScale	scale the available data before plotting, can be "linear" (do not scale, default), "log2" or "zscore"

type	If set to "legend" only the legend/key will be plotted.
error.estimates	Indicates if error estimates are plotted, defaults to TRUE
legend	Indicates if key describing the PlotSetPairs is shown, defaults to TRUE
legend_ext	Indicates if key describing error estimates is shown, defaults to FALSE
legend_pos	The position of main key, defaults to 'topright'
legend_ext_pos	The position of error estimates key, defaults to 'topleft'
cex.axis	Axis numbers font size in points, defaults to 14
cex.lab	Axis labels font size in points, Defaults to 16
cex.main	Main title font size in points, defaults to 20
cex.legend	Keys labels font size in points, defaults to 10
ln.v	Determines if vertical guide line(s) should be plotted (TRUE) or omitted (FALSE). For anchored plots 2 lines indicating the start and end of anchored distance are plotted.
ln.h	Determines if horizontal guide line should be plotted. Numeric value of the parameter indicates the Y-axis position of the line, NULL (default) indicates to omit
colvec	The vector of colours used to plot the lines and error estimate fields. If set value NULL (default) the automatically generated colour values will be used. Accepted values are: vector of any of the three kinds of R colour specifications, i.e., either a colour name (as listed by colors()), a hexadecimal string of the form "#rrggbb" or "#rrggbbaa" (see rgb), or a positive integer i meaning palette()[i]. See col2rgb .
pointsize	The default font point size to be used for plots. Defaults to 12 (1/72 inch).
...	other graphical parameters passed to plot.default (see plot.default , par and section "Details" below)

Details

Relevant parameters passed to [plot.default](#) function:

- log a character string which contains "x" if the x axis is to be logarithmic, "y" if the y axis is to be logarithmic and "xy" or "yx" if both axes are to be logarithmic.
- ann a logical value indicating whether the default annotation (title and x and y axis labels) should appear on the plot.
- axes a logical value indicating whether both axes should be drawn on the plot. Use graphical parameter "xaxt" or "yaxt" to suppress just one of the axes.
- rame.plot a logical indicating whether a box should be drawn around the plot.
- panel.first an "expression" to be evaluated after the plot axes are set up but before any plotting takes place. This can be useful for drawing background grids or scatterplot smooths. Note that this works by lazy evaluation: passing this argument from other plot methods may well not work since it may be evaluated too early.
- panel.last an expression to be evaluated after plotting has taken place but before the axes, title and box are added. See the comments about panel.first.
- asp the y/x aspect ratio, see [plot.window](#).

Value

NULL

Methods (by class)

- list: Method for signature plotset='list'
- PlotSetPair: Method for signature plotset='PlotSetPair'
- PlotSetList: Method for signature plotset='PlotSetList'
- PlotSetArray: Method for signature plotset='PlotSetArray'

Author(s)

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See AlsoOther plotting functions: [getPlotSetArray](#), [plotHeatmap](#), [plot](#)**Examples**

```
# Get the paths of example files
bed1 <- system.file("extdata",
  "Transcripts_ce10_chrI_100Kb.bed", package="seqplots")
bed2 <- system.file("extdata",
  "GSM1208361_chrI_100Kb_PeakCalls.bed", package="seqplots")
bw1 <- system.file("extdata",
  "GSM1208360_chrI_100Kb_q5_sample.bw", package="seqplots")

#If required install C. elegans genomic package from Bioconductor
if(!"BSgenome.Celegans.UCSC.ce10" %in% BSgenome::installed.genomes()) {
  if(.Platform$OS.type != "windows" || .Machine$sizeof.pointer != 4) {
    source("http://bioconductor.org/biocLite.R")
    biocLite("BSgenome.Celegans.UCSC.ce10")
  }
}

#Get getPlotSetArray for track and feature files
if(.Platform$OS.type != "windows" || .Machine$sizeof.pointer != 4) {
  plotset1 <- getPlotSetArray(bw1, c(bed1, bed2), 'ce10')
} else {
  load(system.file("extdata", "precalc_plotset.Rdata", package="seqplots"))
}

plotAverage(plotset1) # equivalent to plot(plotset1) or plotset1$plot()
```

 plotHeatmap

Plot heatmap with optional clustering

Description

Draw the heatmap plot from [PlotSetArray](#), [PlotSetList](#), [PlotSetPair](#) classes or properly formatted [list](#) (see details) in active graphics window. Axes and titles, keys and other plot elements are controlled by function parameters.

