# Package 'SCpubr'

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Type Package

**Title** Generate Publication Ready Visualizations of Single Cell Transcriptomics Data

Version 2.0.2

**Description** A system that provides a streamlined way of generating publication ready plots for known Single-Cell transcriptomics data in a "publication ready" format. This is, the goal is to automatically generate plots with the highest quality possible, that can be used right away or with minimal modifications for a research article.

License GPL-3

URL https://github.com/enblacar/SCpubr/,
 https://enblacar.github.io/SCpubr-book/

BugReports https://github.com/enblacar/SCpubr/issues/

**Depends** R (>= 4.0.0)

Suggests AnnotationDbi, assertthat, AUCell, circlize, cli, cluster, clusterProfiler, colorspace, ComplexHeatmap, covr, decoupleR, dplyr (>= 1.1.0), enrichplot, forcats, ggalluvial, ggbeeswarm, ggdist, ggExtra, ggh4x, ggnewscale, ggplot2 (>= 3.4.0), ggplotify, ggrastr, ggrepel, ggridges, ggsignif, graphics, infercnv, knitr, labeling, magrittr, MASS, Matrix, methods, Nebulosa, org.Hs.eg.db, patchwork, pheatmap, plyr, purrr, qpdf, RColorBrewer, rjags, rlang, rmarkdown, scales, scattermore, Seurat, SeuratObject, sf, stringr, svglite, testthat (>= 3.0.0), tibble, tidyr, UCell, viridis, withr

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100

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do\_AlluvialPlot

Generate Alluvial plots.

#### Description

This function is based on the **ggalluvial** package. It allows you to generate alluvial plots from a given Seurat object.

```
do_AlluvialPlot(
  sample,
  first_group,
  last_group,
 middle_groups = NULL,
  colors.use = NULL,
  plot.title = NULL,
 plot.subtitle = NULL,
  plot.caption = NULL,
  font.size = 14,
  font.type = "sans",
  xlab = NULL,
  ylab = "Number of cells",
  repel = FALSE,
  fill.by = last_group,
  use_labels = FALSE,
  stratum.color = "black",
  stratum.fill = "white",
  stratum.width = 1/3,
  stratum.fill.conditional = FALSE,
  use_geom_flow = FALSE,
  alluvium.color = "white",
  flow.color = "white",
  flip = FALSE,
  label.color = "black",
  curve_type = "sigmoid",
  use_viridis = FALSE,
  viridis.palette = "G",
  viridis.direction = -1,
  sequential.palette = "YlGnBu",
  sequential.direction = 1,
  plot.grid = FALSE,
  grid.color = "grey75",
  grid.type = "dashed",
  na.value = "white",
  legend.position = "right",
  legend.title = NULL,
```

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```
plot.title.face = "bold",
  plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
  legend.title.face = "bold",
  legend.text.face = "plain"
)
```

#### **Arguments**

Seurat | A Seurat object, generated by CreateSeuratObject. sample character | Categorical metadata variable. First group of nodes of the alluvial first\_group plot. last\_group character | Categorical metadata variable. Last group of nodes of the alluvial character | Categorical metadata variable. Vector of groups of nodes of the middle\_groups alluvial plot. colors.use character | Named list of colors corresponding to the unique values in fill.by (which defaults to last\_group). plot.title, plot.subtitle, plot.caption character | Title, subtitle or caption to use in the plot. font.size numeric | Overall font size of the plot. All plot elements will have a size relationship with this font size. character | Base font family for the plot. One of: font.type • mono: Mono spaced font. • serif: Serif font family. • sans: Default font family. xlab, ylab character | Titles for the X and Y axis. repel logical | Whether to repel the text labels. fill.by character | One of first\_group, middle\_groups (one of the values, if multiple mid\_groups) or last\_group. These values will be used to color the alluvium/flow. use\_labels logical | Whether to use labels instead of text for the stratum. stratum.color, alluvium.color, flow.color character | Color for the border of the alluvium (and flow) and stratum. stratum.fill character | Color to fill the stratum. stratum.width logical | Width of the stratum. stratum.fill.conditional logical | Whether to fill the stratum with the same colors as the alluvium/flow. logical | Whether to use geom\_flow instead of geom\_alluvium. Visual results use\_geom\_flow might differ. flip logical | Whether to invert the axis of the displayed plot. label.color character | Color for the text labels.

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curve\_type

character | Type of curve used in geom\_alluvium. One of:

• linear. • cubic. • quintic. • sine. • arctangent. • sigmoid. • xspline. logical | Whether to use viridis color scales. use\_viridis viridis.palette character | A capital letter from A to H or the scale name as in scale\_fill\_viridis. viridis.direction numeric | Either 1 or -1. Controls how the gradient of viridis scale is formed. sequential.palette character | Type of sequential color palette to use. Out of the sequential palettes defined in brewer.pal. sequential.direction numeric | Direction of the sequential color scale. Either 1 or -1. plot.grid logical | Whether to plot grid lines. grid.color character | Color of the grid in the plot. In heatmaps, color of the border of the cells. character | One of the possible linetype options: grid.type • blank. • solid. · dashed. • dotted. • dotdash. • longdash. · twodash. na.value character | Color value for NA. legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. • none: No legend is displayed. character | Title for the legend. legend.title plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title. character | Controls the style of the font for the corresponding theme element. • plain: For normal text. • italic: For text in itallic. • bold: For text in bold.

• bold.italic: For text both in itallic and bold.

#### Value

A ggplot2 object.

#### **Examples**

do\_BarPlot

Create Bar Plots.

## **Description**

Create Bar Plots.

```
do_BarPlot(
    sample,
    group.by,
    order = FALSE,
    add.n = FALSE,
    add.n.face = "bold",
    add.n.expand = c(0, 1.15),
    add.n.size = 4,
    order.by = NULL,
    split.by = NULL,
    facet.by = NULL,
    position = "stack",
    font.size = 14,
```

```
font.type = "sans",
  legend.position = "bottom",
  legend.title = NULL,
  legend.ncol = NULL,
  legend.nrow = NULL,
  legend.byrow = FALSE,
  axis.text.x.angle = 45,
  xlab = NULL,
  ylab = NULL,
  colors.use = NULL,
  flip = FALSE,
  plot.title = NULL,
  plot.subtitle = NULL,
  plot.caption = NULL,
  plot.grid = FALSE,
  grid.color = "grey75",
  grid.type = "dashed",
  plot.title.face = "bold",
  plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
  legend.title.face = "bold",
  legend.text.face = "plain",
  strip.text.face = "bold",
  return_data = FALSE
)
```

#### **Arguments**

order.by

based on its value.

sample Seurat | A Seurat object, generated by CreateSeuratObject. character | Metadata column to compute the counts of. Has to be either a group.by character or factor column. logical | Whether to order the results in descending order of counts. order add.n logical | Whether to add the total counts on top of each bar. add.n.face character | Font face of the labels added by add.n. add.n.expand numeric | Vector of two numerics representing the start and end of the scale. Minimum should be 0 and max should be above 1. This basically expands the Y axis so that the labels fit when flip = TRUE. • stack: Set the bars side by side, displaying the total number of counts. Uses position\_stack. • fill: Set the bars on top of each other, displaying the proportion of counts from the total that each group represents. Uses position\_fill. add.n.size numeric | Size of the labels

character | When split. by is used, value of group. by to reorder the columns

split.by	character   Metadata column to split the values of group.by by. If not used, defaults to the active idents.
facet.by	character   Metadata column to gather the columns by. This is useful if you have other overarching metadata.
position	character   Position function from ggplot2. Either stack or fill.
font.size	numeric   Overall font size of the plot. All plot elements will have a size relationship with this font size.
font.type	character   Base font family for the plot. One of:
	mono: Mono spaced font.
	serif: Serif font family.
	• sans: Default font family.
legend.positio	
	character   Position of the legend in the plot. One of:
	• top: Top of the figure.
	• bottom: Bottom of the figure.
	• left: Left of the figure.
	<ul><li>right: Right of the figure.</li><li>none: No legend is displayed.</li></ul>
1	
legend.title	character   Title for the legend.
legend.ncol	numeric   Number of columns in the legend.
legend.nrow	numeric   Number of rows in the legend.
legend.byrow	logical   Whether the legend is filled by row or not.
axis.text.x.an	numeric   Degree to rotate the X labels. One of: 0, 45, 90.
vlob vlob	-
xlab, ylab	character   Titles for the X and Y axis.
colors.use	named_vector   Named vector of valid color representations (either name of HEX codes) with as many named colors as unique values of group.by. If group.by is not provided, defaults to the unique values of Idents. If not provided, a color scale will be set by default.
flip	logical   Whether to invert the axis of the displayed plot.
plot.title, plo	ot.subtitle, plot.caption
	character   Title, subtitle or caption to use in the plot.
plot.grid	logical   Whether to plot grid lines.
grid.color	character   Color of the grid in the plot. In heatmaps, color of the border of the cells.
grid.type	character   One of the possible linetype options:
	• blank.
	• solid.
	dashed.
	• dotted.
	• dotdash.

- longdash.
- twodash.

plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.character | Controls the style of the font for the corresponding theme element.

One of:

- plain: For normal text.
- italic: For text in itallic.
- bold: For text in bold.
- bold.italic: For text both in itallic and bold.

strip.text.face

character | Controls the style of the font for the strip text. One of:

- plain: For normal text.
- italic: For text in itallic.
- bold: For text in bold.
- bold.italic: For text both in itallic and bold.

return\_data

logical | Returns a data.frame with the count and proportions displayed in the plot.

#### Value

A ggplot2 object containing a Bar plot.

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_BarPlot", passive = TRUE)</pre>
if (isTRUE(value)){
  # Consult the full documentation in https://enblacar.github.io/SCpubr-book/
  # Define your Seurat object.
sample <- readRDS(system.file("extdata/seurat_dataset_example.rds", package = "SCpubr"))</pre>
  # Basic bar plot, horizontal.
  p1 <- SCpubr::do_BarPlot(sample = sample,</pre>
                            group.by = "seurat_clusters",
                            legend.position = "none",
                            plot.title = "Number of cells per cluster")
  # Split by a second variable.
  sample$modified_orig.ident <- sample(x = c("Sample_A", "Sample_B", "Sample_C"),</pre>
                                         size = ncol(sample),
                                         replace = TRUE,
                                         prob = c(0.2, 0.7, 0.1)
  p <- SCpubr::do_BarPlot(sample,</pre>
                           group.by = "seurat_clusters",
                           split.by = "modified_orig.ident",
```

do\_BeeSwarmPlot

BeeSwarm plot.

#### **Description**

BeeSwarm plot.

```
do_BeeSwarmPlot(
  sample,
  feature_to_rank,
  group.by = NULL,
  assay = NULL,
  reduction = NULL,
  slot = NULL,
  continuous_feature = FALSE,
  order = FALSE,
  colors.use = NULL,
  legend.title = NULL,
  legend.type = "colorbar",
  legend.position = "bottom",
  legend.framewidth = 0.5,
  legend.tickwidth = 0.5,
  legend.length = 20,
  legend.width = 1,
  legend.framecolor = "grey50",
  legend.tickcolor = "white",
  legend.ncol = NULL,
  legend.icon.size = 4,
  plot.title = NULL,
  plot.subtitle = NULL,
  plot.caption = NULL,
  xlab = NULL,
 ylab = NULL,
  font.size = 14,
  font.type = "sans",
  remove_x_axis = FALSE,
```

```
remove_y_axis = FALSE,
  flip = FALSE,
  use_viridis = FALSE,
  viridis.palette = "G",
  viridis.direction = 1,
  sequential.palette = "YlGnBu",
  sequential.direction = 1,
  verbose = TRUE,
  raster = FALSE,
  raster.dpi = 300,
  plot_cell_borders = TRUE,
  border.size = 1.5,
  border.color = "black",
  pt.size = 2,
 min.cutoff = NA,
 max.cutoff = NA,
  na.value = "grey75",
  number.breaks = 5,
  plot.title.face = "bold",
  plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
  legend.title.face = "bold",
  legend.text.face = "plain"
)
```

# Arguments

sample Seurat | A Seurat object, generated by CreateSeuratObject.

feature\_to\_rank

character | Feature for which the cells are going to be ranked. Ideal case is that this feature is stored as a metadata column.

character | Metadata variable to group the output by. Has to be a character of group.by

factor column.

character | Assay to use. Defaults to the current assay. assay

reduction character | Reduction to use. Can be the canonical ones such as "umap", "pca",

> or any custom ones, such as "diffusion". If you are unsure about which reductions you have, use Seurat::Reductions(sample). Defaults to "umap" if present or to the last computed reduction if the argument is not provided.

slot character | Data slot to use. Only one of: counts, data, scale.data. Defaults to

"data".

continuous\_feature

logical | Is the feature to rank and color for continuous? I.e: an enrichment

order logical | Whether to reorder the groups based on the median of the ranking.

