Package 'EnhancedVolcano'

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Type Package

Title Publication-ready volcano plots with enhanced colouring and labeling

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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

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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,
  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. 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',
  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)
```

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