Usage

```
plotHeatmap(plotset, main = "", labels = NA, legend = TRUE,
  keepratio = FALSE, plotScale = "no", sortrows = FALSE, clusters = 5L,
  clstmethod = "kmeans", include = NULL, ssomt1 = 2L, ssomt2 = 2L,
  cex.main = 16, cex.lab = 12, cex.axis = 12, cex.legend = 12,
  xlab = "", ylab = "", autoscale = TRUE, zmin = 0, zmax = 10,
  xlim = NULL, ln.v = TRUE, s = 0.01, indi = TRUE, o_min = NA,
  o_max = NA, colvec = NULL, clspace = NULL, pointsize = 12,
  embed = FALSE, ggplot = FALSE, raster = FALSE, ...)
```

```
## S4 method for signature 'list'
```

```
plotHeatmap(plotset, main = "", labels = NA,
  legend = TRUE, keepratio = FALSE, plotScale = "no", sortrows = FALSE,
  clusters = 5L, clstmethod = "kmeans", include = NULL, ssomt1 = 2L,
  ssomt2 = 2L, cex.main = 16, cex.lab = 12, cex.axis = 12,
  cex.legend = 12, xlab = "", ylab = "", autoscale = TRUE, zmin = 0,
  zmax = 10, xlim = NULL, ln.v = TRUE, s = 0.01, indi = TRUE,
  o_min = NA, o_max = NA, colvec = NULL, clspace = NULL,
  pointsize = 12, embed = FALSE, ggplot = FALSE, raster = FALSE, ...)
```

```
## S4 method for signature 'PlotSetPair'
```

```
plotHeatmap(plotset, main = "", labels = NA,
  legend = TRUE, keepratio = FALSE, plotScale = "no", sortrows = FALSE,
  clusters = 5L, clstmethod = "kmeans", include = NULL, ssomt1 = 2L,
  ssomt2 = 2L, cex.main = 16, cex.lab = 12, cex.axis = 12,
  cex.legend = 12, xlab = "", ylab = "", autoscale = TRUE, zmin = 0,
  zmax = 10, xlim = NULL, ln.v = TRUE, s = 0.01, indi = TRUE,
  o_min = NA, o_max = NA, colvec = NULL, clspace = NULL,
  pointsize = 12, embed = FALSE, ggplot = FALSE, raster = FALSE, ...)
```

```
## S4 method for signature 'PlotSetList'
```

```
plotHeatmap(plotset, main = "", labels = NA,
  legend = TRUE, keepratio = FALSE, plotScale = "no", sortrows = FALSE,
  clusters = 5L, clstmethod = "kmeans", include = NULL, ssomt1 = 2L,
  ssomt2 = 2L, cex.main = 16, cex.lab = 12, cex.axis = 12,
  cex.legend = 12, xlab = "", ylab = "", autoscale = TRUE, zmin = 0,
  zmax = 10, xlim = NULL, ln.v = TRUE, s = 0.01, indi = TRUE,
```

```

o_min = NA, o_max = NA, colvec = NULL, clspace = NULL,
pointsize = 12, embed = FALSE, ggplot = FALSE, raster = FALSE, ...)

## S4 method for signature 'PlotSetArray'
plotHeatmap(plotset, main = "", labels = NA,
  legend = TRUE, keepratio = FALSE, plotScale = "no", sortrows = FALSE,
  clusters = 5L, clstmethod = "kmeans", include = NULL, ssomt1 = 2L,
  ssomt2 = 2L, cex.main = 16, cex.lab = 12, cex.axis = 12,
  cex.legend = 12, xlab = "", ylab = "", autoscale = TRUE, zmin = 0,
  zmax = 10, xlim = NULL, ln.v = TRUE, s = 0.01, indi = TRUE,
  o_min = NA, o_max = NA, colvec = NULL, clspace = NULL,
  pointsize = 12, embed = FALSE, ggplot = FALSE, raster = FALSE, ...)