named\_vector | Named vector of valid color representations (either name of colors.use HEX codes) with as many named colors as unique values of group.by. If group.by is not provided, defaults to the unique values of Idents. If not provided, a color scale will be set by default. legend.title character | Title for the legend. character | Type of legend to display. One of: legend.type • normal: Default legend displayed by **ggplot2**. • colorbar: Redefined colorbar legend, using guide\_colorbar. legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. • none: No legend is displayed. legend.framewidth, legend.tickwidth numeric | Width of the lines of the box in the legend. legend.length, legend.width numeric | Length and width of the legend. Will adjust automatically depending on legend side. legend.framecolor character | Color of the lines of the box in the legend. legend.tickcolor character | Color of the ticks of the box in the legend. legend.ncol numeric | Number of columns in the legend. legend.icon.size numeric | Size of the icons in legend. plot.title, plot.subtitle, plot.caption character | Title, subtitle or caption to use in the plot. xlab, ylab character | Titles for the X and Y axis. font.size numeric | Overall font size of the plot. All plot elements will have a size relationship with this font size. font.type character | Base font family for the plot. One of: • mono: Mono spaced font. • serif: Serif font family. • sans: Default font family. remove\_x\_axis, remove\_y\_axis logical | Remove X axis labels and ticks from the plot. flip logical | Whether to invert the axis of the displayed plot. use\_viridis logical | Whether to use viridis color scales. viridis.palette character | A capital letter from A to H or the scale name as in scale\_fill\_viridis.

```
viridis.direction
                  numeric | Either 1 or -1. Controls how the gradient of viridis scale is formed.
sequential.palette
                  character | Type of sequential color palette to use. Out of the sequential
                  palettes defined in brewer.pal.
sequential.direction
                  numeric | Direction of the sequential color scale. Either 1 or -1.
verbose
                  logical | Whether to show extra comments, warnings, etc.
raster
                  logical | Whether to raster the resulting plot. This is recommendable if plotting
                  a lot of cells.
raster.dpi
                  numeric | Pixel resolution for rasterized plots. Defaults to 1024. Only activates
                  on Seurat versions higher or equal than 4.1.0.
plot_cell_borders
                  logical | Whether to plot border around cells.
border.size
                  numeric | Width of the border of the cells.
border.color
                  character | Color for the border of the heatmap body.
                  numeric | Size of the dots.
pt.size
min.cutoff, max.cutoff
                  numeric | Set the min/max ends of the color scale. Any cell/group with a value
                  lower than min.cutoff will turn into min.cutoff and any cell with a value higher
                  than max.cutoff will turn into max.cutoff. In FeaturePlots, provide as many
                  values as features. Use NAs to skip a feature.
na.value
                  character | Color value for NA.
number.breaks
                  numeric | Controls the number of breaks in continuous color scales of ggplot2-
                  based plots.
plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.
                  character | Controls the style of the font for the corresponding theme element.
                  One of:
                     • plain: For normal text.
```

- italic: For text in itallic.
- bold: For text in bold.
- bold.italic: For text both in itallic and bold.

#### Value

A ggplot2 object containing a Bee Swarm plot.

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_BeeSwarmPlot", passive = TRUE)</pre>
if (isTRUE(value)){
  # Consult the full documentation in https://enblacar.github.io/SCpubr-book/
```

```
# Define your Seurat object.
sample <- readRDS(system.file("extdata/seurat_dataset_example.rds", package = "SCpubr"))</pre>
  # Basic Bee Swarm plot - categorical coloring.
  # This will color based on the unique values of seurat_clusters.
  p <- SCpubr::do_BeeSwarmPlot(sample = sample,</pre>
                                feature_to_rank = "PC_1",
                                group.by = "seurat_clusters",
                                continuous_feature = FALSE)
  # Basic Bee Swarm plot - continuous coloring.
  # This will color based on the PC_1 values.
  p <- SCpubr::do_BeeSwarmPlot(sample = sample,</pre>
                                feature_to_rank = "PC_1",
                                group.by = "seurat_clusters",
                                continuous_feature = TRUE)
} else if (base::isFALSE(value)){
  message("This function can not be used without its suggested packages.")
 message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
```

do\_BoxPlot

Generate Box Plots.

## **Description**

Generate Box Plots.

```
do_BoxPlot(
  sample,
  feature,
  group.by = NULL,
  split.by = NULL,
  assay = NULL,
  slot = "data",
  font.size = 14,
  font.type = "sans",
  axis.text.x.angle = 45,
  colors.use = NULL,
  na.value = "grey75",
  plot.title = NULL,
  plot.subtitle = NULL,
  plot.caption = NULL,
  xlab = NULL,
```

```
ylab = NULL,
  legend.title = NULL,
  legend.title.position = "top",
  legend.position = "bottom",
  boxplot.line.color = "black",
  outlier.color = "black",
  outlier.alpha = 0.5,
  boxplot.linewidth = 0.5,
  boxplot.width = NULL,
  plot.grid = TRUE,
 grid.color = "grey75",
  grid.type = "dashed",
  flip = FALSE,
  order = FALSE,
  use_silhouette = FALSE,
  use_test = FALSE,
  comparisons = NULL,
  test = "wilcox.test",
 map_signif_level = TRUE,
 plot.title.face = "bold",
 plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
  legend.title.face = "bold",
  legend.text.face = "plain"
)
```

## **Arguments**

aammla	Source I A Source chiese computed by Create Source Object
sample	Seurat   A Seurat object, generated by CreateSeuratObject.
feature	character   Feature to represent.
group.by	character   Metadata variable to group the output by. Has to be a character of factor column.
split.by	character   Secondary metadata variable to further group (split) the output by. Has to be a character of factor column.
assay	character   Assay to use. Defaults to the current assay.
slot	character   Data slot to use. Only one of: counts, data, scale.data. Defaults to "data".
font.size	numeric   Overall font size of the plot. All plot elements will have a size relationship with this font size.
font.type	character   Base font family for the plot. One of:
	mono: Mono spaced font.
	• serif: Serif font family.
	• sans: Default font family.

axis.text.x.angle numeric | Degree to rotate the X labels. One of: 0, 45, 90. colors.use named\_vector | Named vector of valid color representations (either name of HEX codes) with as many named colors as unique values of group.by. If group.by is not provided, defaults to the unique values of Idents. If not provided, a color scale will be set by default. na.value character | Color value for NA. plot.title, plot.subtitle, plot.caption character | Title, subtitle or caption to use in the plot. character | Titles for the X and Y axis. xlab, ylab legend.title character | Title for the legend. legend.title.position character | Position for the title of the legend. One of: • top: Top of the legend. • bottom: Bottom of the legend. • left: Left of the legend. • right: Right of the legend. legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. • none: No legend is displayed. boxplot.line.color character | Color of the borders of the boxplots if use\_silhouette is FALSE. outlier.color character | Color of the outlier dots. outlier.alpha numeric | Alpha applied to the outliers. boxplot.linewidth numeric | Width of the lines in the boxplots. Also controls the lines of the tests applied if use\_test is set to true. boxplot.width numeric | Width of the boxplots. plot.grid logical | Whether to plot grid lines. grid.color character | Color of the grid in the plot. In heatmaps, color of the border of the cells. grid.type character | One of the possible linetype options: • blank. • solid. · dashed. • dotted. • dotdash. • longdash.

· twodash.

flip logical | Whether to invert the axis of the displayed plot.

order logical | Whether to order the boxplots by average values. Can not be used

alongside split.by.

use\_silhouette logical|Whether to color the borders of the boxplots instead of the inside area.

use\_test logical | Whether to apply a statistical test to a given pair of elements. Can not

be used alongside split.by.

comparisons A list of length-2 vectors. The entries in the vector are either the names of 2

values on the x-axis or the 2 integers that correspond to the index of the columns

of interest.

test the name of the statistical test that is applied to the values of the 2 columns (e.g.

t.test, wilcox.test etc.). If you implement a custom test make sure that it

returns a list that has an entry called p.value.

map\_signif\_level

Boolean value, if the p-value are directly written as annotation or asterisks are used instead. Alternatively one can provide a named numeric vector to create custom mappings from p-values to annotation: For example: c("\*\*\*"=0.001, "\*\*"=0.05). Alternatively, one can provide a function that takes a

numeric argument (the p-value) and returns a string.

plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.

character | Controls the style of the font for the corresponding theme element.

One of:

• plain: For normal text.

• italic: For text in itallic.

• bold: For text in bold.

• bold.italic: For text both in itallic and bold.

#### Value

A ggplot2 object.

```
# Use silhouette style.
  p <- SCpubr::do_BoxPlot(sample = sample,</pre>
                           feature = "nCount_RNA",
                           use_silhouette = TRUE)
  # Order by mean values.
  p <- SCpubr::do_BoxPlot(sample = sample,</pre>
                           feature = "nCount_RNA",
                           order = TRUE)
  p
  # Apply second grouping.
 sample$orig.ident <- ifelse(sample$seurat_clusters %in% c("0", "1", "2", "3"), "A", "B")</pre>
  p <- SCpubr::do_BoxPlot(sample = sample,</pre>
                           feature = "nCount_RNA",
                           split.by = "orig.ident")
  p
  # Apply statistical tests.
  p <- SCpubr::do_BoxPlot(sample = sample,</pre>
                           feature = "nCount_RNA",
                           group.by = "orig.ident",
                           use_test = TRUE,
                           comparisons = list(c("A", "B")))
  р
} else if (base::isFALSE(value)){
  message("This function can not be used without its suggested packages.")
  message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
}
```

do\_CellularStatesPlot Cellular States plot.

## Description

This plot aims to show the relationships between distinct enrichment scores. If 3 variables are provided, the relationship is between the Y axis and the dual X axis. If 4 variables are provided, each corner of the plot represents how enriched the cells are in that given list. How to interpret this? In a 3-variable plot, the Y axis just means one variable. The higher the cells are in the Y axis the more enriched they are in that given variable. The X axis is a dual parameter one. Cells falling into each extreme of the axis are highly enriched for either x1 or x2, while cells falling in between are not enriched for any of the two. In a 4-variable plot, each corner shows the enrichment for one of the 4 given features. Cells will tend to locate in either of the four corners, but there will be cases of cells locating mid-way between two given corners (enriched in both features) or in the middle of the plot (not enriched for any).

```
do_CellularStatesPlot(
  sample,
  input_gene_list,
  x1,
  y1,
  x2 = NULL
  y2 = NULL,
  group.by = NULL,
  colors.use = NULL,
  legend.position = "bottom",
  legend.icon.size = 4,
  legend.ncol = NULL,
  legend.nrow = NULL,
  legend.byrow = FALSE,
  plot.title = NULL,
  plot.subtitle = NULL,
  plot.caption = NULL,
  font.size = 14,
  font.type = "sans",
  xlab = NULL,
  ylab = NULL,
  axis.ticks = TRUE,
  axis.text = TRUE,
  verbose = FALSE,
  enforce_symmetry = FALSE,
  plot_marginal_distributions = FALSE,
  marginal.type = "density",
  marginal.size = 5,
  marginal.group = TRUE,
  plot_cell_borders = TRUE,
  plot_enrichment_scores = FALSE,
  border.size = 2,
  border.color = "black",
  pt.size = 2,
  raster = FALSE,
  raster.dpi = 1024,
  plot_features = FALSE,
  features = NULL,
  use_viridis = TRUE,
  viridis.palette = "G",
  viridis.direction = 1,
  sequential.palette = "YlGnBu",
  sequential.direction = -1,
  nbin = 24,
  ctrl = 100,
  number.breaks = 5,
  plot.title.face = "bold",
```

```
plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
  legend.title.face = "bold"
  legend.text.face = "plain"
)
```

#### **Arguments**

legend.nrow

legend.byrow

plot.title, plot.subtitle, plot.caption

sample Seurat | A Seurat object, generated by CreateSeuratObject. input\_gene\_list named\_list | Named list of lists of genes to be used as input. х1 character | A name of a list from input\_gene\_list. First feature in the X axis. Will go on the right side of the X axis if y2 is not provided and top-right quadrant if provided. character | A name of a list from input\_gene\_list. First feature on the Y axis. y1 Will become the Y axis if y2 is not provided and bottom-right quadrant if procharacter | A name of a list from input\_gene\_list. Second feature on the X х2 axis. Will go on the left side of the X axis if y2 is not provided and top-left quadrant if provided. character | A name of a list from input\_gene\_list. Second feature on the Y y2 axis. Will become the bottom-left quadrant if provided. character | Metadata variable to group the output by. Has to be a character of group.by factor column. colors.use named\_vector | Named vector of valid color representations (either name of HEX codes) with as many named colors as unique values of group.by. If group.by is not provided, defaults to the unique values of Idents. If not provided, a color scale will be set by default. legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. • none: No legend is displayed. legend.icon.size numeric | Size of the icons in legend. legend.ncol numeric | Number of columns in the legend.

numeric | Number of rows in the legend.

logical | Whether the legend is filled by row or not.

character | Title, subtitle or caption to use in the plot.

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numeric | Overall font size of the plot. All plot elements will have a size relafont.size tionship with this font size. font.type character | Base font family for the plot. One of: • mono: Mono spaced font. • serif: Serif font family. • sans: Default font family. xlab, ylab character | Titles for the X and Y axis. axis.ticks logical | Whether to show axis ticks. axis.text logical | Whether to show axis text. verbose logical | Whether to show extra comments, warnings, etc. enforce\_symmetry logical | Whether to enforce the plot to follow a symmetry (3 variables, the X axis has 0 as center, 4 variables, all axis have the same range and the plot is squared). plot\_marginal\_distributions logical | Whether to plot marginal distributions on the figure or not. marginal.type character | One of: • density: Compute density plots on the margins. • histogram: Compute histograms on the margins. • boxplot: Compute boxplot on the margins. • violin: Compute violin plots on the margins. • densigram: Compute densigram plots on the margins. numeric | Size ratio between the main and marginal plots. A value of 5 means marginal.size that the main plot is 5 times bigger than the marginal plots. marginal.group logical | Whether to group the marginal distribution by group.by or current identities. plot\_cell\_borders logical | Whether to plot border around cells. plot\_enrichment\_scores logical | Whether to report enrichment scores for the input lists as plots. numeric | Width of the border of the cells. border.size border.color character | Color for the border of the heatmap body. pt.size numeric | Size of the dots. raster logical | Whether to raster the resulting plot. This is recommendable if plotting a lot of cells. numeric | Pixel resolution for rasterized plots. Defaults to 1024. Only activates raster.dpi on Seurat versions higher or equal than 4.1.0. plot\_features logical | Whether to also report any other feature onto the primary plot. features character | Additional features to plot. use\_viridis logical | Whether to use viridis color scales. viridis.palette character | A capital letter from A to H or the scale name as in scale\_fill\_viridis.

