# Package 'epistack'

March 31, 2025

```
Title Heatmaps of Stack Profiles from Epigenetic Signals
```

**Version** 1.12.0

Description The epistack package main objective is the visualizations of stacks of genomic tracks (such as, but not restricted to, ChIP-seq, ATAC-seq, DNA methyation or genomic conservation data) centered at genomic regions of interest. epistack needs three different inputs: 1) a genomic score objects, such as ChIP-seq coverage or DNA methylation values, provided as a `GRanges` (easily obtained from `bigwig` or `bam` files). 2) a list of feature of interest, such as peaks or transcription start sites, provided as a `GRanges` (easily obtained from `gtf` or `bed` files). 3) a score to sort the features, such as peak height or gene expression value.

License MIT + file LICENSE

**Encoding** UTF-8

LazyData false

**Imports** GenomicRanges, SummarizedExperiment, BiocGenerics, S4Vectors, IRanges, graphics, plotrix, grDevices, stats, methods

**Roxygen** list(markdown = TRUE)

RoxygenNote 7.2.0

**Depends** R (>= 4.1)

**Suggests** testthat (>= 3.0.0), BiocStyle, knitr, rmarkdown, EnrichedHeatmap, biomaRt, rtracklayer, covr, vdiffr, magick

Config/testthat/edition 3

VignetteBuilder knitr

biocViews RNASeq, Preprocessing, ChIPSeq, GeneExpression, Coverage

URL https://github.com/GenEpi-GenPhySE/epistack

git\_url https://git.bioconductor.org/packages/epistack

git\_branch RELEASE\_3\_20

git\_last\_commit bea6e71

git\_last\_commit\_date 2024-10-29

**Repository** Bioconductor 3.20

**Date/Publication** 2025-03-31

2 addBins

# Author SACI Safia [aut],

DEVAILLY Guillaume [cre, aut]

Maintainer DEVAILLY Guillaume <gdevailly@hotmail.com>

# **Contents**

addB	ins addBins()	
Index		18
	stackepi_gr	17
	stackepi	16
	redimMatrix	15
	plotStackProfileLegend	14
	plotStackProfile	13
	plotMetric	12
	plotEpistack	9
	plotBoxMetric	8
	plotBinning	7
	plotAverageProfile	
	meanColor	6
	GRanges2RSE	5
	addMetricAndArrangeRSE	4
	addMetricAndArrangeGRanges	3
	addBins	2

## Description

Add an optional bin metadata column to gr, to serve as annotations for the epistack plots.

# Usage

```
addBins(rse, nbins = 5L, bin = NULL)
```

# Arguments

rse a SummarizedExperiment or a GRanges object.

nbins an integer number, the number of bins.

bin a vector containing pre-determined bins, in the same order as gr.

## **Details**

nbins is taken into account only if bin is NULL. rse should be sorted first, usually with the addMetricAndArrangeGRanges() function. addBin(rse, bin = vec) is equivalent to rse\$bin <- vec, while addBin(rse, nbins = 5) will create 5 bins of equal size based on rse order.

# Value

the RangedSummarizedExperiment or GRanges object with a new bin metadata column

#### See Also

addMetricAndArrangeGRanges plotBinning

#### **Examples**

```
data("stackepi")
addBins(stackepi)

# 3 bins instead of 5
addBins(stackepi, nbins = 3)

# assign bins using a vector
addBins(stackepi, bin = rep(c("a", "b", "c"),
length.out = length(stackepi)))
```

addMetricAndArrangeGRanges

addMetricAndArrangeGRanges()

#### **Description**

Perform an inner join between a GRanges object and a data.frame. Sort the resulting GRanges based on a metric column.

## Usage

```
addMetricAndArrangeGRanges(
   gr,
   order,
   gr_key = "name",
   order_key = "name",
   order_value = "exp",
   shuffle_tie = TRUE
)
```

# **Arguments**

gr a GRanges object.

order a data.frame with at least two columns: keys and values.

gr\_key name of the gr metadata column containing unique names for each genomic region in gr. Usually gene names/id or peak id.

order\_key name of the order column that will be used as key for the inner join.

order\_value name of the order column that contain value used for sorting.