```

Arguments

plotset	The dataset to plot - can be PlotSetArray , PlotSetList , PlotSetPair or properly formatted list
main	The main title of the plot, shown in top-centre part of the figure; defaults to NULL (not visible)
labels	The character vector giving sub-titles of heatmaps (plotted over the heatmap and below the main title). The defaults NULL value indicates that feature/track file names will be used to generate the sub-titles.
legend	if TRUE plot the colour key
keepratio	If TRUE keep 1:1 aspect ratio of the figure; defaults to FALSE
plotScale	scale the available data before plotting, can be "linear" (do not scale, default), "log2" or "zscore"
sortrows	If "increasing" or "decreasing" the rows of heatmap will be sorted by mean value across all heatmaps, defaults to FALSE - not sorted. For backwards compatibility TRUE is synonymous to "increasing".
clusters	The number of cluster for "kmeans" and "hclust", ignored for "ssom", defaults to 5L
clstmethod	Determines the heatmap clustering algorithm "kmeans" for k-means (default, see kmeans), "hclust" (see hclust) for hierarchical clustering, "ssom" for (super) self organising map (see supersom) with torus topology and "none" of FALSE to turn off the clustering
include	The logical vector indicating if given subplot should influence clustering and sorting, if given element is FALSE the sub-heatmap will be still plotted, and the order of data rows will be determined by clustering/sorting other sub-heatmaps, defaults to NULL, which includes all - equivalent to <code>rep(TRUE, length(plotset))</code>
ssomt1	Determines , the dimensionality of SOM - number of neurons in 1st dimension, number of resulting clusters equals <code>ssomt1*ssomt2</code> , defaults to 2L
ssomt2	Determines , the dimensionality of SOM - number of neurons in 2st dimension, number of resulting clusters equals <code>ssomt1*ssomt2</code> , defaults to 2L
cex.main	Main title font size in points, defaults to 16
cex.lab	Axis labels font size in points, Defaults to 12

<code>cex.axis</code>	Axis numbers font size in points, defaults to 12
<code>cex.legend</code>	Keys labels font size in points, defaults to 12
<code>xlab</code>	label below x-axis
<code>ylab</code>	label below y-axis
<code>autoscale</code>	if TRUE the colour keys will be auto scaled
<code>zmin</code>	global minimum value on colour key, ignored if <code>autoscale</code> is TRUE
<code>zmax</code>	global maximum value on colour key, ignored if <code>autoscale</code> is TRUE
<code>xlim</code>	the x limits (<code>x1</code> , <code>x2</code>) of the plot. Note that <code>x1 > x2</code> is allowed and leads to a "reversed axis". The default value, NULL, indicates that the whole range present in <code>plotset</code> will be plotted.
<code>ln.v</code>	Determines if vertical guide line(s) should be plotted (TRUE) or omitted (FALSE). For anchored plots 2 lines indicating the start and end of anchored distance are plotted.
<code>s</code>	The saturation value used to auto scale colour key limits, defaults to 0.01
<code>indi</code>	If TRUE (defaults) the independent colour keys will be plotted below heatmaps, if FALSE the common colour key is shown rightmost
<code>o_min</code>	vector of length equal to number of sub heatmaps determining minimum value on color key for each sub plot, if NULL (default) or NA the global settings are used, ignored in <code>indi</code> is FALSE
<code>o_max</code>	vector of length equal to number of sub heatmaps determining maximum value on color key for each sub plot, if NULL (default) or NA the global settings are used, ignored in <code>indi</code> is FALSE
<code>colvec</code>	The vector or list of colour values used generate sub-heatmaps colorspace. If NULL (default) the automatically generated colour values will be used for all sub-heatmaps. If single color is provided, the sequential colorspace reging from given color to white will be created. If the vector of colors is provided, the continous pallete will be created using these colors. NA value indicates default color pallete to be used for give sub-heatmap. Accepted values are: vector of any of the three kinds of R colour specifications, i.e., either a color name (as listed by <code>colors()</code>), a hexadecimal string of the form "#rrggbb" or "#rrggbbaa" (see <code>rgb</code>), or a positive integer <code>i</code> meaning <code>palette()[i]</code> . See col2rgb .
<code>clspace</code>	The colours pace of the heatmap, see grDevices
<code>pointsize</code>	The default font point size to be used for plots. Defaults to 12 (1/72 inch).
<code>embed</code>	If TRUE plot single (first) heatmap without using grid system. Useful to embed heatmap in complex layouts, see layout and par for details. Defaults to FALSE.
<code>ggplot</code>	Use <code>ggplot2</code> package instead of standard R graphics, defaults to FALSE
<code>raster</code>	The bitmap raster is used to plot the heatmap image, see "useRaster" option in image function and geom_raster function for details, defaults to FALSE
<code>...</code>	parameters passed to internal plotting function

Value

The cluster report data.frame, giving cluster assignments and sorting order for each feature. It contains following columns:

