

Package ‘seqplots’

April 15, 2020

Type Package

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

Version 1.24.0

Date 2014-09-15

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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.

License GPL-3

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

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

Depends R (>= 3.2.0)

Imports methods, IRanges, BSgenome, digest, rtracklayer, GenomicRanges, Biostrings, shiny ($\geq 0.13.0$), DBI, RSQLite, plotrix, fields, grid, kohonen, parallel, GenomeInfoDb, class, S4Vectors, ggplot2, reshape2, gridExtra, jsonlite, DT ($\geq 0.1.0$), RColorBrewer, Rsamtools, GenomicAlignments, BiocManager

Suggests testthat, BiocStyle, knitr, rmarkdown, covr

VignetteBuilder knitr

biocViews ImmunoOncology, ChIPSeq, RNASeq, Sequencing, Software, Visualization

LazyLoad yes

RoxygenNote 6.1.1

Collate 'MotifSetup-class.R' 'PlotSetPair-class.R' 'PlotSetArray-class.R' 'PlotSetList-class.R' 'add_local_files.R' 'deployServerInstance.R' 'generic_methods.R' 'getPlotSetArray.R' 'helper_functions.R' 'int_getSF.R' 'int_ggHeatmapPlotWrapper.R' 'int_heatmapPlotWrapper.R' 'int_imPlot2.R' 'int_plotMext.R' 'plotAverage.R' 'plotHeatmap.R' 'run.R' 'seqplots-package.R' 'zzz.R'

git_url <https://git.bioconductor.org/packages/seqplots>

git_branch RELEASE_3_10

git_last_commit 95b0a96

git_last_commit_date 2019-10-29

Date/Publication 2020-04-14

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R topics documented:

| | |
|------------------------------|----|
| add_local_file | 3 |
| getPlotSetArray | 3 |
| getREF | 6 |
| import.narrowPeak | 6 |
| MotifSetup-class | 7 |
| plot | 8 |
| plotAverage | 9 |
| plotHeatmap | 12 |
| PlotSetArray-class | 17 |
| PlotSetList-class | 18 |
| PlotSetPair-class | 18 |
| run | 19 |
| seqplots | 20 |
| seqplots-generic | 22 |

| | |
|--------------|-----------|
| Index | 24 |
|--------------|-----------|

add_local_file *Add files to local SeqPlots installation*

Description

Add files to local SeqPlots installation

Usage

```
add_local_file(x, name = deparse(substitute(x)), file_genome = "ce11",
  file_user = "user", file_comment = "",
  root = file.path(path.expand("~"), "SeqPlots_data"))
```

Arguments

| | |
|--------------|--------------------------------------------------------|
| x | file path, GRanges, SimpleRleList or BigWigFile object |
| name | neme of the file |
| file_genome | genome version |
| file_user | user name |
| file_comment | comment |
| root | local installation directory |

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", lv11m = message, lv12m = 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 addMotiff 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) {
    if (!requireNamespace("BiocManager", quietly=TRUE))
      install.packages("BiocManager")
    BiocManager::install("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

```

getREF

Get reference genome

Description

Get reference genome

Usage

```
getREF(genome)
```

Arguments

genome The filename of FASTA file or genome code for BSgenome

Value

DNAStrngSet

import.narrowPeak

Import function for narrowPeak format

Description

Import function for narrowPeak format

Usage

