Package 'EnhancedVolcano'

April 3, 2025

Type Package

Title Publication-ready volcano plots with enhanced colouring and labeling

Version 1.25.0

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Description Volcano plots represent a useful way to visualise the results of differential expression analyses. Here, we present a highly-configurable function that produces publication-ready volcano plots. EnhancedVolcano will attempt to fit as many point labels in the plot window as possible, thus avoiding 'clogging' up the plot with labels that could not otherwise have been read. Other functionality allows the user to identify up to 4 different types of attributes in the same plot space via colour, shape, size, and shade parameter configurations.

License GPL-3

Depends ggplot2, ggrepel

Imports methods

Suggests ggalt, ggrastr, RUnit, BiocGenerics, knitr, DESeq2, pasilla, airway, org.Hs.eg.db, gridExtra, magrittr, rmarkdown

URL https://github.com/kevinblighe/EnhancedVolcano

biocViews RNASeq, GeneExpression, Transcription, DifferentialExpression, ImmunoOncology

VignetteBuilder knitr

RoxygenNote 7.1.2

git_url https://git.bioconductor.org/packages/EnhancedVolcano

git_branch devel

git_last_commit 88a8e6a

git_last_commit_date 2024-10-29

Repository Bioconductor 3.21

Date/Publication 2025-04-03

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Description

Volcano plots represent a useful way to visualise the results of differential expression analyses. Here, we present a highly-configurable function that produces publication-ready volcano plots [@EnhancedVolcano]. EnhancedVolcano will attempt to fit as many variable names in the plot window as possible, thus avoiding 'clogging' up the plot with labels that could not otherwise have been read.

Usage

```
EnhancedVolcano(
  toptable,
  lab,
 Х,
 у,
  selectLab = NULL,
 xlim = c(min(toptable[[x]], na.rm = TRUE) - 1.5, max(toptable[[x]], na.rm = TRUE) +
 ylim = c(0, max(-log10(toptable[[y]]), na.rm = TRUE) + 5),
 xlab = bquote(~Log[2] ~ "fold change"),
 ylab = bquote(~-Log[10] ~ italic(P)),
  axisLabSize = 18,
  title = "Volcano plot",
  subtitle = bquote(italic(EnhancedVolcano)),
  caption = paste0("total = ", nrow(toptable), " variables"),
  titleLabSize = 18,
  subtitleLabSize = 14,
  captionLabSize = 14,
  pCutoff = 1e-05,
  pCutoffCol = y,
  FCcutoff = 1,
  cutoffLineType = "longdash",
  cutoffLineCol = "black",
```

```
cutoffLineWidth = 0.4,
pointSize = 2,
labSize = 5,
labCol = "black",
labFace = "plain";
boxedLabels = FALSE,
parseLabels = FALSE,
shape = 19,
shapeCustom = NULL,
col = c("grey30", "forestgreen", "royalblue", "red2"),
colCustom = NULL,
colAlpha = 1/2,
colGradient = NULL,
colGradientBreaks = c(pCutoff, 1),
colGradientLabels = c("0", "1.0"),
colGradientLimits = c(0, 1),
legendLabels = c("NS", expression(Log[2] \sim FC), "p-value", expression(p - value ~ and
  ~ log[2] ~ FC)),
legendPosition = "top",
legendLabSize = 14,
legendIconSize = 5,
legendDropLevels = TRUE,
encircle = NULL,
encircleCol = "black",
encircleFill = "pink",
encircleAlpha = 3/4,
encircleSize = 2.5,
shade = NULL,
shadeFill = "grey",
shadeAlpha = 1/2,
shadeSize = 0.01,
shadeBins = 2,
drawConnectors = FALSE,
widthConnectors = 0.5,
typeConnectors = "closed",
endsConnectors = "first",
lengthConnectors = unit(0.01, "npc"),
colConnectors = "grey10",
max.overlaps = 15,
maxoverlapsConnectors = NULL,
min.segment.length = 0,
directionConnectors = "both",
arrowheads = TRUE.
hline = NULL,
hlineType = "longdash",
hlineCol = "black",
hlineWidth = 0.4,
vline = NULL,
```

```
vlineType = "longdash",
vlineCol = "black",
vlineWidth = 0.4,
gridlines.major = TRUE,
gridlines.minor = TRUE,
border = "partial",
borderWidth = 0.8,
borderColour = "black",
raster = FALSE
)
```