- **originalOrder** - number of feature (row) in GFF/BED, can be used to restore original order after sorting on cluster ID
- **ClusterID** - the numeric ID of the cluster. The topmost cluster on the heatmap is annotated with 1, and the bottom cluster with k, where k equals to number of clusters selected, exported only if clustering is enabled
- **SortingOrder** - the order imposed on heatmap by sorting by mean row(s) values, exported only if sorting is enabled
- **FinalOrder** - the final order of heatmap's rows, this can be influenced by sorting and clustering; 1 indicates topmost row

Methods (by class)

- list: Method for signature `list` with following format: `list[[FEATURE]][[TRACK/MOTIF]][[KEY_VALUE]]`
- PlotSetPair: Method for signature `PlotSetPair`
- PlotSetList: Method for signature `PlotSetList`
- PlotSetArray: Method for signature `PlotSetArray`

See Also

Other plotting functions: [getPlotSetArray](#), [plotAverage](#), [plot](#)

Examples

```
# Get the paths of example files
bed1 <- system.file("extdata",
  "Transcripts_ce10_chrI_100Kb.bed", package="seqplots")
bed2 <- system.file("extdata",
  "GSM1208361_chrI_100Kb_PeakCalls.bed", package="seqplots")
bw1 <- system.file("extdata",
  "GSM1208360_chrI_100Kb_q5_sample.bw", package="seqplots")

#If required install C. elegans genomic package from Bioconductor
if(!"BSgenome.Celegans.UCSC.ce10" %in% BSgenome::installed.genomes()) {
  if(.Platform$OS.type != "windows" || .Machine$sizeof.pointer != 4) {
    source("http://bioconductor.org/biocLite.R")
    biocLite("BSgenome.Celegans.UCSC.ce10")
  }
}

#Get getPlotSetArray for track and feature files
if(.Platform$OS.type != "windows" || .Machine$sizeof.pointer != 4) {
  plotset1 <- getPlotSetArray(bw1, c(bed1, bed2), 'ce10')
} else {
  load(system.file("extdata", "precalc_plotset.Rdata", package="seqplots"))
}
```

```
# equivalent to plot(plotset1, what='h') or plotset1$plot(what='h')
plotHeatmap(plotset1[[1]])
```

PlotSetArray-class *PlotSetArray Reference Class*

Description

PlotSetArray Reference Class

Fields

data a nested list holding the data
 annotations list of annotations

Methods

anno(n) Extracts the genomic locations for nth feature as GRanges
 as.array(x, ...) Converts PlotSetArray calss to matrix of PlotSeqPairs
 get(i, j) Subsetting method, returns PlotSetArray
 getByID(i) Subsetting method, returns PlotSeqList
 getPairs(i) Subsetting method, takes pair IDs list, returns PlotSetList
 getRow(i) Subsetting method, get row of data as list
 info() Outputs data.frame describing the content of PlotSetList
 nfeatures() Outputs the number of features in the PlotSetArray
 ntracks() Outputs the number of tracks in the PlotSetArray
 pairind() Outputs the list of pair IDs
 plot(...) Plot the PlotSetArray, i.e. all PlotSetPairs within class. See [plot](#) for details.
 subset(i, j) Subsetting method, get PlotSetPair as list
 unlist() Flattens PlotSetArray to PlotSetList

Subsetting

x is an object of PlotSetArray class:

- `x[1:2, 1:2]` produces [PlotSetArray](#) with 2 feature(s) and 2 tracks.
- `x[1:2]` produces [PlotSetList](#) with 2 feature/tracks pairs.
- `x[[1]]` produces single [PlotSetPair](#).
- `unlist(x)` produces [PlotSetList](#) with all feature/tracks pairs.
- `x$as.array()` produces the matrix of [PlotSetPair](#) classes with all feature/tracks pairs.

See Also

Other classes: [MotifSetup-class](#), [PlotSetList-class](#), [PlotSetPair-class](#)

PlotSetList-class *PlotSetList Reference Class*

Description

PlotSetList Reference Class

Fields

data a nested list holding the data
 annotations list of annotations

Methods

get(i) Subsetting method
 info() Outputs data.frame describing the content of PlotSetList
 npaires() Outputs the number (integer) of PlotSetPairs in the PlotSetList
 plot(what = "a", ...) Plot the PlotSetList, i.e. all PlotSetPairs in the list. See [plot](#) for details.

Subsetting

x is an object of PlotSetList class:

- x[1:2] produces [PlotSetList](#) with 2 feature/tracks pairs.
- x[[1]] produces single [PlotSetPair](#).