```
import.narrowPeak(x)
```

Arguments

x the path or connection to .narrowPeak file

Value

GenomicRanges

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"

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)

Przemyslaw Stempor

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) {
    if (!requireNamespace("BiocManager", quietly=TRUE))
      install.packages("BiocManager")
    BiocManager::install("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, clsmethod = "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, plotz = TRUE, FO = NULL,
CL = NA, sort_by = NULL, sort_mids = FALSE, sort_dst = 0L,
clst_mids = FALSE, ...)

## S4 method for signature 'list'
plotHeatmap(plotset, main = "", labels = NA,
  legend = TRUE, kepratio = 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, plotz = TRUE, FO = NULL,
  CL = NA, sort_by = NULL, sort_mids = FALSE, sort_dst = 0L,
  clst_mids = FALSE, ...)

## S4 method for signature 'PlotSetPair'
plotHeatmap(plotset, main = "", labels = NA,
  legend = TRUE, kepratio = 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, plotz = TRUE, FO = NULL,
  CL = NA, sort_by = NULL, sort_mids = FALSE, sort_dst = 0L,
  clst_mids = FALSE, ...)

## S4 method for signature 'PlotSetList'
plotHeatmap(plotset, main = "", labels = NA,
  legend = TRUE, kepratio = 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, plotz = TRUE, FO = NULL,
  CL = NA, sort_by = NULL, sort_mids = FALSE, sort_dst = 0L,
  clst_mids = FALSE, ...)

## S4 method for signature 'PlotSetArray'
plotHeatmap(plotset, main = "", labels = NA,
  legend = TRUE, kepratio = 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, plotz = TRUE, FO = NULL,
CL = NA, sort_by = NULL, sort_mids = FALSE, sort_dst = 0L,
clst_mids = 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, "bed_scores" to use scores stored in BED/GFF file as cluster indicates, 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 |
| cex.axis | Axis numbers font size in points, defaults to 12 |
| cex.legend | Keys labels font size in points, defaults to 12 |
| xlab | label below x-axis |
| ylab | label below y-axis |
| autoscale | if TRUE the colour keys will be auto scaled |
| zmin | global minimum value on colour key, ignored if autoscale is TRUE |
| zmax | global maximum value on colour key, ignored if autoscale is TRUE |

| | |
|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 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. |
| 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. |
| s | The saturation value used to auto scale colour key limits, defaults to 0.01 |
| indi | If TRUE (defaults) the independent colour keys will be plotted below heatmaps, if FALSE the common colour key is shown rightmost |
| o_min | 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 indi is FALSE |
| o_max | 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 indi is FALSE |
| colvec | 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 colors()), a hexadecimal string of the form "#rrggbb" or "#rrggbbaa" (see rgb), or a positive integer i meaning palette()[i]. See col2rgb . |
| clspace | The colours pace of the heatmap, see grDevices |
| pointsize | The default font point size to be used for plots. Defaults to 12 (1/72 inch). |
| embed | 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. |
| ggplot | Use ggplot2 package instead of standard R graphics, defaults to FALSE |
| raster | 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 |
| plotz | if FALSE the functions returns a list of plot parameters, rather than actual plot |
| F0 | If set, this vector determines a final order of features in the heatmap |
| CL | If set, this vector determines a class assignment of features in the heatmap |
| sort_by | Set which subplots contribute to sorting |
| sort_mids | For anchored plot, sort using only middle values |
| sort_dst | For anchored plot, the flanking distance that should be added to sorting calculation |
| clst_mids | For anchored plot, cluster using only middle values |
| ... | 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) {
    if (!requireNamespace("BiocManager", quietly=TRUE))
      install.packages("BiocManager")
    BiocManager::install("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
 npairs() 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
 stdev 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 initiation

Description

This function initiates 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://fontawesome.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.
- Thomas Williams, Colin Kelley and others (2010). Gnuplot 4.4: an interactive plotting program. URL <http://www.R-project.org/>.
- Kent, W.J., Sugnet, C.W., Furey, T.S., Roskin, K.M., Pringle, T.H., Zahler, A.M. and Haussler, a. D. (2002). **The Human Genome Browser at UCSC**. *Genome Research*. 12:996-1006.
- Kent WJ, Zweig AS, Barber G, Hinrichs AS, Karolchik D. (2010). **BigWig and BigBed: enabling browsing of large distributed datasets**. *Bioinformatics*. 1;26(17):2204-7
- 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 <code>i</code> |
| <code>drop</code> | For matrices and arrays. If <code>TRUE</code> 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](#)

Index

*Topic **seqplots**

- run, [19](#)
- [,PlotSetArray,ANY,missing,ANY-method (seqplots-generic), [22](#)
- [,PlotSetArray,ANY,vector,ANY-method (seqplots-generic), [22](#)
- [,PlotSetList,ANY,ANY,ANY-method (seqplots-generic), [22](#)
- [[,PlotSetArray,ANY,ANY-method (seqplots-generic), [22](#)
- [[,PlotSetList,ANY,ANY-method (seqplots-generic), [22](#)

- add_local_file, [3](#)
- as.integer, [22](#)
- available.genomes, [5](#)

- BSgenome, [5](#)

- col2rgb, [11](#), [15](#)

- dimnames, [22](#)
- drop, [23](#)

- Extract, [23](#)

- geom_raster, [15](#)
- getPlotSetArray, [3](#), [8](#), [12](#), [16](#)
- getREF, [6](#)
- grDevices, [15](#)

- hclust, [14](#)

- image, [15](#)
- import.narrowPeak, [6](#)
- installed.genomes, [5](#)

- kmeans, [14](#)

- layout, [15](#)
- list, [9](#), [10](#), [12](#), [14](#), [16](#)

- message, [4](#)
- MotifSetup, [3](#), [4](#)
- MotifSetup (MotifSetup-class), [7](#)
- MotifSetup-class, [7](#)

- names, [22](#)

- par, [11](#), [15](#)
- plot, [5](#), [8](#), [8](#), [12](#), [16–19](#)
- plot,PlotSetArray,ANY-method (plot), [8](#)
- plot,PlotSetList,ANY-method (plot), [8](#)
- plot,PlotSetPair,ANY-method (plot), [8](#)
- plot.default, [11](#)
- plotAverage, [5](#), [8](#), [9](#), [16](#)
- plotAverage,list-method (plotAverage), [9](#)
- plotAverage,PlotSetArray-method (plotAverage), [9](#)
- plotAverage,PlotSetList-method (plotAverage), [9](#)
- plotAverage,PlotSetPair-method (plotAverage), [9](#)
- plotHeatmap, [5](#), [8](#), [12](#), [12](#)
- plotHeatmap,list-method (plotHeatmap), [12](#)
- plotHeatmap,PlotSetArray-method (plotHeatmap), [12](#)
- plotHeatmap,PlotSetList-method (plotHeatmap), [12](#)
- plotHeatmap,PlotSetPair-method (plotHeatmap), [12](#)
- PlotSetArray, [4](#), [5](#), [8–10](#), [12](#), [14](#), [16](#), [17](#), [22](#), [23](#)
- PlotSetArray (PlotSetArray-class), [17](#)
- PlotSetArray-class, [17](#)
- PlotSetList, [8–10](#), [12](#), [14](#), [16–18](#), [22](#), [23](#)
- PlotSetList (PlotSetList-class), [18](#)
- PlotSetList-class, [18](#)
- PlotSetPair, [8–10](#), [12](#), [14](#), [16–18](#), [22](#), [23](#)
- PlotSetPair (PlotSetPair-class), [18](#)
- PlotSetPair-class, [18](#)

- run, [19](#)
- runApp, [19](#)

- seqplots, [20](#)
- seqplots-generic, [22](#)
- seqplots-package (seqplots), [20](#)
- supersom, [14](#)