 $shuffle\_tie \qquad a \ boolean \ Value \ (TRUE \ / \ FALSE). \ When \ TRUE, \ shuffle \ the \ GRanges \ before$ 

sorting, mixing the ties.

# **Details**

This utility function allow the addition of a metric column to genomic regions of interest. One of its common use case is to add gene expression values on a set of transcription start sites. The resulting GRanges object will only contain regions presents in both gr and order.

#### Value

a GRanges sorted in descending order.

#### **Examples**

```
data("stackepi_gr")
ramdomOrder <- data.frame(gene_id = stackepi_gr$gene_id,
    value = rnorm(length(stackepi_gr)))
addMetricAndArrangeGRanges(stackepi_gr,
    ramdomOrder, gr_key = "gene_id",
    order_key = "gene_id", order_value = "value")</pre>
```

addMetricAndArrangeRSE

addMetricAndArrangeRSE()

# Description

Perform an inner join between a rangedSummarizedExperiment object and a data.frame. Sort the resulting rangedSummarizedExperiment based on a metric column.

## Usage

```
addMetricAndArrangeRSE(
    rse,
    order,
    rse_key = "name",
    order_key = "name",
    order_value = "exp",
    shuffle_tie = TRUE
)
```

# **Arguments**

rse a rangedSummarizedExperiment object.

order a data.frame with at least two columns: keys and values.

rse\_key name of the gr metadata column containing unique names for each genomic

region in rowRanges(rse). Usually gene names/id or peak id.

order\_key name of the order column that will be used as key for the inner join.

order\_value name of the order column that contain value used for sorting.

shuffle\_tie a boolean Value (TRUE / FALSE). When TRUE, shuffle the GRanges before

sorting, mixing the ties.

## **Details**

This utility function allow the addition of a metric column to genomic regions of interest. One of its common use case is to add gene expression values on a set of transcription start sites. The resulting GRanges object will only contain regions presents in both rse and order.

GRanges2RSE 5

#### Value

a rangedSummarizedExperiment sorted in descending order.

## **Examples**

```
data("stackepi")
ramdomOrder <- data.frame(
    gene_id = SummarizedExperiment::rowRanges(stackepi)$gene_id,
    value = rnorm(length(stackepi))
)
addMetricAndArrangeRSE(stackepi,
    ramdomOrder, rse_key = "gene_id",
    order_key = "gene_id", order_value = "value")</pre>
```

GRanges2RSE

GRanges2RSE()

## **Description**

Convert objects from the old input format (GRanges object) to the new recommanded input format RangedSummarizedExperiment.

## Usage

```
GRanges2RSE(gr, patterns, names = patterns)
```

# **Arguments**

gr a GRanges object with matrix embeded as metadata columns.

patterns A character vector of column prefixes (can be regular expressions) that should

match columns of gr.

names specify the desired names of the assays (if different from patterns).

#### **Details**

Mostly used for backward compatibilities and unit testing.

# Value

 $a \; {\tt RangedSummarizedExperiment}.$ 

#### **Examples**

```
data("stackepi_gr")
GRanges2RSE(stackepi_gr, patterns = c("window"))
GRanges2RSE(stackepi_gr, patterns = c("^window_"), names = c("DNAme"))
```

6 plotAverageProfile

meanColor

meanColor

## **Description**

Return the average color of a vector of colors, computed in the RGB space.

# Usage

```
meanColor(colors)
```

# Arguments

colors

a vector of colors

## **Details**

Input colors can be either in html or color name formats. The alpha channel is supported but optional.

#### Value

a single color value

## See Also

redimMatrix

# **Examples**

```
meanColor(c("#000000FF", "#FFFFFF00", "#FFFF00FF", "#FF0000FF"))

# works with color names
meanColor(c("blue", "red"))

# Mix color names and HTML codes
meanColor(c("blue", "red", "#FFFF00FF"))

# works without alpha channel in inputs (but outputs an alpha channel):
meanColor(c("#000000", "#FFFFFFF", "#FFFF000", "#FF0000"))
```

 ${\tt plotAverageProfile}$ 

plotAverageProfile()

# Description

Plot the average stack profiles +/- error (sd or sem). If a bin column is present in rowRanges(rse), one average profile is drawn for each bin.