Arguments

toptable A data-frame of test statistics (if not, a data frame, an attempt will be made to

convert it to one). Requires at least the following: column for variable names (can be rownames); a column for log2 fold changes; a column for nominal or

adjusted p-value.

lab A column name in toptable containing variable names. Can be rownames (toptable).

x A column name in toptable containing log2 fold changes.

y A column name in toptable containing nominal or adjusted p-values.

selectLab A vector containing a subset of lab.

xlim Limits of the x-axis.
ylim Limits of the y-axis.
xlab Label for x-axis.
ylab Label for y-axis.

axisLabSize Size of x- and y-axis labels.

title Plot title.
subtitle Plot subtitle.
caption Plot caption.
titleLabSize Size of plot title.

subtitleLabSize

Size of plot subtitle.

captionLabSize Size of plot caption.

pCutoff Cut-off for statistical significance. A horizontal line will be drawn at -log10(pCutoff).

pCutoffCol Column name of statistical significance values to be used as the cut-off. A typ-

ical usage situation would be to pass nominal [un-adjusted] p-values as 'y', but adjusted p-values as pCutoffCol. In this way, a plot is generated via -

log10(unadjusted p-value), but cut-offs based on adjusted p-values.

FCcutoff Cut-off for absolute log2 fold-change. Vertical lines will be drawn at the nega-

tive and positive values of log2FCcutoff.

cutoffLineType Line type for FCcutoff and pCutoff ('blank', 'solid', 'dashed', 'dotted', 'dot-

dash', 'longdash', 'twodash').

cutoffLineCol Line colour for FCcutoff and pCutoff.

cutoffLineWidth

Line width for FCcutoff and pCutoff.

pointSize Size of plotted points for each variable. Can be a single value or a vector of

sizes.

labSize Size of labels for each variable.

Colour of labels for each variable.

labFace Font face of labels for each variable.

boxedLabels Logical, indicating whether or not to draw labels in boxes.

parseLabels Logical, indicating whether or not to parse expressions in labels

shape Shape of the plotted points. Either a single value for all points, or 4 values

corresponding to the default 4 legend labels specified by legendLabels.

shapeCustom Named vector / key-value pairs that will over-ride the default shape scheme. The

order must match that of toptable. Names / keys relate to groups / categories;

values relate to shape encodings.

col Colour shading for plotted points, corresponding to the default 4 legend labels

specified by legendLabels.

colCustom Named vector / key-value pairs that will over-ride the default colour scheme.

The order must match that of toptable. Names / keys relate to groups / cate-

gories; values relate to colour.

colAlpha Alpha for purposes of controlling colour transparency of variable points.

colGradient If activated, over-rides the default discrete colour scheme and replaces it with a

continous scheme that shades based on nominal or adjusted p-value specified by

y. For example, c('red2', 'blue2').

colGradientBreaks

Break-points for the two colours specified by colGradient.

colGradientLabels

Labels for the break-points specified by colGradientBreaks.

colGradientLimits

Limits of the colour scheme specified by colGradient, i.e., max and min possible

p-values.

legendLabels Plot legend text labels.

legendPosition Position of legend ('top', 'bottom', 'left', 'right').

legendLabSize Size of plot legend text.

legendIconSize Size of plot legend icons / symbols.

legendDropLevels

Logical, drop unused factor levels from legend.

encircle A vector of variable names to encircle. Requires installation of package ggalt.

encircleCol Colour of the encircled line.

encircleFill Colour fill of the encircled region.

encircleAlpha Alpha for purposes of controlling colour transparency of encircled region.

encircleSize Line width of the encircled line.

shade A vector of variable names to shade.

shadeFill Colour of shaded regions.

shadeAlpha Alpha for purposes of controlling colour transparency of shaded region.

shadeSize Size of the shade contour lines.

shadeBins Number of bins for the density of the shade.