```
viridis.direction
                  numeric | Either 1 or -1. Controls how the gradient of viridis scale is formed.
sequential.palette
                  character | Type of sequential color palette to use. Out of the sequential
                  palettes defined in brewer.pal.
sequential.direction
                  numeric | Direction of the sequential color scale. Either 1 or -1.
nbin
                  numeric | Number of bins to use in AddModuleScore.
ctrl
                  numeric | Number of genes in the control set to use in AddModuleScore.
                  numeric | Controls the number of breaks in continuous color scales of ggplot2-
number.breaks
                  based plots.
plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.
                  character | Controls the style of the font for the corresponding theme element.
                  One of:
                    • plain: For normal text.
                    • italic: For text in itallic.
                    • bold: For text in bold.
```

#### **Details**

This plots are based on the following publications:

• Neftel, C. *et al.* An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma. Cell 178, 835-849.e21 (2019). doi:10.1016/j.cell.2019.06.024

• bold.italic: For text both in itallic and bold.

• Tirosh, I., Venteicher, A., Hebert, C. *et al.* Single-cell RNA-seq supports a developmental hierarchy in human oligodendroglioma. Nature 539, 309–313 (2016). doi:10.1038/nature20123

#### Value

A ggplot2 object containing a butterfly plot.

do\_ChordDiagramPlot

```
"D" = rownames(sample)[31:40])
  # Using two variables: A scatter plot X vs Y.
  p <- SCpubr::do_CellularStatesPlot(sample = sample,</pre>
                                      input_gene_list = gene_set,
                                      x1 = "A",
                                      y1 = "B",
                                      nbin = 1,
                                      ctrl = 10)
 р
  # Using three variables. Figure from: https://www.nature.com/articles/nature20123.
  p <- SCpubr::do_CellularStatesPlot(sample = sample,</pre>
                                      input_gene_list = gene_set,
                                      x1 = "A",
                                      y1 = "B",
                                      x2 = "C",
                                      nbin = 1,
                                      ctrl = 10)
 р
  # Using four variables. Figure from: https://pubmed.ncbi.nlm.nih.gov/31327527/
  p <- SCpubr::do_CellularStatesPlot(sample = sample,</pre>
                                      input_gene_list = gene_set,
                                      x1 = "A"
                                      y1 = "C",
                                      x2 = "B",
                                      y2 = "D",
                                      nbin = 1,
                                      ctrl = 10)
} else if (base::isFALSE(value)){
 message("This function can not be used without its suggested packages.")
 message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
}
```

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do\_ChordDiagramPlot Generate a Chord diagram.

#### **Description**

Generate a Chord diagram.

```
do_ChordDiagramPlot(
```

```
sample = NULL,
  from = NULL,
  to = NULL,
  colors.from = NULL,
  colors.to = NULL,
  big.gap = 10,
  small.gap = 1,
  link.border.color = NA,
  link.border.width = 1,
  highlight_group = NULL,
  alpha.highlight = 25,
  link.sort = NULL,
  link.decreasing = TRUE,
  z_{index} = FALSE,
  self.link = 1,
  symmetric = FALSE,
  directional = 1,
  direction.type = c("diffHeight", "arrows"),
  link.arr.type = "big.arrow",
  scale = FALSE,
  alignment = "default",
  annotationTrack = c("grid", "axis"),
  padding_labels = 4,
)
```

#### **Arguments**

```
sample
                  Seurat | A Seurat object, generated by CreateSeuratObject.
                  character | Categorical metadata variable to be used as origin and end points
from, to
                  of the interactions.
colors.from, colors.to
                  named_vector | Named vector of colors corresponding to the unique values of
                  "from" and "to".
big.gap
                  numeric | Space between the groups in "from" and "to".
                  numeric | Space within the groups.
small.gap
link.border.color
                  character | Color for the border of the links. NA = no color.
link.border.width
                  numeric | Width of the border line of the links.
highlight_group
                  character | A value from from that will be used to highlight only the links
                  coming from it.
alpha.highlight
                  numeric | A value between 00 (double digits) and 99 to depict the alpha of the
                  highlighted links. No transparency needs "FF"
link.sort
                  pass\ to\ chord \texttt{DiagramFromMatrix}\ or\ chord \texttt{DiagramFromDataFrame}
```

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link.decreasing

pass to chordDiagramFromMatrix or chordDiagramFromDataFrame

z\_index logical | Whether to bring the bigger links to the top.

self.link numeric | Behavior of the links. One of:

• 1: Prevents self linking.

• 2: Allows self linking.

symmetric pass to chordDiagramFromMatrix

directional numeric | Set the direction of the links. One of:

• 0: Non-directional data.

- 1: Links go from "from" to "to".
- -1: Links go from "to" to "from".
- 2: Links go in both directions.

direction.type character | How to display the directions. One of:

- diffHeight: Sets a line at the origin of the group showing to how many groups and in which proportion this group is linked to.
- arrows: Sets the connection as arrows.
- both: Sets up both behaviors. Use as: c("diffHeight", "arrows").

link.arr.type character | Sets the appearance of the arrows. One of:

- triangle: Arrow with a triangle tip at the end displayed on top of the link.
- big.arrow: The link itself ends in a triangle shape.

scale logical | Whether to put all nodes the same width.

alignment character | How to align the diagram. One of:

- default: Allows circlize to set up the plot as it sees fit.
- horizontal: Sets the break between "from" and "to" groups on the horizontal axis.
- vertical: Sets the break between "from" and "to" groups on the vertical axis.

annotationTrack

pass to chordDiagramFromMatrix or chordDiagramFromDataFrame

padding\_labels numeric | Number of extra padding (white spaces) of the labels so that they do not overlap with the scales.

... For internal use only.

#### Value

A circlize plot.

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_ChordDiagramPlot", passive = TRUE)</pre>
```

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```
if (isTRUE(value)){
  # Consult the full documentation in https://enblacar.github.io/SCpubr-book/
  # Define your Seurat object.
 sample <- readRDS(system.file("extdata/seurat_dataset_example.rds", package = "SCpubr"))</pre>
  # Basic chord diagram.
  sample$assignment <- ifelse(sample$seurat_clusters %in% c("0", "4", "7"), "A", "B")</pre>
  sample$assignment[sample$seurat_clusters %in% c("1", "2")] <- "C"</pre>
  sample$assignment[sample$seurat_clusters %in% c("10", "5")] <- "D"</pre>
  sample sassignment [sample seurat\_clusters \%in\% c("8", "9")] <- "E"
  p <- SCpubr::do_ChordDiagramPlot(sample = sample,</pre>
                                     from = "seurat_clusters",
                                     to = "assignment")
  p
} else if (base::isFALSE(value)){
  message("This function can not be used without its suggested packages.")
  message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
}
```

do\_ColorPalette

Generate color scales based on a value.

## **Description**

This function is an adaptation of colortools package. As the package was removed from CRAN on 23-06-2022, this utility function came to existence in order to cover the gap. It is, on its basis, an adaptation of the package into a single function. Original code, developed by Gaston Sanchez, can be found in: https://github.com/gastonstat/colortools

```
do_ColorPalette(
  colors.use,
  n = 12,
  opposite = FALSE,
  adjacent = FALSE,
  triadic = FALSE,
  split_complementary = FALSE,
  tetradic = FALSE,
  square = FALSE,
  complete_output = FALSE,
  plot = FALSE,
  font.size = 14,
```

do\_ColorPalette 27

```
font.type = "sans"
)
```

#### **Arguments**

colors.use character | One color upon which generate the color scale. Can be a name or a HEX code. numeric | Number of colors to include in the color wheel. Use it when all other n options are FALSE, otherwise, it becomes 12. opposite logical | Return the opposing color to the one provided. adjacent logical | Return the adjacent colors to the one provided. triadic logical | Return the triadic combination of colors to the one provided. split\_complementary logical | Return the split complementary combination of colors to the one provided. tetradic logical | Return the tetradic combination of colors to the one provided. square logical | Return the square combination of colors to the one provided. complete\_output logical | Runs all the previous options and returns all the outputs as a list that contains all color vectors, all plots and a combined plot with everything. plot logical | Whether to also return a plot displaying the values instead of a vector with the color. font.size numeric | Overall font size of the plot. All plot elements will have a size relationship with this font size. font.type character | Base font family for the plot. One of: • mono: Mono spaced font. • serif: Serif font family. • sans: Default font family.

#### Value

A character vector with the desired color scale.

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```
colors <- SCpubr::do_ColorPalette(colors.use = "steelblue",</pre>
                                      opposite = TRUE)
  p <- SCpubr::do_ColorPalette(colors.use = "steelblue",</pre>
                                 opposite = TRUE,
                                 plot = TRUE)
  # Generate a trio of adjacent colors based on a given one.
  colors <- SCpubr::do_ColorPalette(colors.use = "steelblue",</pre>
                                      adjacent = TRUE)
  p <- SCpubr::do_ColorPalette(colors.use = "steelblue",</pre>
                                 adjacent = TRUE,
                                 plot = TRUE)
  # Generate a trio of triadic colors based on a given one.
  colors <- SCpubr::do_ColorPalette(colors.use = "steelblue",</pre>
                                      triadic = TRUE)
  p <- SCpubr::do_ColorPalette(colors.use = "steelblue",</pre>
                                 triadic = TRUE,
                                 plot = TRUE)
  # Generate a trio of split complementary colors based on a given one.
  colors <- SCpubr::do_ColorPalette(colors.use = "steelblue",</pre>
                                      split_complementary = TRUE)
  p <- SCpubr::do_ColorPalette(colors.use = "steelblue",</pre>
                                 split_complementary = TRUE,
                                 plot = TRUE)
  # Generate a group of tetradic colors based on a given one.
  colors <- SCpubr::do_ColorPalette(colors.use = "steelblue",</pre>
                                      tetradic = TRUE)
  p <- SCpubr::do_ColorPalette(colors.use = "steelblue",</pre>
                                 tetradic = TRUE,
                                 plot = TRUE)
  # Generate a group of square colors based on a given one.
  colors <- SCpubr::do_ColorPalette(colors.use = "steelblue",</pre>
                                      square = TRUE)
  p <- SCpubr::do_ColorPalette(colors.use = "steelblue",</pre>
                                 square = TRUE,
                                 plot = TRUE)
  # Retrieve the output of all options.
  out <- SCpubr::do_ColorPalette(colors.use = "steelblue",</pre>
                                   complete_output = TRUE)
  ## Retrieve the colors.
  colors <- out$colors</pre>
  ## Retrieve the plots.
  plots <- out$plots</pre>
  ## Retrieve a combined plot with all the options.
  p <- out$combined_plot</pre>
} else if (base::isFALSE(value)){
  message("This function can not be used without its suggested packages.")
```

```
\label{lem:message} \begin{tabular}{ll} message("Check out which ones are needed using `SCpubr::state\_dependencies()`.") \\ \end{tabular}
```

do\_CopyNumberVariantPlot

Display CNV scores from inferCNV as Feature Plots.

## **Description**

Display CNV scores from inferCNV as Feature Plots.

```
do_CopyNumberVariantPlot(
  sample,
  infercnv_object,
  chromosome_locations,
  group.by = NULL,
  using_metacells = FALSE,
 metacell_mapping = NULL,
  legend.type = "colorbar",
  legend.position = "bottom",
  legend.length = 20,
  legend.width = 1,
  legend.framewidth = 0.5,
  legend.tickwidth = 0.5,
  legend.framecolor = "grey50",
  legend.tickcolor = "white",
  font.size = 14,
  pt.size = 1,
  font.type = "sans",
  axis.text.x.angle = 45,
  enforce_symmetry = TRUE,
  legend.title = NULL,
  na.value = "grey75",
  viridis.palette = "G",
  viridis.direction = 1,
  verbose = FALSE,
 min.cutoff = NA,
 max.cutoff = NA,
  number.breaks = 5,
  diverging.palette = "RdBu",
  diverging.direction = -1,
  sequential.palette = "YlGnBu",
  sequential.direction = -1,
  use_viridis = TRUE,
```

```
return_object = FALSE,
      grid.color = "white",
      border.color = "black",
      flip = FALSE,
      plot.title.face = "bold",
      plot.subtitle.face = "plain",
      plot.caption.face = "italic",
      axis.title.face = "bold",
      axis.text.face = "plain"
      legend.title.face = "bold",
      legend.text.face = "plain"
    )
Arguments
    sample
                     Seurat | A Seurat object, generated by CreateSeuratObject.
    infercnv_object
                      infercny | Output inferCNV object run on the same Seurat object.
    chromosome_locations
                      tibble | Tibble containing the chromosome regions to use. Can be obtained
                     using utils::data("human_chr_locations", package = "SCpubr").
                     character | Metadata variable to group the output by. Has to be a character of
    group.by
                     factor column.
    using_metacells
                      logical | Whether inferCNV was run using metacells or not.
    metacell_mapping
                      named_vector | Vector or cell - metacell mapping.
    legend.type
                     character | Type of legend to display. One of:
                        • normal: Default legend displayed by ggplot2.
                        • colorbar: Redefined colorbar legend, using guide_colorbar.
    legend.position
                     character | Position of the legend in the plot. One of:
                        • top: Top of the figure.
                        • bottom: Bottom of the figure.
                        • left: Left of the figure.
                        • right: Right of the figure.
                        • none: No legend is displayed.
    legend.length, legend.width
                     numeric | Length and width of the legend. Will adjust automatically depending
                     on legend side.
    legend.framewidth, legend.tickwidth
                     numeric | Width of the lines of the box in the legend.
    legend.framecolor
                      character | Color of the lines of the box in the legend.
    legend.tickcolor
                     character | Color of the ticks of the box in the legend.
```

grid.color

cells.