7 plotBinning

#### Usage

```
plotAverageProfile(
  rse,
  assay = NULL,
  x_labels = c("Before", "Anchor", "After"),
  palette = colorRampPalette(c("#DF536B", "black", "#61D04F")),
  alpha_for_se = 0.25,
  error_type = c("sd", "sem", "ci95"),
  reversed_z_order = FALSE,
  ylim = NULL,
  y_title = NULL,
  pattern = NULL
```

# **Arguments** rse

(for backward compatibility, pattern will be required). specify the name of the assay to plot, that should match one of assayNames(rse). assay x\_labels x-axis labels. palette A vector of colors, or a function that returns a palette of n colors. alpha\_for\_se the transparency (alpha) value for the error band.

can be either "sd" (standard deviation), "sem" (standard error of the mean), or error\_type

"ci95" (95% confidence interval). Default: "sd".

reversed\_z\_order

should the z-order of the curves be reversed (i.e. first or last bin on top?)

a RangedSummarizedExperiment input. Aletrnatively: can be a GRanges object

ylim a vector of two numbers corresponding to the y-limits of the plot.

y\_title the y-axis title.

only if rse is of class GRanges. A single character that should match metadata pattern

of rse (can be a regular expression).

## Value

Display a plot.

## **Examples**

```
data("stackepi")
plotAverageProfile(stackepi)
```

plotBinning plotBinning()

# **Description**

Plot a vertical color bar of the bin column.

8 plotBoxMetric

#### Usage

```
plotBinning(
   rse,
   target_height = 650,
   palette = colorRampPalette(c("#DF536B", "black", "#61D04F"))
)
```

## **Arguments**

rse a RangedSummarizedExperiment input with a column bin in rowRanges(rse).

Alternatively (for backward compatibility), a GRanges object or any object such

as rse\$bin exists.

target\_height an integer, the approximate height (in pixels) of the final plot. Used to avoid

overplotting artefacts.

palette A vector of colors, or a function that returns a palette of n colors.

#### Value

Display a plot.

## **Examples**

```
data("stackepi")
rse <- stackepi
rse <- addBins(rse, nbins = 3)
plotBinning(rse)

gr2 <- data.frame(bin = rep(c(1,2,3,4), each = 5))
plotBinning(gr2, palette = colorRampPalette(c("blue4", "forestgreen", "coral3", "goldenrod"))))</pre>
```

plotBoxMetric

plotBoxMetric()

## **Description**

Plot distribution of a metric values as boxplots depending of bins. If the bin is absent from gr, a single boxplot is drawn.

# Usage

```
plotBoxMetric(
    rse,
    metric = "expr",
    title = "Metric",
    trans_func = function(x) x,
    ylim = NULL,
    ylab = "metric",
    palette = colorRampPalette(c("#DF536B", "black", "#61D04F"))
)
```

plotEpistack 9

## **Arguments**

rse	a RangedSummarizedExperiment input. Aletrnatively: can be a GRanges object (for backward compatibility).
metric	name of the column in rse metadata containing scores.
title	title of the plot.
trans_func	A function to transform value of x before ploting. Useful to apply $\log 10$ transformation (i.e. with trans_func = function(x) $\log 10(x+1)$ ).
ylim	limit of the y axis; format: ylim = c(min, max)
ylab	y-axis title
palette	A vector of colors, or a function that returns a palette of n colors.

#### Value

Display a plot.

## **Examples**

plotEpistack

plotEpistack()

## **Description**

Given a list of genomic regions, epigenetic signals surrounding these regions, and a score for each regions, plot epigenetic stacks depending on the score. An optional bin column allow the grouping of several genomic regions to produce average profiles per bins.