drawConnectors Logical, indicating whether or not to connect plot labels to their corresponding

points by line connectors.

widthConnectors

Line width of connectors.

typeConnectors Have the arrow head open ('open') or filled ('closed')?

endsConnectors Which end of connectors to draw arrow head? ('last', 'first', 'both').

lengthConnectors

Length (size) of the connector arrowheads.

colConnectors Line colour of connectors and line segments.

max.overlaps Equivalent of max.overlaps in ggrepel. Set to 'Inf' to always display all labels

when drawConnectors = TRUE.

maxoverlapsConnectors

See max.overlaps.

min.segment.length

When drawConnectors = TRUE, specifies the minimum length of the connector

line segments.

directionConnectors

direction in which to draw connectors. 'both', 'x', or 'y'.

arrowheads Logical, indicating whether or not to draw arrow heads or or just have straight

lines.

hline Draw one or more horizontal lines passing through this/these values on y-axis.

For single values, only a single numerical value is necessary. For multiple lines,

pass these as a vector, e.g., c(60,90).

hlineType Line type for hline ('blank', 'solid', 'dashed', 'dotted', 'dotdash', 'longdash',

'twodash').

hlineCol Colour of hline. hlineWidth Width of hline.

vline Draw one or more vertical lines passing through this/these values on x-axis. For

single values, only a single numerical value is necessary. For multiple lines,

pass these as a vector, e.g., c(60,90).

vlineType Line type for vline ('blank', 'solid', 'dashed', 'dotted', 'dotdash', 'longdash',

'twodash').

vlineCol Colour of vline. vlineWidth Width of vline.

gridlines.major

Logical, indicating whether or not to draw major gridlines.

gridlines.minor

Logical, indicating whether or not to draw minor gridlines.

border Add a border for just the x and y axes ('partial') or the entire plot grid ('full')?

borderWidth Width of the border on the x and y axes.

borderColour Colour of the border on the x and y axes.

raster Logical, indicating whether to rasterize the geom_point layer. Requires instal-

lation of ggrastr.

Details

Volcano plots represent a useful way to visualise the results of differential expression analyses. Here, we present a highly-configurable function that produces publication-ready volcano plots [@EnhancedVolcano]. EnhancedVolcano will attempt to fit as many variable names in the plot window as possible, thus avoiding 'clogging' up the plot with labels that could not otherwise have been read.

Value

A ggplot2 object.

Author(s)

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Examples

```
library('pasilla')
pasCts <- system.file('extdata', 'pasilla_gene_counts.tsv',</pre>
  package='pasilla', mustWork=TRUE)
pasAnno <- system.file('extdata', 'pasilla_sample_annotation.csv',</pre>
  package='pasilla', mustWork=TRUE)
cts <- as.matrix(read.csv(pasCts,sep='\t',row.names='gene_id'))</pre>
coldata <- read.csv(pasAnno, row.names=1)</pre>
coldata <- coldata[,c('condition','type')]</pre>
rownames(coldata) <- sub('fb', '', rownames(coldata))</pre>
cts <- cts[, rownames(coldata)]</pre>
library('DESeq2')
dds <- DESeqDataSetFromMatrix(countData = cts,</pre>
  colData = coldata,
  design = \sim condition)
featureData <- data.frame(gene=rownames(cts))</pre>
mcols(dds) <- DataFrame(mcols(dds), featureData)</pre>
dds <- DESeq(dds)
res <- results(dds)</pre>
EnhancedVolcano(res,
  lab = rownames(res),
  x = 'log2FoldChange',
  y = 'pvalue',
```

```
pCutoff = 10e-4,
FCcutoff = 1.333,
xlim = c(-5.5, 5.5),
ylim = c(0, -log10(10e-12)),
pointSize = 1.5,
labSize = 2.5,
title = 'DESeq2 results',
subtitle = 'Differential expression',
caption = 'FC cutoff, 1.333; p-value cutoff, 10e-4',
legendPosition = "right",
legendLabSize = 14,
col = c('grey30', 'forestgreen', 'royalblue', 'red2'),
colAlpha = 0.9,
drawConnectors = TRUE,
hline = c(10e-8),
widthConnectors = 0.5)
```

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