numeric | Overall font size of the plot. All plot elements will have a size relafont.size tionship with this font size. numeric | Size of the dots. pt.size font.type character | Base font family for the plot. One of: • mono: Mono spaced font. • serif: Serif font family. • sans: Default font family. axis.text.x.angle numeric | Degree to rotate the X labels. One of: 0, 45, 90. enforce\_symmetry logical | Return a symmetrical plot axes-wise or continuous color scale-wise, when applicable. legend.title character | Title for the legend. character | Color value for NA. na.value viridis.palette character | A capital letter from A to H or the scale name as in scale\_fill\_viridis. viridis.direction numeric | Either 1 or -1. Controls how the gradient of viridis scale is formed. verbose logical | Whether to show extra comments, warnings, etc. min.cutoff, max.cutoff numeric | Set the min/max ends of the color scale. Any cell/group with a value lower than min.cutoff will turn into min.cutoff and any cell with a value higher than max.cutoff will turn into max.cutoff. In FeaturePlots, provide as many values as features. Use NAs to skip a feature. number.breaks numeric | Controls the number of breaks in continuous color scales of ggplot2based plots. diverging.palette character | Type of symmetrical color palette to use. Out of the diverging palettes defined in brewer.pal. diverging.direction numeric | Either 1 or -1. Direction of the divering palette. This basically flips the two ends. sequential.palette character | Type of sequential color palette to use. Out of the sequential palettes defined in brewer.pal. sequential.direction numeric | Direction of the sequential color scale. Either 1 or -1. use\_viridis logical | Whether to use viridis color scales. return\_object logical | Returns the Seurat object with the modifications performed in the function. Nomally, this contains a new assay with the data that can then be used for any other visualization desired.

character | Color of the grid in the plot. In heatmaps, color of the border of the

```
flip logical | Whether to invert the axis of the displayed plot.

plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.

character | Controls the style of the font for the corresponding theme element.

One of:
```

- plain: For normal text.italic: For text in itallic.
- bold: For text in bold.
- bold.italic: For text both in itallic and bold.

#### Value

A list containing Feature Plots for different chromosome regions and corresponding dot plots by groups..

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_CopyNumberVariantPlot", passive = TRUE)</pre>
if (isTRUE(value)){
  # Consult the full documentation in https://enblacar.github.io/SCpubr-book/
  # This function expects that you have run inferCNV on your
  # own and you have access to the output object.
  # Define your Seurat object.
  sample <- readRDS(system.file("extdata/seurat_dataset_example.rds",</pre>
                                 package = "SCpubr"))
  # Define your inferCNV object.
  infercnv_object <- readRDS(system.file("extdata/infercnv_object_example.rds",</pre>
                                           package = "SCpubr"))
  # Get human chromosome locations.
  chromosome_locations = SCpubr::human_chr_locations
  # Compute for a all chromosomes.
  p <- SCpubr::do_CopyNumberVariantPlot(sample = sample,</pre>
                                          infercnv_object = infercnv_object,
                                          using_metacells = FALSE,
                                          chromosome_locations = chromosome_locations)
} else if (base::isFALSE(value)){
  message("This function can not be used without its suggested packages.")
  message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
}
```

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do\_CorrelationPlot

Create correlation matrix heatmaps.

#### Description

Create correlation matrix heatmaps.

```
do_CorrelationPlot(
  sample = NULL,
  input_gene_list = NULL,
  cluster = TRUE,
  remove.diagonal = TRUE,
 mode = "hvg",
  assay = NULL,
  group.by = NULL,
  legend.title = "Pearson coef.",
  enforce_symmetry = ifelse(mode == "hvg", TRUE, FALSE),
  font.size = 14,
  font.type = "sans".
  na.value = "grey75",
  legend.width = 1,
  legend.length = 20,
  legend.framewidth = 0.5,
  legend.tickwidth = 0.5,
  legend.framecolor = "grey50",
  legend.tickcolor = "white",
  legend.type = "colorbar",
  legend.position = "bottom",
 min.cutoff = NA,
 max.cutoff = NA,
  number.breaks = 5,
  plot.title = NULL,
  plot.subtitle = NULL,
  plot.caption = NULL,
  diverging.palette = "RdBu",
  diverging.direction = -1,
  use_viridis = FALSE,
  viridis.palette = "G",
  viridis.direction = -1,
  sequential.palette = "YlGnBu",
  sequential.direction = 1,
  axis.text.x.angle = 45,
  grid.color = "white",
  border.color = "black"
  plot.title.face = "bold",
```

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```
plot.subtitle.face = "plain",
      plot.caption.face = "italic",
      axis.title.face = "bold",
      axis.text.face = "plain",
      legend.title.face = "bold"
      legend.text.face = "plain"
    )
Arguments
    sample
                      Seurat | A Seurat object, generated by CreateSeuratObject.
    input_gene_list
                      named_list | Named list of lists of genes to be used as input.
    cluster
                      logical | Whether to cluster the elements in the heatmap or not.
    remove.diagonal
                      logical | Whether to convert diagnoal to NA. Normally this value would be 1,
                      heavily shifting the color scale.
                      character | Different types of correlation matrices can be computed. Right
    mode
                      now, the only possible value is "hvg", standing for Highly Variable Genes. The
                      sample is subset for the HVG and the data is re-scaled. Scale data is used for the
                      correlation.
    assay
                      character | Assay to use. Defaults to the current assay.
                      character | Metadata variable to group the output by. Has to be a character of
    group.by
                      factor column.
    legend.title
                      character | Title for the legend.
    enforce_symmetry
                      logical | Return a symmetrical plot axes-wise or continuous color scale-wise,
                      when applicable.
    font.size
                      numeric | Overall font size of the plot. All plot elements will have a size rela-
                      tionship with this font size.
    font.type
                      character | Base font family for the plot. One of:
                         • mono: Mono spaced font.
                         • serif: Serif font family.
                         • sans: Default font family.
    na.value
                      character | Color value for NA.
    legend.length, legend.width
                      numeric | Length and width of the legend. Will adjust automatically depending
                      on legend side.
    legend.framewidth, legend.tickwidth
                      numeric | Width of the lines of the box in the legend.
    legend.framecolor
                      character | Color of the lines of the box in the legend.
    legend.tickcolor
```

character | Color of the ticks of the box in the legend.

• normal: Default legend displayed by **ggplot2**.

character | Type of legend to display. One of:

legend.type

• colorbar: Redefined colorbar legend, using guide\_colorbar. legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. • none: No legend is displayed. min.cutoff, max.cutoff numeric | Set the min/max ends of the color scale. Any cell/group with a value lower than min.cutoff will turn into min.cutoff and any cell with a value higher than max.cutoff will turn into max.cutoff. In FeaturePlots, provide as many values as features. Use NAs to skip a feature. number.breaks numeric | Controls the number of breaks in continuous color scales of ggplot2based plots. plot.title, plot.subtitle, plot.caption character | Title, subtitle or caption to use in the plot. diverging.palette character | Type of symmetrical color palette to use. Out of the diverging palettes defined in brewer.pal. diverging.direction numeric | Either 1 or -1. Direction of the divering palette. This basically flips the two ends. logical | Whether to use viridis color scales. use\_viridis viridis.palette character | A capital letter from A to H or the scale name as in scale\_fill\_viridis. viridis.direction numeric | Either 1 or -1. Controls how the gradient of viridis scale is formed. sequential.palette character | Type of sequential color palette to use. Out of the sequential palettes defined in brewer.pal. sequential.direction numeric | Direction of the sequential color scale. Either 1 or -1. axis.text.x.angle numeric | Degree to rotate the X labels. One of: 0, 45, 90. grid.color character | Color of the grid in the plot. In heatmaps, color of the border of the cells. border.color character | Color for the border of the heatmap body. plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title. character | Controls the style of the font for the corresponding theme element. One of:

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- plain: For normal text.
- italic: For text in itallic.
- bold: For text in bold.
- bold.italic: For text both in itallic and bold.

#### Value

A ggplot2 object.

# **Examples**

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_CorrelationPlot", passive = TRUE)

if (isTRUE(value)){
    # Consult the full documentation in https://enblacar.github.io/SCpubr-book/

    # Define your Seurat object.
sample <- readRDS(system.file("extdata/seurat_dataset_example.rds", package = "SCpubr"))

# Default values.
p <- SCpubr::do_CorrelationPlot(sample = sample)
p

} else if (base::isFALSE(value)){
    message("This function can not be used without its suggested packages.")
    message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
}</pre>
```

do\_DimPlot

Wrapper for DimPlot.

## **Description**

Wrapper for DimPlot.

```
do_DimPlot(
   sample,
   reduction = NULL,
   group.by = NULL,
   split.by = NULL,
   colors.use = NULL,
   shuffle = TRUE,
   order = NULL,
```

do\_DimPlot

```
raster = FALSE,
pt.size = 1,
label = FALSE,
label.color = "black",
label.fill = "white",
label.size = 4,
label.box = TRUE,
repel = FALSE,
cells.highlight = NULL,
idents.highlight = NULL,
idents.keep = NULL,
sizes.highlight = 1,
ncol = NULL,
plot.title = NULL,
plot.subtitle = NULL,
plot.caption = NULL,
legend.title = NULL,
legend.position = "bottom",
legend.title.position = "top",
legend.ncol = NULL,
legend.nrow = NULL,
legend.icon.size = 4,
legend.byrow = FALSE,
raster.dpi = 2048,
dims = c(1, 2),
font.size = 14,
font.type = "sans",
na.value = "grey75",
plot_cell_borders = TRUE,
border.size = 2,
border.color = "black",
border.density = 1,
plot_marginal_distributions = FALSE,
marginal.type = "density",
marginal.size = 5,
marginal.group = TRUE,
plot.axes = FALSE,
plot_density_contour = FALSE,
contour.position = "bottom",
contour.color = "grey90",
contour.lineend = "butt",
contour.linejoin = "round",
contour_expand_axes = 0.25,
plot.title.face = "bold",
plot.subtitle.face = "plain",
plot.caption.face = "italic",
axis.title.face = "bold",
axis.text.face = "plain",
```

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```
legend.title.face = "bold",
  legend.text.face = "plain"
)
```

# Arguments

sample	Seurat   A Seurat object, generated by CreateSeuratObject.
reduction	character   Reduction to use. Can be the canonical ones such as "umap", "pca", or any custom ones, such as "diffusion". If you are unsure about which reductions you have, use Seurat::Reductions(sample). Defaults to "umap" if present or to the last computed reduction if the argument is not provided.
group.by	character   Metadata variable to group the output by. Has to be a character of factor column.
split.by	character   Secondary metadata variable to further group (split) the output by. Has to be a character of factor column.
colors.use	named_vector   Named vector of valid color representations (either name of HEX codes) with as many named colors as unique values of group.by. If group.by is not provided, defaults to the unique values of Idents. If not provided, a color scale will be set by default.
shuffle	logical   Whether to shuffle the cells or not, so that they are not plotted clusterwise. Recommended.
order	character   Vector of identities to be plotted. Either one with all identities or just some, which will be plotted last.
raster	logical   Whether to raster the resulting plot. This is recommendable if plotting a lot of cells.
pt.size	numeric   Size of the dots.
label	logical   Whether to plot the cluster labels in the UMAP. The cluster labels will have the same color as the cluster colors.
label.color	character   Color of the labels in the plot.
label.fill	character   Color to fill the labels. Has to be a single color, that will be used for all labels. If NULL, the colors of the clusters will be used instead.
label.size	numeric   Size of the labels in the plot.
label.box	logical   Whether to plot the plot labels as $geom\_text$ (FALSE) or $geom\_label$ (TRUE).
repel	logical   Whether to repel the text labels.
cells.highlight, idents.highlight	
	<pre>character   Vector of cells/identities to focus into. The identities have to much those in Seurat::Idents(sample) The rest of the cells will be grayed out. Both parameters can be used at the same time.</pre>
idents.keep	character   Vector of identities to keep. This will effectively set the rest of the cells that do not match the identities provided to NA, therefore coloring them according to na.value parameter.
sizes.highlight	
	numeric   Point size of highlighted cells using cells.highlight parameter.

ncol numeric | Number of columns used in the arrangement of the output plot using "split.by" parameter. plot.title, plot.subtitle, plot.caption character | Title, subtitle or caption to use in the plot. legend.title character | Title for the legend. legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. none: No legend is displayed. legend.title.position character | Position for the title of the legend. One of: • top: Top of the legend. • bottom: Bottom of the legend. • left: Left of the legend. • right: Right of the legend. legend.ncol numeric | Number of columns in the legend. legend.nrow numeric | Number of rows in the legend. legend.icon.size numeric | Size of the icons in legend. legend.byrow logical | Whether the legend is filled by row or not. raster.dpi numeric | Pixel resolution for rasterized plots. Defaults to 1024. Only activates on Seurat versions higher or equal than 4.1.0. dims numeric | Vector of 2 numerics indicating the dimensions to plot out of the selected reduction. Defaults to c(1, 2) if not specified. font.size numeric | Overall font size of the plot. All plot elements will have a size relationship with this font size. font.type character | Base font family for the plot. One of: • mono: Mono spaced font. • serif: Serif font family. • sans: Default font family. na.value character | Color value for NA. plot\_cell\_borders logical | Whether to plot border around cells. border.size numeric | Width of the border of the cells. character | Color for the border of the heatmap body. border.color border.density numeric | Controls the number of cells used when plot\_cell\_borders = TRUE. Value between 0 and 1. It computes a 2D kernel density and based on this cells that have a density below the specified quantile will be used to generate the cluster contour. The lower this number, the less cells will be selected, thus reducing the overall size of the plot but also potentially preventing all the contours to be properly drawn.