See Also

Other classes: [MotifSetup-class](#), [PlotSetArray-class](#), [PlotSetPair-class](#)

PlotSetPair-class *PlotSetPair Reference Class*

Description

PlotSetPair Reference Class

Fields

means numeric vector of means
 stderr numeric vector of standard errors
 conint numeric vector of 95% confidence intervals
 all_ind numeric vector giving the relative position of the bins in the genome
 numeric value giving the length of anchored distance, NULL for point feature plots
 desc character string describing the PlotSetPair
 heatmap numeric matrix used for plotting the heatmap

Methods

`as.list()` Convert to PlotSetPair list.

`plot(what = "a", ...)` Plot the PlotSetPair class. See [plot](#) for details.

See Also

Other classes: [MotifSetup-class](#), [PlotSetArray-class](#), [PlotSetList-class](#)

run

SeqPlots innitiation

Description

This function initate SeqPlots and opens web browser with graphical user interface (GUI).

Usage

```
run(root = file.path(path.expand("~"), "SeqPlots_data"), debug = FALSE, ...)
```

Arguments

<code>root</code>	the path to data directory, it will be created if not existing
<code>debug</code>	run the SeqPlots in debug mode, i.e. with R console embedded in web interface
<code>...</code>	arguments sent to runApp function

Details

The default data directory is `"~/SeqPlots_data"`.

Value

Normally returns nothing (NULL), returns an error if one accrued. Usage messages are shown in R console.

Author(s)

Przemyslaw Stempor

Examples

```
run()
```

seqplots

SeqPlots - An interactive tool for visualizing NGS signals and sequence motif densities along genomic features using average plots and heatmaps.

Description

SeqPlots is a tool for plotting next generation sequencing (NGS) based experiments' signal tracks, e.g. reads coverage from ChIP-seq, RNA-seq and DNA accessibility assays like DNase-seq and MNase-seq, over user specified genomic features, e.g. promoters, gene bodies, etc. It can also calculate sequence motif density profiles from reference genome. The data are visualized as average signal profile plot, with error estimates (standard error and 95% of heatmaps that can be sorted and clustered using hierarchical clustering, k-means algorithm and self organising maps. Plots can be prepared using R programming language or web browser based graphical user interface (GUI) implemented using Shiny framework. The dual-purpose implementation allows running the software locally on desktop or deploying it on server. SeqPlots is useful for both for exploratory data analyses and preparing replicable, publication quality plots. Other features of the software include collaboration and data sharing capabilities, as well as ability to store pre-calculated result matrixes, that combine many sequencing experiments and in-silico generated tracks with multiple different features. These binaries can be further used to generate new combination plots on fly, run automated batch operations or share with colleagues, who can adjust their plotting parameters without loading actual tracks and recalculating numeric values. SeqPlots relays on Bioconductor packages, mainly on rtracklayer for data input and BSgenome packages for reference genome sequence and annotations.

Details

Useful links:

- Project web page: <https://github.com/Przemol/seqplots>
- Online documentation: <http://przemol.github.io/seqplots/>
- Project wiki: <https://github.com/przemol/seqplots/wiki>
- Issue tracker: <http://github.com/przemol/seqplots/issues>
- Bug reports: <http://github.com/przemol/seqplots/issues/new>

Author(s)

Przemyslaw Stempor

References

R project and Bioconductor:

- R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.

- Bioconductor: Open software development for computational biology and bioinformatics R. Gentleman, V. J. Carey, D. M. Bates, B. Bolstad, M. Dettling, S. Dudoit, B. Ellis, L. Gautier, Y. Ge, and others 2004, Genome Biology, Vol. 5, R80. URL <http://www.bioconductor.org/>.
- RStudio and Inc. (2014). shiny: Web Application Framework for R. R package version 0.10.1. <http://shiny.rstudio.com/>.
- Other CRAN packages: digest, DBI, RSQLite, jsonlite, plotrix, fields, grid, kohonen, Cairo, parallel
- Bioconductor packages: IRanges, BSgenome, Rsamtools, rtracklayer, GenomicRanges and Biostrings