# Usage

```
plotEpistack(
    rse,
    assays = NULL,
    tints = "gray",
    titles = NULL,
    legends = "",
    main = NULL,
    x_labels = c("Before", "Anchor", "After"),
    zlim = c(0, 1),
    ylim = NULL,
    metric_col = "exp",
    metric_title = "Metric",
    metric_label = "metric",
    metric_ylab = NULL,
```

10 plotEpistack

```
metric_transfunc = function(x) x,
bin_palette = colorRampPalette(c("#DF536B", "black", "#61D04F")),
npix_height = 650,
n_core = 1,
high_mar = c(2.5, 0.6, 4, 0.6),
low_mar = c(2.5, 0.6, 0.3, 0.6),
error_type = c("ci95", "sd", "sem"),
reversed_z_order = FALSE,
rel_widths = c(score = 0.35, bin = 0.08, assays = 0.35),
rel_heights = c(1, 0.14, 0.3),
patterns = NULL,
...
)
```

#### Arguments

npix\_height

a RangedSummarizedExperiment input. Aletrnatively: can be a GRanges object rse (for backward compatibility, patterns will be required). specify the name(s) and order of assay(s) to plot. A vector of names that should assays match assayNames(rse). a vector of colors to tint the heatmaps. Can alos be a function returning n colors, tints or a list of such palette functions. titles titles of each heatmap. Defaults to assays. legends legend names for the epistacks. main Main title for the figure. x\_labels a character vector of length 3 used as x-axis labels. zlim the minimum and maximum z values the heatmap. Format:  $z \lim = c(\min,$ max). zlim can also be specified of as a list of pairs of limits, on for each assay. ylim limits of the y axis for bottom plots. ylim can also be specified of as a list of pairs of limits, on for each assay. Format: ylim = c(min, max) metric\_col a character, name of a column in gr such as expression value, peak height, pvalue, fold change, etc. title to be display on the leftmost plots. metric\_title metric\_label label of the leftmost plots. metric\_ylab y axis label of the top left plot. metric\_transfunc a function to transform value of metric\_col before plotting. Useful to apply log10 transformation (i.e. with trans\_func = function(x) log10(x+1)). bin\_palette A vector of colors, or a function that returns a palette of n colors. Used to color

average profiles per bin in the bottom plots.

The matrix height is reduced to this number of rows before plotting. Useful to limit overplotting artefacts. It should roughtly be set to the pixel height in the

final heatmaps

n\_core number of core used to speedup the martrix resizing.

high\_mar a vector of numerical values corresponding to the margins of the top figures.

c(bottom, left, top, right)

plotEpistack 11

low\_mar a vector of numerical values corresponding to the margins of the bottom figures.

c(bottom, left, top, right)

error\_type error\_type, can be either "sd" (standard deviation), "sem" (standard error of the

mean), or "ci95" (95% confidence interval). Default: "ci95".

reversed\_z\_order

For the bottom panels: should the z-order of the curves be reversed (i.e. first or

last bin on top)?

rel\_widths A named vector of three elements of relative panel widths: score is the left-

most panel, bin is the optionnal binning panels, and assays are the panels of the stacked-matrices. Default to c(score = .35, bin = .08, assays = .35)

rel\_heights A vector of three elements of relative panel heights. Default to c(1, .14, .3)

patterns only if rse is of class GRanges. A character vector of column prefixes (can be

only if 1 se is of class orkanges. A character vector of column prefixes

regular expressions) that should match columns of rse.

... Arguments to be passed to par such as cex

#### **Details**

This function produce a comprehensive figure including epigenetic heatmaps and average epigenetic profiles from a well formated RangedSummarizedExperiment object with expected rowData metadata columns. It scales resonably well up to hundreds of thousands of genomic regions.

The visualisation is centered on an anchor, a set of genomic coordinated that can be transcription start sites or peak center for example. Anchor coordinates are those of the GRanges used as a rowData in the input RangedSummarizedExperiment object (hereafter rse).

Anchors are plotted from top to bottom in the same order as in rse. One should sort rse before plotting if needed.

rse's rowData should have a metric column that is used in the leftmost plots. The name of the metric column must be specified to metric\_col. The metric can be transformed before plotting if needed using the metric\_transfunc parameter.