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```
logical | Whether to plot marginal distributions on the figure or not.
marginal.type
                  character | One of:
                    • density: Compute density plots on the margins.
                    • histogram: Compute histograms on the margins.
                    • boxplot: Compute boxplot on the margins.
                    • violin: Compute violin plots on the margins.
                    • densigram: Compute densigram plots on the margins.
marginal.size
                  numeric | Size ratio between the main and marginal plots. A value of 5 means
                  that the main plot is 5 times bigger than the marginal plots.
marginal.group logical | Whether to group the marginal distribution by group.by or current
                  identities.
plot.axes
                  logical | Whether to plot axes or not.
plot_density_contour
                  logical | Whether to plot density contours in the UMAP.
contour.position
                  character | Whether to plot density contours on top or at the bottom of the
                  visualization layers, thus overlapping the clusters/cells or not.
contour.color
                  character | Color of the density lines.
contour.lineend
                  character | Line end style (round, butt, square).
contour.linejoin
                  character | Line join style (round, mitre, bevel).
contour_expand_axes
                  numeric | To make the contours fit the plot, the limits of the X and Y axis are
                  expanding a given percentage from the min and max values for each axis. This
                  controls such percentage.
plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.
                  character | Controls the style of the font for the corresponding theme element.
                    • plain: For normal text.
                    • italic: For text in itallic.
                    • bold: For text in bold.
```

#### Value

A ggplot2 object containing a DimPlot.

plot\_marginal\_distributions

# Examples

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_DimPlot", passive = TRUE)</pre>
```

• bold.italic: For text both in itallic and bold.

```
if (isTRUE(value)){
  # Consult the full documentation in https://enblacar.github.io/SCpubr-book/
  # Define your Seurat object.
 sample <- readRDS(system.file("extdata/seurat_dataset_example.rds", package = "SCpubr"))</pre>
  # Basic DimPlot.
  p <- SCpubr::do_DimPlot(sample = sample)</pre>
  # Restrict the amount of identities displayed.
  p <- SCpubr::do_DimPlot(sample = sample,</pre>
                           idents.keep = c("1", "3", "5"))
  # Group by another variable rather than `Seurat::Idents(sample)`
  p <- SCpubr::do_DimPlot(sample = sample,</pre>
                           group.by = "seurat_clusters")
  # Split the output in as many plots as unique identities.
  p <- SCpubr::do_DimPlot(sample = sample,</pre>
                           split.by = "seurat_clusters")
  # Highlight given identities
  p <- SCpubr::do_DimPlot(sample,</pre>
                           idents.highlight = c("1", "3"))
} else if (base::isFALSE(value)){
  message("This function can not be used without its suggested packages.")
 message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
}
```

do\_DotPlot

This function is a wrapper for DotPlot. It provides most of its functionalities while adding extra. You can

### **Description**

This function is a wrapper for DotPlot. It provides most of its functionalities while adding extra. You can

```
do_DotPlot(
  sample,
  features,
  assay = NULL,
```

```
group.by = NULL,
  scale = FALSE,
  legend.title = NULL,
  legend.type = "colorbar",
  legend.position = "bottom",
  legend.framewidth = 0.5,
  legend.tickwidth = 0.5,
  legend.length = 20,
  legend.width = 1,
  legend.framecolor = "grey50",
  legend.tickcolor = "white",
  colors.use = NULL,
  dot.scale = 6,
  plot.title = NULL,
  plot.subtitle = NULL,
  plot.caption = NULL,
  xlab = NULL,
 ylab = NULL,
  font.size = 14,
  font.type = "sans",
  cluster = FALSE,
  flip = FALSE,
  axis.text.x.angle = 45,
  scale.by = "size",
  use_viridis = FALSE,
  viridis.palette = "G",
  viridis.direction = -1,
  sequential.palette = "YlGnBu",
  sequential.direction = 1,
  na.value = "grey75",
  dot_border = TRUE,
  plot.grid = TRUE,
  grid.color = "grey75",
  grid.type = "dashed",
  number.breaks = 5,
  plot.title.face = "bold",
  plot.subtitle.face = "plain",
 plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
  legend.title.face = "bold",
  legend.text.face = "plain"
)
```

# Arguments

sample Seurat | A Seurat object, generated by CreateSeuratObject.

features character | Features to represent.

character | Assay to use. Defaults to the current assay. assay character | Metadata variable to group the output by. Has to be a character of group.by factor column. scale logical | Whether the data should be scaled or not. Non-scaled data allows for comparison across genes. Scaled data allows for an easier comparison along the same gene. legend.title character | Title for the legend. character | Type of legend to display. One of: legend.type • normal: Default legend displayed by **ggplot2**. • colorbar: Redefined colorbar legend, using guide\_colorbar. legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. • none: No legend is displayed. legend.framewidth, legend.tickwidth numeric | Width of the lines of the box in the legend. legend.length, legend.width numeric | Length and width of the legend. Will adjust automatically depending on legend side. legend.framecolor character | Color of the lines of the box in the legend. legend.tickcolor character | Color of the ticks of the box in the legend. named\_vector | Named vector of valid color representations (either name of colors.use HEX codes) with as many named colors as unique values of group.by. If group.by is not provided, defaults to the unique values of Idents. If not provided, a color scale will be set by default. dot.scale numeric | Scale the size of the dots. plot.title, plot.subtitle, plot.caption character | Title, subtitle or caption to use in the plot. character | Titles for the X and Y axis. xlab, ylab font.size numeric | Overall font size of the plot. All plot elements will have a size relationship with this font size. font.type character | Base font family for the plot. One of: • mono: Mono spaced font. • serif: Serif font family.

• sans: Default font family.

tures.

logical | Whether to cluster the identities based on the expression of the fea-

cluster

```
logical | Whether to invert the axis of the displayed plot.
flip
axis.text.x.angle
                  numeric | Degree to rotate the X labels. One of: 0, 45, 90.
scale.by
                  character | How to scale the size of the dots. One of:
                     • radius: use radius aesthetic.
                     • size: use size aesthetic.
use_viridis
                  logical | Whether to use viridis color scales.
viridis.palette
                  character | A capital letter from A to H or the scale name as in scale_fill_viridis.
viridis.direction
                  numeric | Either 1 or -1. Controls how the gradient of viridis scale is formed.
sequential.palette
                  character | Type of sequential color palette to use. Out of the sequential
                  palettes defined in brewer.pal.
sequential.direction
                  numeric | Direction of the sequential color scale. Either 1 or -1.
na.value
                  character | Color value for NA.
dot_border
                  logical | Whether to plot a border around dots.
plot.grid
                  logical | Whether to plot grid lines.
grid.color
                  character | Color of the grid in the plot. In heatmaps, color of the border of the
                  cells.
grid.type
                  character | One of the possible linetype options:
                    • blank.
                     • solid.
                    · dashed.
                     • dotted.
                     • dotdash.
                     • longdash.
                     · twodash.
                  numeric | Controls the number of breaks in continuous color scales of ggplot2-
number.breaks
                  based plots.
plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.
                  character | Controls the style of the font for the corresponding theme element.
                  One of:
                    • plain: For normal text.
                    • italic: For text in itallic.
```

### Value

A ggplot2 object containing a Dot Plot.

• bold: For text in bold.

• bold.italic: For text both in itallic and bold.

### **Examples**

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_DotPlot", passive = TRUE)</pre>
if (isTRUE(value)){
  # Define your Seurat object.
sample <- readRDS(system.file("extdata/seurat_dataset_example.rds", package = "SCpubr"))</pre>
  # Basic Dot plot.
  p <- SCpubr::do_DotPlot(sample = sample,</pre>
                           features = "EPC1")
  # Querying multiple features.
  genes <- rownames(sample)[1:14]</pre>
  p <- SCpubr::do_DotPlot(sample = sample,</pre>
                           features = genes)
  # Inverting the axes.
  p <- SCpubr::do_DotPlot(sample = sample,</pre>
                           features = genes,
                           cluster = TRUE,
                           plot.title = "Clustered",
                           flip = TRUE)
  # Modifying default colors.
  # Two colors to generate a gradient.
  p <- SCpubr::do_DotPlot(sample = sample,</pre>
                           features = genes,
                           colors.use = c("#001219", "#e9d8a6"))
  # Querying multiple features as a named list - splitting by each item in list.
  # Genes have to be unique.
  genes <- list("Naive CD4+ T" = rownames(sample)[1:2],</pre>
                 "EPC1+ Mono" = rownames(sample)[3:4],
                 "Memory CD4+" = rownames(sample)[5],
                 "B" = rownames(sample)[6],
                 "CD8+ T" = rownames(sample)[7],
                 "FCGR3A+ Mono" = rownames(sample)[8:9],
                 "NK" = rownames(sample)[10:11],
                 "DC" = rownames(sample)[12:13],
                 "Platelet" = rownames(sample)[14])
  p <- SCpubr::do_DotPlot(sample = sample,</pre>
                           features = genes)
  # Clustering the identities.
  p <- SCpubr::do_DotPlot(sample = sample,</pre>
                           features = genes,
                           cluster = TRUE,
                           plot.title = "Clustered")
} else if (base::isFALSE(value)){
```

```
message("This function can not be used without its suggested packages.")
message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
}
```

do\_EnrichmentHeatmap Create enrichment scores heatmaps.

# Description

This function computes the enrichment scores for the cells using AddModuleScore and then aggregates the scores by the metadata variables provided by the user and displays it as a heatmap, computed by Heatmap.

```
do_EnrichmentHeatmap(
  sample,
  input_gene_list,
  features.order = NULL,
  groups.order = NULL,
  cluster = TRUE,
  scale_scores = TRUE,
  assay = NULL,
  slot = NULL,
  reduction = NULL,
  group.by = NULL,
  verbose = FALSE,
  na.value = "grey75",
  legend.position = "bottom",
  use_viridis = FALSE,
  viridis.palette = "G",
  viridis.direction = 1,
  legend.framewidth = 0.5,
  legend.tickwidth = 0.5,
  legend.length = 20,
  legend.width = 1,
  legend.framecolor = "grey50",
  legend.tickcolor = "white",
  legend.type = "colorbar",
  font.size = 14,
  font.type = "sans".
  axis.text.x.angle = 45,
  enforce_symmetry = FALSE,
  nbin = 24,
  ctrl = 100,
  flavor = "Seurat",
```

```
legend.title = NULL,
ncores = 1,
storeRanks = TRUE,
min.cutoff = NA,
max.cutoff = NA,
pt.size = 1,
plot_cell_borders = TRUE,
border.size = 2,
return_object = FALSE,
number.breaks = 5,
sequential.palette = "YlGnBu",
diverging.palette = "RdBu",
diverging.direction = -1,
sequential.direction = 1,
flip = FALSE,
grid.color = "white",
border.color = "black"
plot.title.face = "bold",
plot.subtitle.face = "plain",
plot.caption.face = "italic",
axis.title.face = "bold",
axis.text.face = "plain",
legend.title.face = "bold",
legend.text.face = "plain"
```

### Arguments

)

Seurat | A Seurat object, generated by CreateSeuratObject. sample input\_gene\_list named\_list | Named list of lists of genes to be used as input. features.order character | Should the gene sets be ordered in a specific way? Provide it as a vector of characters with the same names as the names of the gene sets. groups.order named\_list | Should the groups in theheatmaps be ordered in a specific way? Provide it as a named list (as many lists as values in group.by) with the order for each of the elements in the groups. cluster logical | Whether to perform clustering of rows and columns. scale\_scores logical | Whether to transform the scores to a range of 0-1 for plotting. assay character | Assay to use. Defaults to the current assay. slot character | Data slot to use. Only one of: counts, data, scale.data. Defaults to reduction character | Reduction to use. Can be the canonical ones such as "umap", "pca", or any custom ones, such as "diffusion". If you are unsure about which reductions you have, use Seurat::Reductions(sample). Defaults to "umap" if present or to the last computed reduction if the argument is not provided. group.by character | Metadata variable to group the output by. Has to be a character of factor column.

verbose logical | Whether to show extra comments, warnings, etc. character | Color value for NA. na.value legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. • none: No legend is displayed. use\_viridis logical | Whether to use viridis color scales. viridis.palette character | A capital letter from A to H or the scale name as in scale\_fill\_viridis. viridis.direction numeric | Either 1 or -1. Controls how the gradient of viridis scale is formed. legend.framewidth, legend.tickwidth numeric | Width of the lines of the box in the legend. legend.length, legend.width numeric | Length and width of the legend. Will adjust automatically depending on legend side. legend.framecolor character | Color of the lines of the box in the legend. legend.tickcolor character | Color of the ticks of the box in the legend. character | Type of legend to display. One of: legend.type • normal: Default legend displayed by **ggplot2**. • colorbar: Redefined colorbar legend, using guide\_colorbar. font.size numeric | Overall font size of the plot. All plot elements will have a size relationship with this font size. font.type character | Base font family for the plot. One of: • mono: Mono spaced font. • serif: Serif font family. • sans: Default font family. axis.text.x.angle numeric | Degree to rotate the X labels. One of: 0, 45, 90. enforce\_symmetry logical | Whether the geyser and feature plot has a symmetrical color scale. numeric | Number of bins to use in AddModuleScore. nbin ctrl numeric | Number of genes in the control set to use in AddModuleScore. character | One of: Seurat, UCell. Compute the enrichment scores using Adflavor dModuleScore or AddModuleScore\_UCell. character | Title for the legend. legend.title numeric | Number of cores used to run UCell scoring. ncores

storeRanks logical | Whether to store the ranks for faster UCell scoring computations. Might require large amounts of RAM. min.cutoff, max.cutoff numeric | Set the min/max ends of the color scale. Any cell/group with a value lower than min.cutoff will turn into min.cutoff and any cell with a value higher than max.cutoff will turn into max.cutoff. In FeaturePlots, provide as many values as features. Use NAs to skip a feature. numeric | Size of the dots. pt.size plot\_cell\_borders logical | Whether to plot border around cells. border.size numeric | Width of the border of the cells. logical | Return the Seurat object with the enrichment scores stored. return\_object numeric | Controls the number of breaks in continuous color scales of ggplot2number.breaks based plots. sequential.palette character | Type of sequential color palette to use. Out of the sequential palettes defined in brewer.pal. diverging.palette character | Type of symmetrical color palette to use. Out of the diverging palettes defined in brewer.pal. diverging.direction numeric | Either 1 or -1. Direction of the divering palette. This basically flips the two ends. sequential.direction numeric | Direction of the sequential color scale. Either 1 or -1. flip logical | Whether to invert the axis of the displayed plot. grid.color character | Color of the grid in the plot. In heatmaps, color of the border of the cells. border.color character | Color for the border of the heatmap body. plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title. character | Controls the style of the font for the corresponding theme element. One of:

- plain: For normal text.
- italic: For text in itallic.
- bold: For text in bold.
- bold.italic: For text both in itallic and bold.

#### Value

A ggplot2 object.

#### **Examples**

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_EnrichmentHeatmap", passive = TRUE)</pre>
if (isTRUE(value)){
  # Consult the full documentation in https://enblacar.github.io/SCpubr-book/
  # Define your Seurat object.
 sample <- readRDS(system.file("extdata/seurat_dataset_example.rds", package = "SCpubr"))</pre>
  # Genes have to be unique.
  genes <- list("A" = rownames(sample)[1:5],</pre>
                 "B" = rownames(sample)[6:10],
                 "C" = rownames(sample)[11:15])
  # Default parameters.
  p <- SCpubr::do_EnrichmentHeatmap(sample = sample,</pre>
                                      input_gene_list = genes,
                                      nbin = 1,
                                      ctrl = 10)
  p
} else if (base::isFALSE(value)){
  message("This function can not be used without its suggested packages.")
  message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
}
```

do\_ExpressionHeatmap Create heatmaps of averaged expression by groups.

