Package 'EnhancedVolcano'

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Type Package

Title Publication-ready volcano plots with enhanced colouring and labeling

Version 1.31.0

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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

Encoding UTF-8

Depends ggplot2, ggrepel

Imports methods, scales, grid, grDevices

Suggests RUnit, ggrastr, 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

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Contents

`EnhancedVolcano` *Publication-ready volcano plots with enhanced colouring and labeling.*

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,
  x,
  y,
  selectLab = NULL,
 xlim = c(min(toptable[[x]], na.rm = TRUE) - 1.5, max(toptable[[x]], na.rm = TRUE) +
    1.5),
  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] ~ 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 typical 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 negative and positive values of log2FCcutoff.
cutoffLineType	Line type for FCcutoff and pCutoff ('blank', 'solid', 'dashed', 'dotted', 'dotdash', '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.
labCol	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 / categories; 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.
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 installation 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)

Kevin Blighe <kevin@clinicalbioinformatics.co.uk>

Examples

```
library('pasilla')
pasCts <- system.file('extdata', 'pasilla_gene_counts.tsv',
  package='pasilla', mustWork=TRUE)
pasAnno <- system.file('extdata', 'pasilla_sample_annotation.csv',
  package='pasilla', mustWork=TRUE)
cts <- as.matrix(read.csv(pasCts,sep='\t',row.names='gene_id'))
coldata <- read.csv(pasAnno, row.names=1)
coldata <- coldata[,c('condition','type')]
rownames(coldata) <- sub('fb', '', rownames(coldata))
cts <- cts[, rownames(coldata)]
library('DESeq2')
dds <- DESeqDataSetFromMatrix(countData = cts,
  colData = coldata,
  design = ~ condition)

featureData <- data.frame(gene=rownames(cts))
mcols(dds) <- DataFrame(mcols(dds), featureData)
dds <- DESeq(dds)
res <- results(dds)

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)
```

GeomEncircle *Custom Geom for Encircling Points in ggplot2*

Description

Custom Geom for Encircling Points in ggplot2

Author(s)

Jared Andrews, heavily based on ggalt code from Ben Bolker (https://github.com/hrbrmstr/ggalt/blob/master/R/geom_encircle.r)

`geom_encircle` *Automatically enclose points in a polygon*

Description

Creates a smooth encircling polygon around a set of points using convex hull calculation and xspline smoothing. Useful for highlighting groups of points in scatter plots.

Usage

```
geom_encircle(
  mapping = NULL,
  data = NULL,
  stat = "identity",
  position = "identity",
  na.rm = FALSE,
  show.legend = NA,
  inherit.aes = TRUE,
  ...
)
```

Arguments

`mapping`	Set of aesthetic mappings created by `aes`. If specified and `inherit.aes = TRUE` (the default), it is combined with the default mapping at the top level of the plot.
`data`	The data to be displayed in this layer. If `NULL`, the default, the data is inherited from the plot data as specified in the call to `ggplot`.
`stat`	The statistical transformation to use on the data for this layer, as a string.
`position`	Position adjustment, either as a string, or the result of a call to a position adjustment function.
`na.rm`	If `FALSE`, the default, missing values are removed with a warning. If `TRUE`, missing values are silently removed.
`show.legend`	Logical. Should this layer be included in the legends? `NA`, the default, includes if any aesthetics are mapped.
`inherit.aes`	If `FALSE`, overrides the default aesthetics, rather than combining with them.
`...`	Other arguments passed on to `layer`. These are often aesthetics, used to set an aesthetic to a fixed value, like `colour = "red"` or `size = 3`. They may also be parameters to the paired geom/stat. Additional parameters include: **s_shape** Controls the shape of the spline (default = 0.5). **s_open** Logical indicating whether the spline should be open (default = FALSE). **expand** Amount to expand the encircling polygon outward (default = 0.05). **spread** Spread factor for single or double point sets (default = 0.1).

Value

A ggplot2 layer that can be added to a plot.

Author(s)

Jared Andrews, heavily based on ggalt code from Ben Bolker (https://github.com/hrbrmstr/ggalt/blob/master/R/geom_encircle.r)

Examples

```
## Not run:
library(ggplot2)

d <- data.frame(x=c(1,1,2),y=c(1,2,2)*100)

gg <- ggplot(d,aes(x,y))
gg <- gg + scale_x_continuous(expand=c(0.5,1))
gg <- gg + scale_y_continuous(expand=c(0.5,1))

gg + geom_encircle(s_shape=1, expand=0) + geom_point()

gg + geom_encircle(s_shape=1, expand=0.1, colour="red") + geom_point()

gg + geom_encircle(s_shape=0.5, expand=0.1, colour="purple") + geom_point()

gg + geom_encircle(data=subset(d, x==1), colour="blue", spread=0.02) +
  geom_point()

gg + geom_encircle(data=subset(d, x==2), colour="cyan", spread=0.04) +
  geom_point()

gg <- ggplot(mpg, aes(displ, hwy))
gg + geom_encircle(data=subset(mpg, hwy>40)) + geom_point()
gg + geom_encircle(aes(group=manufacturer)) + geom_point()
gg + geom_encircle(aes(group=manufacturer,fill=manufacturer),alpha=0.4)+
       geom_point()
gg + geom_encircle(aes(group=manufacturer,colour=manufacturer))+
       geom_point()

ss <- subset(mpg,hwy>31 & displ<2)

gg + geom_encircle(data=ss, colour="blue", s_shape=0.9, expand=0.07) +
  geom_point() + geom_point(data=ss, colour="blue")

## End(Not run)
```

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