JavaScript and CSS

- jQuery framework - <http://jquery.com>
- Bootstrap - <http://getbootstrap.com>
- DataTables, Table plug-in for jQuery - <http://www.datatables.net>
- jQuery File Upload Plugin - <https://github.com/blueimp/jQuery-File-Upload>
- jQuery throttle - <http://benalman.com/projects/jquery-throttle-debounce-plugin/>
- jQuery Cookie Plugin - <https://github.com/carhartl/jquery-cookie>
- Modernizer JS library - <http://modernizr.com>
- JavaScript Templates - <https://github.com/blueimp/JavaScript-Templates>
- JavaScript Color Picker - <http://jscolor.com>
- md5-js - <https://github.com/wbond/md5-js>
- Font Awesome - <http://fortawesome.github.io/Font-Awesome>
- Google Fonts - <https://www.google.com/fonts>
- jQuery user interface - <http://jqueryui.com> (documentation)
- jquery.tocify.js: jQuery Table of Contents - <https://github.com/gfranko/jquery.tocify.js> (documentation)
- Strapdown <https://github.com/arturadib/strapdown> (documentation)
- Bootswatch themes - <http://bootswatch.com> (documentation)
- google-code-prettify - <https://code.google.com/p/google-code-prettify> (documentation)
- marked - <https://github.com/chjj/marked> (documentation)

Important conceptual contribution to the project

- Liu T, Ortiz J, Taing L, Meyer C, Lee B, Zhang Y, Shin H, Wong S, Ma J, Lei Y, et al. 2011. [Cistrome: an integrative platform for transcriptional regulation studies](#). Genome Biology 12: R83.
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- Nicol, J.W., Helt, G.A., Blanchard, S.G., Raja, A. and Loraine, A.E. (2009). **The Integrated Genome Browser: free software for distribution and exploration of genome-scale datasets**. *Bioinformatics* (Oxford, England). 25:2730-1.
- Thorvaldsdottir, H., Robinson, J.T. and Mesirov, J.P. (2012). **Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration**. *Briefings in bioinformatics*. bbs017

Server deployment

- Shiny Server - <https://github.com/rstudio/shiny-server>
- ShinyApps - <https://github.com/rstudio/shinyapps>

Publications containing figures made by SeqPlots

- Chen RA, Stempor P, Down TA, Zeiser E, Feuer SK, Ahringer J. **Extreme HOT regions are CpG-dense promoters in *C. elegans* and humans**. *Genome Res* 24(7):1138-1146 Jul 2014

seqplots-generic

SeqPlots generic methods

Description

Generic operators working with [PlotSetArray](#), [PlotSetList](#) and [PlotSetPair](#) to subset or flatten the data structure.

Usage

```
## S4 method for signature 'PlotSetList,ANY,ANY,ANY'
x[i, j, ..., drop = TRUE]

## S4 method for signature 'PlotSetList,ANY,ANY'
x[[i, j, ...]]

## S4 method for signature 'PlotSetArray,ANY,missing,ANY'
x[i, j, ..., drop = TRUE]

## S4 method for signature 'PlotSetArray,ANY,vector,ANY'
x[i, j]

## S4 method for signature 'PlotSetArray,ANY,ANY'
x[[i, j, ...]]
```

Arguments

x	an object of class PlotSetArray , PlotSetList or PlotSetPair
i	indices specifying elements to extract or replace. Indices are numeric or character vectors or empty (missing) or NULL. Numeric values are coerced to integer as by as.integer (and hence truncated towards zero). Character vectors will be matched to the names of the object (or for matrices/arrays, the dimnames): see ‘Character indices’ below for further details. For [-indexing only: i, j, ... can be logical vectors, indicating elements/slices to select. Such vectors are recycled if necessary to match the corresponding extent. i, j, ... can also be negative integers, indicating elements/slices to leave out of the selection. When indexing arrays by [a single argument i can be a matrix with as many columns as there are dimensions of x; the result is then a vector with elements corresponding to the sets of indices in each row of i. An index value of NULL is treated as if it were <code>integer(0)</code> .
j	see description for i
...	see description for i
drop	For matrices and arrays. If TRUE the result is coerced to the lowest possible dimension (see the examples). This only works for extracting elements, not for the replacement. See drop for further details.

Value

If x is [PlotSetArray](#) class:

- `x[1:2, 1:2]` produces [PlotSetArray](#) with 2 feature(s) and 2 tracks.
- `x[1:2]` produces [PlotSetList](#) with 2 feature/tracks pairs.
- `x[[1]]` produces single [PlotSetPair](#).
- `x$as.array()` produces the matrix of [PlotSetPair](#) classes with all feature/tracks pairs.

If x is [PlotSetList](#) class:

- `x[1:2]` produces [PlotSetList](#) with 2 feature/tracks pairs.
- `x[[1]]` produces single [PlotSetPair](#).

See Also

[Extract](#)

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