# Description

This function generates a heatmap with averaged expression values by the unique groups of the metadata variables provided by the user.

```
do_ExpressionHeatmap(
    sample,
    features,
    group.by = NULL,
    assay = NULL,
    cluster = TRUE,
    features.order = NULL,
    groups.order = NULL,
    slot = "data",
    legend.title = "Avg. Expression",
```

```
na.value = "grey75",
  legend.position = "bottom",
  legend.width = 1,
  legend.length = 20,
  legend.framewidth = 0.5,
  legend.tickwidth = 0.5,
  legend.framecolor = "grey50",
  legend.tickcolor = "white",
  legend.type = "colorbar",
  font.size = 14,
  font.type = "sans",
  axis.text.x.angle = 45,
  enforce_symmetry = FALSE,
 min.cutoff = NA,
 max.cutoff = NA,
  diverging.palette = "RdBu",
  diverging.direction = -1,
  sequential.palette = "YlGnBu",
  sequential.direction = 1,
  number.breaks = 5,
  use_viridis = FALSE,
  viridis.palette = "G",
  viridis.direction = -1,
  flip = FALSE,
  grid.color = "white",
  border.color = "black",
  plot.title.face = "bold",
  plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
  legend.title.face = "bold",
  legend.text.face = "plain"
)
```

#### **Arguments**

sample Seurat | A Seurat object, generated by CreateSeuratObject. features character | Features to represent. character | Metadata variable to group the output by. Has to be a character of group.by factor column. character | Assay to use. Defaults to the current assay. assay logical | Whether to perform clustering of rows and columns. cluster character | Should the gene sets be ordered in a specific way? Provide it as a features.order vector of characters with the same names as the names of the gene sets. groups.order named\_list | Should the groups in theheatmaps be ordered in a specific way? Provide it as a named list (as many lists as values in group.by) with the order for each of the elements in the groups.

slot character | Data slot to use. Only one of: counts, data, scale.data. Defaults to

"data".

legend.title character | Title for the legend.

na.value character | Color value for NA.

legend.position

character | Position of the legend in the plot. One of:

- top: Top of the figure.
- bottom: Bottom of the figure.
- left: Left of the figure.
- right: Right of the figure.
- none: No legend is displayed.

legend.length, legend.width

numeric | Length and width of the legend. Will adjust automatically depending on legend side.

legend.framewidth, legend.tickwidth

numeric | Width of the lines of the box in the legend.

legend.framecolor

character | Color of the lines of the box in the legend.

legend.tickcolor

character | Color of the ticks of the box in the legend.

legend.type character | Type of legend to display. One of:

- normal: Default legend displayed by **ggplot2**.
- colorbar: Redefined colorbar legend, using guide\_colorbar.

font.size numeric | Overall font size of the plot. All plot elements will have a size rela-

tionship with this font size.

font.type character | Base font family for the plot. One of:

- mono: Mono spaced font.
- serif: Serif font family.
- sans: Default font family.

axis.text.x.angle

numeric | Degree to rotate the X labels. One of: 0, 45, 90.

enforce\_symmetry

logical | Return a symmetrical plot axes-wise or continuous color scale-wise, when applicable.

min.cutoff, max.cutoff

numeric | Set the min/max ends of the color scale. Any cell/group with a value lower than min.cutoff will turn into min.cutoff and any cell with a value higher than max.cutoff will turn into max.cutoff. In FeaturePlots, provide as many values as features. Use NAs to skip a feature.

diverging.palette

character | Type of symmetrical color palette to use. Out of the diverging palettes defined in brewer.pal.

```
diverging.direction
                  numeric | Either 1 or -1. Direction of the divering palette. This basically flips
                  the two ends.
sequential.palette
                  character | Type of sequential color palette to use. Out of the sequential
                  palettes defined in brewer.pal.
sequential.direction
                  numeric | Direction of the sequential color scale. Either 1 or -1.
                  numeric | Controls the number of breaks in continuous color scales of ggplot2-
number.breaks
                  based plots.
use_viridis
                  logical | Whether to use viridis color scales.
viridis.palette
                  character | A capital letter from A to H or the scale name as in scale_fill_viridis.
viridis.direction
                  numeric | Either 1 or -1. Controls how the gradient of viridis scale is formed.
flip
                  logical | Whether to invert the axis of the displayed plot.
grid.color
                  character | Color of the grid in the plot. In heatmaps, color of the border of the
                  cells.
border.color
                  character | Color for the border of the heatmap body.
plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.
                  character | Controls the style of the font for the corresponding theme element.
                  One of:
                     • plain: For normal text.
                     • italic: For text in itallic.
                    • bold: For text in bold.
```

# Value

A ggplot2 object.

#### **Examples**

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_ExpressionHeatmap", passive = TRUE)

if (isTRUE(value)){
    # Consult the full documentation in https://enblacar.github.io/SCpubr-book/

    # Define your Seurat object.
    sample <- readRDS(system.file("extdata/seurat_dataset_example.rds", package = "SCpubr"))

# Define list of genes.
    genes <- rownames(sample)[1:10]

# Default parameters.</pre>
```

• bold.italic: For text both in itallic and bold.

do\_FeaturePlot

Wrapper for FeaturePlot.

### **Description**

Wrapper for FeaturePlot.

```
do_FeaturePlot(
  sample,
  features,
  assay = NULL,
  reduction = NULL,
  slot = NULL,
 order = FALSE,
  group.by = NULL,
  group.by.colors.use = NULL,
  group.by.legend = NULL,
 group.by.show.dots = TRUE,
  group.by.dot.size = 8,
  group.by.cell_borders = FALSE,
  group.by.cell_borders.alpha = 0.1,
  split.by = NULL,
  idents.keep = NULL,
  cells.highlight = NULL,
  idents.highlight = NULL,
  dims = c(1, 2),
  enforce_symmetry = FALSE,
  symmetry.type = "absolute",
  symmetry.center = NA,
  pt.size = 1,
  font.size = 14,
  font.type = "sans",
  legend.title = NULL,
  legend.type = "colorbar",
  legend.position = "bottom",
```

```
legend.framewidth = 0.5,
legend.tickwidth = 0.5,
legend.length = 20,
legend.width = 1,
legend.framecolor = "grey50",
legend.tickcolor = "white",
legend.ncol = NULL,
legend.nrow = NULL,
legend.byrow = FALSE,
plot.title = NULL,
plot.subtitle = NULL,
plot.caption = NULL,
individual.titles = NULL,
individual.subtitles = NULL,
individual.captions = NULL,
ncol = NULL,
use_viridis = FALSE,
viridis.palette = "G",
viridis.direction = 1,
raster = FALSE,
raster.dpi = 1024,
plot_cell_borders = TRUE,
border.size = 2,
border.color = "black",
border.density = 1,
na.value = "grey75",
verbose = TRUE,
plot.axes = FALSE,
min.cutoff = rep(NA, length(features)),
max.cutoff = rep(NA, length(features)),
plot_density_contour = FALSE,
contour.position = "bottom",
contour.color = "grey90",
contour.lineend = "butt",
contour.linejoin = "round",
contour_expand_axes = 0.25,
label = FALSE,
label.color = "black",
label.size = 4,
number.breaks = 5,
diverging.palette = "RdBu",
diverging.direction = -1,
sequential.palette = "YlGnBu",
sequential.direction = 1,
plot.title.face = "bold",
plot.subtitle.face = "plain",
plot.caption.face = "italic",
axis.title.face = "bold",
```

```
axis.text.face = "plain",
  legend.title.face = "bold",
  legend.text.face = "plain"
)
```

#### **Arguments**

sample Seurat | A Seurat object, generated by CreateSeuratObject.

character | Features to represent. features

character | Assay to use. Defaults to the current assay. assay

reduction character | Reduction to use. Can be the canonical ones such as "umap", "pca",

> or any custom ones, such as "diffusion". If you are unsure about which reductions you have, use Seurat::Reductions(sample). Defaults to "umap" if

present or to the last computed reduction if the argument is not provided.

slot character | Data slot to use. Only one of: counts, data, scale.data. Defaults to

"data".

order logical | Whether to order the cells based on expression.

character | Metadata variable based on which cells are grouped. This will efgroup.by

> fectively introduce a big dot in the center of each cluster, colored using a categorical color scale or with the values provided by the user in group.by.colors.use.

It will also displays a legend.

group.by.colors.use

character | Colors to use for the group dots.

group.by.legend

character | Title for the legend when group.by is used. Use NA to disable it and NULL to use the default column title provided in group.by.

group.by.show.dots

logical | Controls whether to place in the middle of the groups.

group.by.dot.size

numeric | Size of the dots placed in the middle of the groups.

group.by.cell\_borders

logical | Plots another border around the cells displaying the same color code of the dots displayed with group.by. Legend is shown always with alpha = 1regardless of the alpha settings.

group.by.cell\_borders.alpha

numeric | Controls the transparency of the new borders drawn by group.by.cell\_borders.

split.by character | Secondary metadata variable to further group (split) the output by.

Has to be a character of factor column.

idents.keep character | Vector of identities to plot. The gradient scale will also be subset

to only the values of such identities.

cells.highlight, idents.highlight

character | Vector of cells/identities to focus into. The identities have to much those in Seurat::Idents(sample) The rest of the cells will be grayed out.

Both parameters can be used at the same time.

numeric | Vector of 2 numerics indicating the dimensions to plot out of the dims selected reduction. Defaults to c(1, 2) if not specified. enforce\_symmetry logical | Return a symmetrical plot axes-wise or continuous color scale-wise, when applicable. symmetry.type character | Type of symmetry to be enforced. One of: • absolute: The highest absolute value will be taken into a account to generate the color scale. Works after min.cutoff and max.cutoff. • centered: Centers the scale around the provided value in symmetry.center. Works after min.cutoff and max.cutoff. symmetry.center numeric | Value upon which the scale will be centered. pt.size numeric | Size of the dots. font.size numeric | Overall font size of the plot. All plot elements will have a size relationship with this font size. font.type character | Base font family for the plot. One of: • mono: Mono spaced font. • serif: Serif font family. • sans: Default font family. legend.title character | Title for the legend. character | Type of legend to display. One of: legend.type • normal: Default legend displayed by **ggplot2**. • colorbar: Redefined colorbar legend, using guide\_colorbar. legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. • none: No legend is displayed. legend.framewidth, legend.tickwidth numeric | Width of the lines of the box in the legend. legend.length, legend.width numeric | Length and width of the legend. Will adjust automatically depending on legend side. legend.framecolor character | Color of the lines of the box in the legend. legend.tickcolor character | Color of the ticks of the box in the legend. legend.ncol numeric | Number of columns in the legend. legend.nrow numeric | Number of rows in the legend. legend.byrow logical | Whether the legend is filled by row or not.

plot.title, plot.subtitle, plot.caption character | Title, subtitle or caption to use in the plot. individual.titles, individual.subtitles, individual.captions character | Titles or subtitles. for each feature if needed. Either NULL or a vector of equal length of features. ncol numeric | Number of columns used in the arrangement of the output plot using "split.by" parameter. use\_viridis logical | Whether to use viridis color scales. viridis.palette character | A capital letter from A to H or the scale name as in scale fill viridis. viridis.direction numeric | Either 1 or -1. Controls how the gradient of viridis scale is formed. logical | Whether to raster the resulting plot. This is recommendable if plotting raster a lot of cells. numeric | Pixel resolution for rasterized plots. Defaults to 1024. Only activates raster.dpi on Seurat versions higher or equal than 4.1.0. plot\_cell\_borders logical | Whether to plot border around cells. border.size numeric | Width of the border of the cells. border.color character | Color for the border of the heatmap body. numeric | Controls the number of cells used when plot\_cell\_borders = TRUE. border.density Value between 0 and 1. It computes a 2D kernel density and based on this cells that have a density below the specified quantile will be used to generate the cluster contour. The lower this number, the less cells will be selected, thus reducing the overall size of the plot but also potentially preventing all the contours to be properly drawn. na.value character | Color value for NA. verbose logical | Whether to show extra comments, warnings, etc. plot.axes logical | Whether to plot axes or not. min.cutoff, max.cutoff numeric | Set the min/max ends of the color scale. Any cell/group with a value lower than min.cutoff will turn into min.cutoff and any cell with a value higher than max.cutoff will turn into max.cutoff. In FeaturePlots, provide as many values as features. Use NAs to skip a feature. plot\_density\_contour logical | Whether to plot density contours in the UMAP. contour.position character | Whether to plot density contours on top or at the bottom of the visualization layers, thus overlapping the clusters/cells or not. contour.color character | Color of the density lines. contour.lineend character | Line end style (round, butt, square). contour.linejoin character | Line join style (round, mitre, bevel).