The matrix or matrices used to display the heatmap(s) should be passed as assay(s) in rse. Such matrix can be obtained using EnrichedHeatmap::normalizeToMatrix() for example. The assay names are then specified through assays.

If an optionnal bin column is present in rse's rowData, it will be used to group genomic regions to performed average profile per bins in the bottom plots.

Epistack are multipanel plots build using layout(). Margins for the panels can be specified using high\_mar and low\_mar parameters if needed, especially to avoid text overlaps. The default value should be appropriate in most situations. Individual component can be plotted using severa epistack functions such has plotStackProfile() or plotAverageProfile().

Plotting more than > 1000 regions can lead to overplotting issued as well as some plotting artefacts (such as horizontal white strips). Both issues can be resolved with fidling with the npix\_height parameter. npix\_height should be smaller than the number of regions, and in the same order of magnitude of the final heatmap height in pixels. Last minutes call to the redimMatrix() function will hapen before plotting using npix\_height as target height. Parameter n\_core is passed to redimMatrix() to speed up the down-scaling.

The input can also be a GRanges object for backward compatibility. See GRanges2RSE. patterns would then be required.

## Value

Display a plot.

12 plotMetric

#### See Also

 $\verb|plotStackProfile|, plotAverageProfile|, redimMatrix|, normalizeToMatrix|, addMetricAndArrangeGRanges|, addBins|$ 

# **Examples**

```
data("stackepi")
plotEpistack(stackepi,
   metric_col = "exp",
   ylim = c(0, 1),
   metric_transfunc = function(x) log10(x+1))
```

plotMetric

plotMetric()

# **Description**

Plot a vertical line chart of the metric column, in the same order as the input.

# Usage

```
plotMetric(
    x,
    trans_func = function(x) x,
    title = "Metric",
    ylim = NULL,
    xlab = NULL,
    ylab = NULL
)
```

## **Arguments**

#### Value

Display a plot.

## See Also

```
plotEpistack, plotBoxMetric
```

plotStackProfile 13

## **Examples**

```
data("stackepi")
plotMetric(SummarizedExperiment::rowRanges(stackepi)$exp)
```

plotStackProfile plotStackProfile()

# Description

Display a heatmap of an epigenetic track centered at genomic anchors such as Transcription Start Sites or peak center.

# Usage

```
plotStackProfile(
    rse,
    assay = NULL,
    x_labels = c("Before", "Anchor", "After"),
    title = "",
    zlim = NULL,
    palette = function(n) grDevices::hcl.colors(n, rev = TRUE),
    target_height = 650,
    summary_func = function(x) mean(x, na.rm = TRUE),
    n_core = 1,
    pattern = NULL
)
```

## **Arguments**

rse	a RangedSummarizedExperiment input. Aletrnatively: can be a GRanges object (for backward compatibility, pattern will be required).
assay	specify the name of the assay to plot, that should match one of ${\tt assayNames(rse)}.$
x_labels	a character vectors of length 3 used to label the x-axis.
title	The title of the heatmap
zlim	The minimum and maximum z values to match color to values. Format: $zlim = c (min, max)$
palette	a palette of color, (i.e. a function of parameter n that should retrun n colors).
target_height	The matrix height is reduced to this number of rows before plotting. Useful to limit overplotting artefacts. It should roughtly be set to the pixel height in the final heatmap.
summary_func	function passed to redimMatrix(). Usualy mean, but can be set to median or max for sparse matrices.
n_core	multicore option, passed to redimMatrix().
pattern	only if rse is of class GRanges. A character vector of length 1 of a column prefixe (can be regular expressions) that should match columns of rse.

#### **Details**

The visualisation is centered on an anchor, a set of genomic coordinated that can be transcription start sites or peak center for example. Anchor coordinates are those of the RangedSummarizedExperiment object used as an input (hereafter rse).

Anchors are plotted from top to bottom in the same order as in rse. One should sort rse before plotting if needed.

The matrix used to display the heatmap should be passed as assay of rse. Such matrix can be obtained using EnrichedHeatmap::normalizeToMatrix() for example.