```
contour_expand_axes
                  numeric | To make the contours fit the plot, the limits of the X and Y axis are
                  expanding a given percentage from the min and max values for each axis. This
                  controls such percentage.
label
                  logical | Whether to plot the cluster labels in the UMAP. The cluster labels will
                  have the same color as the cluster colors.
label.color
                  character | Color of the labels in the plot.
label.size
                  numeric | Size of the labels in the plot.
number.breaks
                  numeric | Controls the number of breaks in continuous color scales of ggplot2-
                  based plots.
diverging.palette
                  character | Type of symmetrical color palette to use. Out of the diverging
                  palettes defined in brewer.pal.
diverging.direction
                  numeric | Either 1 or -1. Direction of the divering palette. This basically flips
                  the two ends.
sequential.palette
                  character | Type of sequential color palette to use. Out of the sequential
                  palettes defined in brewer.pal.
sequential.direction
                  numeric | Direction of the sequential color scale. Either 1 or -1.
plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.
                  character | Controls the style of the font for the corresponding theme element.
                  One of:
                    • plain: For normal text.
```

- italic: For text in itallic.
- bold: For text in bold.
- bold.italic: For text both in itallic and bold.

#### Value

A ggplot2 object containing a Feature Plot.

#### **Examples**

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_FeaturePlot", passive = TRUE)</pre>
if (isTRUE(value)){
  # Consult the full documentation in https://enblacar.github.io/SCpubr-book/
  # Define your Seurat object.
 sample <- readRDS(system.file("extdata/seurat_dataset_example.rds", package = "SCpubr"))</pre>
  # Regular FeaturePlot.
  p <- SCpubr::do_FeaturePlot(sample = sample,</pre>
```

```
features = "nCount_RNA")
  # FeaturePlot with a subset of identities
  # (in Seurat::Idents(sample)) maintaining the original UMAP shape.
  idents.use <- levels(sample)[!(levels(sample) %in% c("2", "5", "8"))]</pre>
  p <- SCpubr::do_FeaturePlot(sample = sample,</pre>
                               idents.highlight = idents.use,
                               features = c("EPC1"))
  # Splitting the FeaturePlot by a variable and
  # maintaining the color scale and the UMAP shape.
  p <- SCpubr::do_FeaturePlot(sample = sample,</pre>
                               features = "EPC1"
                               split.by = "seurat_clusters")
} else if (base::isFALSE(value)){
  message("This function can not be used without its suggested packages.")
 message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
}
```

do\_FunctionalAnnotationPlot

Compute functional annotation plots using GO or KEGG ontologies

### **Description**

Compute functional annotation plots using GO or KEGG ontologies

```
do_FunctionalAnnotationPlot(
  genes,
 org.db,
  organism = "hsa",
  database = "GO",
  GO_ontology = "BP",
 min.overlap = NULL,
  p.adjust.cutoff = 0.05,
  pAdjustMethod = "BH",
 minGSSize = 10,
 maxGSSize = 500,
  font.size = 10,
  font.type = "sans",
  axis.text.x.angle = 45,
  xlab = NULL,
  ylab = NULL,
  plot.title = NULL,
```

```
plot.subtitle = NULL,
  plot.caption = NULL,
  legend.type = "colorbar",
  legend.position = "bottom",
  legend.framewidth = 0.5,
  legend.tickwidth = 0.5,
  legend.length = 10,
  legend.width = 1,
  legend.framecolor = "grey50",
  legend.tickcolor = "white",
  number.breaks = 5,
  return_matrix = FALSE,
  grid.color = "white",
  border.color = "black"
  plot.title.face = "bold",
  plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
  legend.title.face = "bold",
  legend.text.face = "plain"
)
```

• BH.

#### Arguments

```
genes
                  character | Vector of gene symbols to query for functional annotation.
org.db
                  OrgDB | Database object to use for the query.
                  character | Supported KEGG organism.
organism
database
                  character | Database to run the analysis on. One of:
                    • GO.
                    • KEGG.
GO_ontology
                  character | GO ontology to use. One of:
                    • BP: For Biological Process.
                    • MF: For Molecular Function.
                    • CC: For Cellular Component.
                  numeric | Filter the output result to the terms which are supported by this many
min.overlap
                  genes.
p.adjust.cutoff
                  numeric | Significance cutoff used to filter non-significant terms.
pAdjustMethod
                  character | Method to adjust for multiple testing. One of:
                    • holm.
                    · hochberg.
                     • hommel.
                    • bonferroni.
```

border.color

• BY. • fdr. • none. minGSSize numeric | Minimal size of genes annotated by Ontology term for testing. maxGSSize numeric | Maximal size of genes annotated for testing. numeric | Overall font size of the plot. All plot elements will have a size relafont.size tionship with this font size. font.type character | Base font family for the plot. One of: • mono: Mono spaced font. • serif: Serif font family. • sans: Default font family. axis.text.x.angle numeric | Degree to rotate the X labels. One of: 0, 45, 90. character | Titles for the X and Y axis. xlab, ylab plot.title, plot.subtitle, plot.caption character | Title, subtitle or caption to use in the plot. legend.type character | Type of legend to display. One of: • normal: Default legend displayed by **ggplot2**. • colorbar: Redefined colorbar legend, using guide colorbar. legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. • none: No legend is displayed. legend.framewidth, legend.tickwidth numeric | Width of the lines of the box in the legend. legend.length, legend.width numeric | Length and width of the legend. Will adjust automatically depending on legend side. legend.framecolor character | Color of the lines of the box in the legend. legend.tickcolor character | Color of the ticks of the box in the legend. number.breaks numeric | Controls the number of breaks in continuous color scales of ggplot2based plots. return\_matrix logical | Returns the matrices with the enriched Terms for further use. character | Color of the grid in the plot. In heatmaps, color of the border of the grid.color cells.

character | Color for the border of the heatmap body.

plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title. character | Controls the style of the font for the corresponding theme element. One of:

- plain: For normal text.
- italic: For text in itallic.
- bold: For text in bold.
- bold.italic: For text both in itallic and bold.

#### Value

A list containing a heatmap of the presence/absence of the genes in the enriched term, as well as a bar plot, dot plot and tree plot of the enriched terms.

#### **Examples**

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_FunctionalAnnotationPlot", passive = TRUE)</pre>
if (isTRUE(value)){
  # Consult the full documentation in https://enblacar.github.io/SCpubr-book/
  # Need to load this library or equivalent.
  suppressMessages(library("org.Hs.eg.db"))
  # Define list of genes to query.
  genes.use <- c("CCR7", "CD14", "LYZ",</pre>
                  "S100A4", "MS4A1",
"MS4A7", "GNLY", "NKG7", "FCER1A",
                   "CST3", "PPBP")
  # Compute the grouped GO terms.
  out <- SCpubr::do_FunctionalAnnotationPlot(genes = genes.use,</pre>
                                                org.db = org.Hs.eg.db)
} else if (base::isFALSE(value)){
  message("This function can not be used without its suggested packages.")
  message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
```

do\_GeyserPlot

Generate a Geyser plot.

### **Description**

A Geyser plot is a custom plot in which we plot continuous values on the Y axis grouped by a categorical value in the X. This is plotted as a dot plot, jittered so that the dots span all the way to the other groups. On top of this, the mean and .66 and .95 of the data is plotted, depicting the

overall distribution of the dots. The cells can, then, be colored by a continuous variable (same as Y axis or different) or a categorical one (same as X axis or different).

```
do_GeyserPlot(
  sample,
  features,
  assay = NULL,
  slot = "data",
  group.by = NULL,
  split.by = NULL,
  enforce_symmetry = FALSE,
  scale_type = "continuous",
  order = TRUE,
 plot_cell_borders = TRUE,
  jitter = 0.45,
  pt.size = 1,
 border.size = 2,
  border.color = "black",
  legend.position = "bottom",
  legend.width = 1,
  legend.length = 20,
  legend.framewidth = 0.5,
  legend.tickwidth = 0.5,
  legend.framecolor = "grey50",
  legend.tickcolor = "white",
  legend.type = "colorbar",
  font.size = 14,
  font.type = "sans",
  axis.text.x.angle = 45,
  viridis.palette = "G",
  viridis.direction = 1,
  colors.use = NULL,
  na.value = "grey75",
  legend.ncol = NULL,
  legend.nrow = NULL,
  legend.icon.size = 4,
  legend.byrow = FALSE,
  legend.title = NULL,
  plot.title = NULL,
  plot.subtitle = NULL,
 plot.caption = NULL,
  xlab = "Groups",
 ylab = feature,
  flip = FALSE,
 min.cutoff = rep(NA, length(features)),
 max.cutoff = rep(NA, length(features)),
 number.breaks = 5,
```

```
diverging.palette = "RdBu",
  diverging.direction = -1,
  sequential.palette = "YlGnBu",
  sequential.direction = -1,
  use_viridis = TRUE,
  plot.title.face = "bold",
  plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
  legend.title.face = "bold",
  legend.text.face = "plain")
```

### **Arguments**

sample Seurat | A Seurat object, generated by CreateSeuratObject. features character | Features to represent. character | Assay to use. Defaults to the current assay. assay slot character | Data slot to use. Only one of: counts, data, scale.data. Defaults to "data". group.by character | Metadata variable to group the output by. Has to be a character of factor column. split.by character | Secondary metadata variable to further group (split) the output by. Has to be a character of factor column. enforce\_symmetry logical | Return a symmetrical plot axes-wise or continuous color scale-wise, when applicable. character | Type of color scale to use. One of: scale\_type • categorical: Use a categorical color scale based on the values of "group.by". • continuous: Use a continuous color scale based on the values of "feature". logical | Whether to order the groups by the median of the data (highest to order lowest). plot\_cell\_borders logical | Whether to plot border around cells. numeric | Amount of jitter in the plot along the X axis. The lower the value, the jitter more compacted the dots are. numeric | Size of the dots. pt.size border.size numeric | Width of the border of the cells. border.color character | Color for the border of the heatmap body. legend.position character | Position of the legend in the plot. One of: • top: Top of the figure.

• bottom: Bottom of the figure.

```
• left: Left of the figure.
                     • right: Right of the figure.
                     • none: No legend is displayed.
legend.length, legend.width
                  numeric | Length and width of the legend. Will adjust automatically depending
                  on legend side.
legend.framewidth, legend.tickwidth
                  numeric | Width of the lines of the box in the legend.
legend.framecolor
                  character | Color of the lines of the box in the legend.
legend.tickcolor
                  character | Color of the ticks of the box in the legend.
legend.type
                  character | Type of legend to display. One of:
                     • normal: Default legend displayed by ggplot2.
                     • colorbar: Redefined colorbar legend, using guide colorbar.
font.size
                  numeric | Overall font size of the plot. All plot elements will have a size rela-
                  tionship with this font size.
font.type
                  character | Base font family for the plot. One of:

    mono: Mono spaced font.

                     • serif: Serif font family.
                     • sans: Default font family.
axis.text.x.angle
                  numeric | Degree to rotate the X labels. One of: 0, 45, 90.
viridis.palette
                  character | A capital letter from A to H or the scale name as in scale_fill_viridis.
viridis.direction
                  numeric | Either 1 or -1. Controls how the gradient of viridis scale is formed.
colors.use
                  character | Named vector of colors to use. Has to match the unique values of
                  group.by when scale_type is set to categorical.
na.value
                  character | Color value for NA.
legend.ncol
                  numeric | Number of columns in the legend.
legend.nrow
                  numeric | Number of rows in the legend.
legend.icon.size
                  numeric | Size of the icons in legend.
legend.byrow
                  logical | Whether the legend is filled by row or not.
legend.title
                  character | Title for the legend.
plot.title, plot.subtitle, plot.caption
                  character | Title, subtitle or caption to use in the plot.
                  character | Titles for the X and Y axis.
xlab, ylab
flip
                  logical | Whether to invert the axis of the displayed plot.
```

```
min.cutoff, max.cutoff
                  numeric | Set the min/max ends of the color scale. Any cell/group with a value
                  lower than min.cutoff will turn into min.cutoff and any cell with a value higher
                  than max.cutoff will turn into max.cutoff. In FeaturePlots, provide as many
                  values as features. Use NAs to skip a feature.
                  numeric | Controls the number of breaks in continuous color scales of ggplot2-
number.breaks
                  based plots.
diverging.palette
                  character | Type of symmetrical color palette to use. Out of the diverging
                  palettes defined in brewer.pal.
diverging.direction
                  numeric | Either 1 or -1. Direction of the divering palette. This basically flips
                  the two ends.
sequential.palette
                  character | Type of sequential color palette to use. Out of the sequential
                  palettes defined in brewer.pal.
sequential.direction
                  numeric | Direction of the sequential color scale. Either 1 or -1.
                  logical | Whether to use viridis color scales.
use_viridis
plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.
                  character | Controls the style of the font for the corresponding theme element.
                  One of:
                    • plain: For normal text.
```

- italic: For text in itallic.
- bold: For text in bold.
- bold.italic: For text both in itallic and bold.

#### **Details**

Special thanks to Christina Blume for coming up with the name of the plot.

# Value

Either a plot of a list of plots, depending on the number of features provided.

### **Examples**

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_GeyserPlot", passive = TRUE)

if (isTRUE(value)){
    # Consult the full documentation in https://enblacar.github.io/SCpubr-book/

    # Define your Seurat object.
sample <- readRDS(system.file("extdata/seurat_dataset_example.rds", package = "SCpubr"))

# Geyser plot with categorical color scale.</pre>
```

do\_GroupedGOTermPlot Compute an overview of the GO terms associated with the input list of genes.