This function scale reasonnably wells up to hundred thousands of regions. Overplotting issues are solved by last-minute reduction of the matrix size using redimMatrix().

#### Value

Display a plot.

## See Also

```
plotAverageProfile, plotEpistack, normalizeToMatrix, plotStackProfileLegend
```

#### **Examples**

```
plotStackProfileLegend
```

plotStackProfileLegend()

#### **Description**

Utility function to plot the corresponding legend key of plotStackProfile()'s plots.

## Usage

```
plotStackProfileLegend(
  zlim,
  palette = colorRampPalette(c("white", "grey", "black")),
  title = NA
)
```

# Arguments

```
zlim the limits of the values to be displayed. Format: c(min, max)
palette a palette of color, (i.e. a function of parameter n that should retrun n colors).
title an optionnal title to be display bellow the color legend.
```

redimMatrix 15

#### Value

Display a plot.

## See Also

```
plotStackProfile
```

#### **Examples**

```
plotStackProfileLegend(zlim = c(0, 2),
    palette = colorRampPalette(c("white", "grey", "black")))
```

redimMatrix

redimMatrix()

### **Description**

Reduce the input matrix size by applying a summary function on cells to be fused.

## Usage

```
redimMatrix(
  mat,
  target_height = 100,
  target_width = 100,
  summary_func = function(x) mean(x, na.rm = TRUE),
  output_type = 0,
  n_core = 1
)
```

# **Arguments**

mat the input matrix.

target\_height height of the output matrix (should be smaller than or equal to nrow(mat)).

target\_width width of the output matrix (should be smaller than or equal to ncol(mat)).

summary\_func how to summerize cells? A function such has mean, median, max, or meanColors.

output\_type Type of the output, to be passed to vapply's FUN.VALUE.

n\_core number of core to use for parallel processing.

## **Details**

This function is used to reduce matrix right before plotting them in order to avoid overplotting issues as well as other plotting artefacts.

## Value

a resized matrix of size target\_width x target\_height where the summary\_fun was apply to adjacent cells.

16 stackepi

#### See Also

meanColor

## **Examples**

stackepi

epistack example and test dataset

# Description

DNA methylation profiles (from MBD-seq data) arround transcription start sites of the 693 chr18 genes annotated on the pig genome (Sscrofa11.1), as well as gene expression levels in Transcript Per Million (TPM) measured by RNA-seq in the same duodenum sample.

## Usage

```
data("stackepi")
```

#### **Format**

A RangedSummarizedExperiment of the 693 rows, 2 rows metadata columns, and one assay containing the DNA methylation signal.

## Source

This dataset was generated from ENSEMBL annotation data and data generated by our lab (publicly available soon).

stackepi\_gr 17

stackepi\_gr

epistack backward compatibility dataset

# Description

DNA methylation profiles (from MBD-seq data) arround transcription start sites of the 693 chr18 genes annotated on the pig genome (Sscrofa11.1), as well as gene expression levels in Transcript Per Million (TPM) measured by RNA-seq in the same duodenum sample.

# Usage

```
data("stackepi_gr")
```

#### **Format**

A GRanges of the 693 rows and 54 metadata columns, kept for unit-testing backward-compatibility.

## Source

This dataset was generated from ENSEMBL annotation data and data generated by our lab (publicly available soon).

#### See Also

GRanges2RSE

# **Index**

```
* datasets
    stackepi, 16
    stackepi_gr, 17
addBins, 2, 12
addMetricAndArrangeGRanges, 3, 3, 12
addMetricAndArrangeRSE, 4
GRanges2RSE, 5, 11, 17
meanColor, 6, 16
normalizeToMatrix, 12, 14
par, 11
plotAverageProfile, 6, 12, 14
plotBinning, 3, 7
plotBoxMetric, 8, 12
plotEpistack, 9, 12, 14
plotMetric,\, {\color{red}12}
plotStackProfile, 12, 13, 15
plotStackProfileLegend, 14, 14
redimMatrix, 6, 12, 15
stackepi, 16
stackepi_gr, 17
```