### **Description**

Compute an overview of the GO terms associated with the input list of genes.

```
do_GroupedGOTermPlot(
  genes,
  org.db,
  levels.use = NULL,
  GO_ontology = "BP",
 min.overlap = 3,
  flip = TRUE,
  colors.use = c(Present = "#1e3d59", Absent = "#bccbcd"),
  legend.position = "bottom",
  reverse.levels = TRUE,
  axis.text.x.angle = 45,
  font.size = 10,
  font.type = "sans",
  plot.title = paste0("GO | ", GO_ontology),
  plot.subtitle = NULL,
  plot.caption = NULL,
  verbose = FALSE,
  return_matrices = FALSE,
  grid.color = "white",
```

border.color = "black",
plot.title.face = "bold",

plot.title, plot.subtitle, plot.caption

```
plot.subtitle.face = "plain",
      plot.caption.face = "italic",
      axis.title.face = "bold",
      axis.text.face = "plain",
      legend.title.face = "bold",
      legend.text.face = "plain"
    )
Arguments
    genes
                      character | Vector of gene symbols to query for functional annotation.
    org.db
                      OrgDB | Database object to use for the query.
    levels.use
                      numeric | Vector of numerics corresponding to the GO ontology levels to plot.
                      If NULL will compute all recursively until there are no results.
                      character | GO ontology to use. One of:
    GO_ontology
                         • BP: For Biological Process.
                         • MF: For Molecular Function.
                         • CC: For Cellular Component.
    min.overlap
                      numeric | Filter the output result to the terms which are supported by this many
    flip
                      logical | Whether to invert the axis of the displayed plot.
    colors.use
                      character | Named vector with two colors assigned to the names Present and
    legend.position
                      character | Position of the legend in the plot. One of:
                         • top: Top of the figure.
                         • bottom: Bottom of the figure.
                         • left: Left of the figure.
                         • right: Right of the figure.
                         • none: No legend is displayed.
    reverse.levels logical | Whether to place the higher levels first when computing the joint
                      heatmap.
    axis.text.x.angle
                      numeric | Degree to rotate the X labels. One of: 0, 45, 90.
    font.size
                      numeric | Overall font size of the plot. All plot elements will have a size rela-
                      tionship with this font size.
    font.type
                      character | Base font family for the plot. One of:
                         • mono: Mono spaced font.
                         • serif: Serif font family.
                         • sans: Default font family.
```

character | Title, subtitle or caption to use in the plot.

```
verbose
                  logical | Whether to show extra comments, warnings, etc.
return_matrices
                  logical | Returns the matrices of grouped GO terms.
grid.color
                  character | Color of the grid in the plot. In heatmaps, color of the border of the
border.color
                  character | Color for the border of the heatmap body.
plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.
                  character | Controls the style of the font for the corresponding theme element.
                    • plain: For normal text.
                    • italic: For text in itallic.
```

• bold: For text in bold.

• bold.italic: For text both in itallic and bold.

#### Value

A list containing all the matrices for the respective GO levels and all the individual and combined heatmaps.

#### **Examples**

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_GroupedGOTermPlot", passive = TRUE)</pre>
if (isTRUE(value)){
  # Consult the full documentation in https://enblacar.github.io/SCpubr-book/
  # Need to load this library or equivalent.
  suppressMessages(library("org.Hs.eg.db"))
  # Define list of genes to query.
  genes.use <- c("CCR7", "CD14", "LYZ",
                  "S100A4", "MS4A1",
                  "MS4A7", "GNLY", "NKG7", "FCER1A",
                  "CST3", "PPBP")
  # Compute the grouped GO terms.
  out <- SCpubr::do_GroupedGOTermPlot(genes = genes.use,</pre>
                                       org.db = org.Hs.eg.db)
} else if (base::isFALSE(value)){
  message("This function can not be used without its suggested packages.")
  message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
}
```

do\_GroupwiseDEPlot 71

do\_GroupwiseDEPlot

Compute a heatmap with the results of a group-wise DE analysis.

#### **Description**

Compute a heatmap with the results of a group-wise DE analysis.

```
do_GroupwiseDEPlot(
  sample,
  de_genes,
  group.by = NULL,
  number.breaks = 5,
  top\_genes = 5,
  use_viridis = FALSE,
  viridis.direction = -1,
  viridis.palette.pvalue = "C",
  viridis.palette.logfc = "E",
  viridis.palette.expression = "G",
  sequential.direction = 1,
  sequential.palette.pvalue = "YlGn",
  sequential.palette.logfc = "Y10rRd",
  sequential.palette.expression = "YlGnBu",
  assav = NULL,
  slot = "data",
  legend.position = "bottom",
  legend.width = 1,
  legend.length = 20,
  legend.framewidth = 0.5,
  legend.tickwidth = 0.5,
  legend.framecolor = "grey50",
  legend.tickcolor = "white",
  legend.type = "colorbar",
  font.size = 14,
  font.type = "sans",
  axis.text.x.angle = 45,
 min.cutoff = NA,
 max.cutoff = NA,
  na.value = "grey75",
  grid.color = "white",
  border.color = "black"
  plot.title.face = "bold",
  plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
```

```
legend.title.face = "bold",
legend.text.face = "plain"
)
```

#### **Arguments**

legend.tickcolor

sample Seurat | A Seurat object, generated by CreateSeuratObject. de\_genes tibble | DE genes matrix resulting of running Seurat::FindAllMarkers(). group.by character | Metadata variable to group the output by. Has to be a character of factor column. numeric | Controls the number of breaks in continuous color scales of ggplot2number.breaks based plots. top\_genes numeric | Top N differentially expressed (DE) genes by group to retrieve. use viridis logical | Whether to use viridis color scales. viridis.direction numeric | Either 1 or -1. Controls how the gradient of viridis scale is formed. viridis.palette.pvalue, viridis.palette.logfc, viridis.palette.expression character | Viridis color palettes for the p-value, logfc and expression heatmaps. A capital letter from A to H or the scale name as in scale\_fill\_viridis. sequential.direction numeric | Direction of the sequential color scale. Either 1 or -1. sequential.palette.pvalue, sequential.palette.expression, sequential.palette.logfc character | Sequential palettes for p-value, logfc and expression heatmaps. Type of sequential color palette to use. Out of the sequential palettes defined in brewer.pal. character | Assay to use. Defaults to the current assay. assay slot character | Data slot to use. Only one of: counts, data, scale.data. Defaults to "data". legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. • none: No legend is displayed. legend.length, legend.width numeric | Length and width of the legend. Will adjust automatically depending on legend side. legend.framewidth, legend.tickwidth numeric | Width of the lines of the box in the legend. legend.framecolor character | Color of the lines of the box in the legend.

character | Color of the ticks of the box in the legend.

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```
legend.type
                  character | Type of legend to display. One of:
                     • normal: Default legend displayed by ggplot2.
                     • colorbar: Redefined colorbar legend, using guide_colorbar.
font.size
                  numeric | Overall font size of the plot. All plot elements will have a size rela-
                  tionship with this font size.
                  character | Base font family for the plot. One of:
font.type
                     • mono: Mono spaced font.
                    • serif: Serif font family.
                     • sans: Default font family.
axis.text.x.angle
                  numeric | Degree to rotate the X labels. One of: 0, 45, 90.
min.cutoff, max.cutoff
                  numeric | Set the min/max ends of the color scale. Any cell/group with a value
                  lower than min.cutoff will turn into min.cutoff and any cell with a value higher
                  than max.cutoff will turn into max.cutoff. In FeaturePlots, provide as many
                  values as features. Use NAs to skip a feature.
na.value
                  character | Color value for NA.
                  character | Color of the grid in the plot. In heatmaps, color of the border of the
grid.color
                  cells.
border.color
                  character | Color for the border of the heatmap body.
plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.
                  character | Controls the style of the font for the corresponding theme element.
                  One of:
                     • plain: For normal text.
                     • italic: For text in itallic.
```

- bold: For text in bold.
- bold.italic: For text both in itallic and bold.

#### Value

A heatmap composed of 3 main panels: -log10(adjusted p-value), log2(FC) and mean expression by cluster.

## **Examples**

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_GroupwiseDEPlot", passive = TRUE)

if (isTRUE(value)){
    # Consult the full documentation in https://enblacar.github.io/SCpubr-book/

    # Define your Seurat object.
sample <- readRDS(system.file("extdata/seurat_dataset_example.rds", package = "SCpubr"))

# Compute DE genes and transform to a tibble.</pre>
```

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do\_NebulosaPlot

Wrapper for Nebulosa::plot\_density in Seurat.

# **Description**

Wrapper for Nebulosa::plot\_density in Seurat.

```
do_NebulosaPlot(
  sample,
  features,
  slot = NULL,
  dims = c(1, 2),
 pt.size = 1,
  reduction = NULL,
  combine = TRUE,
 method = c("ks", "wkde"),
  joint = FALSE,
  return_only_joint = FALSE,
  plot.title = NULL,
  plot.subtitle = NULL,
  plot.caption = NULL,
  legend.type = "colorbar",
  legend.framewidth = 0.5,
  legend.tickwidth = 0.5,
  legend.length = 20,
  legend.width = 1,
  legend.framecolor = "grey50",
  legend.tickcolor = "white",
  font.size = 14,
  font.type = "sans",
  legend.position = "bottom",
  plot_cell_borders = TRUE,
```

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```
border.size = 2,
  border.color = "black",
  viridis.palette = "G",
  viridis.direction = 1,
  verbose = TRUE,
  na.value = "grey75",
  plot.axes = FALSE,
  number.breaks = 5,
  use_viridis = FALSE,
  sequential.palette = "YlGnBu",
  sequential.direction = 1,
  plot.title.face = "bold",
  plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain"
  legend.title.face = "bold",
  legend.text.face = "plain"
)
```

#### **Arguments**

sample Seurat | A Seurat object, generated by CreateSeuratObject.

features character | Features to represent.

slot character | Data slot to use. Only one of: counts, data, scale.data. Defaults to

"data".

dims numeric | Vector of 2 numerics indicating the dimensions to plot out of the

selected reduction. Defaults to c(1, 2) if not specified.

pt.size numeric | Size of the dots.

reduction character | Reduction to use. Can be the canonical ones such as "umap", "pca",

or any custom ones, such as "diffusion". If you are unsure about which reductions you have, use Seurat::Reductions(sample). Defaults to "umap" if present or to the last computed reduction if the argument is not provided.

combine logical | Whether to create a single plot out of multiple features.

method Kernel density estimation method:

- ks: Computes density using the kde function from the ks package.
- wkde: Computes density using a modified version of the kde2d function from the MASS package to allow weights. Bandwidth selection from the ks package is used instead.

joint logical | Whether to plot different features as joint density.

return\_only\_joint

logical | Whether to only return the joint density panel.

plot.title, plot.subtitle, plot.caption

character | Title, subtitle or caption to use in the plot.

legend.type character | Type of legend to display. One of:

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• normal: Default legend displayed by ggplot2.

• colorbar: Redefined colorbar legend, using guide\_colorbar.

legend.framewidth, legend.tickwidth

numeric | Width of the lines of the box in the legend.

legend.length, legend.width

numeric | Length and width of the legend. Will adjust automatically depending on legend side.

legend.framecolor

character | Color of the lines of the box in the legend.

legend.tickcolor

character | Color of the ticks of the box in the legend.

font.size numeric | Overall font size of the plot. All plot elements will have a size rela-

tionship with this font size.

font.type character | Base font family for the plot. One of:

• mono: Mono spaced font.

• serif: Serif font family.

• sans: Default font family.

legend.position

character | Position of the legend in the plot. One of:

• top: Top of the figure.

• bottom: Bottom of the figure.

• left: Left of the figure.

• right: Right of the figure.

• none: No legend is displayed.

plot\_cell\_borders

logical | Whether to plot border around cells.

border.size numeric | Width of the border of the cells.

border.color character | Color for the border of the heatmap body.

viridis.palette

character | A capital letter from A to H or the scale name as in scale\_fill\_viridis.

viridis.direction

numeric | Either 1 or -1. Controls how the gradient of viridis scale is formed.

verbose logical | Whether to show extra comments, warnings, etc.

na.value character | Color value for NA.

plot.axes logical | Whether to plot axes or not.

number.breaks numeric | Controls the number of breaks in continuous color scales of ggplot2-

based plots.

use\_viridis logical | Whether to use viridis color scales.

sequential.palette

character | Type of sequential color palette to use. Out of the sequential palettes defined in brewer.pal.

```
sequential.direction

numeric | Direction of the sequential color scale. Either 1 or -1.

plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.

character | Controls the style of the font for the corresponding theme element.

One of:

plain: For normal text.

italic: For text in itallic.

bold: For text in bold.
```

#### Value

A ggplot2 object containing a Nebulosa plot.

## **Examples**

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_NebulosaPlot", passive = TRUE)</pre>
if (isTRUE(value)){
  # Consult the full documentation in https://enblacar.github.io/SCpubr-book/
  # Define your Seurat object.
sample <- readRDS(system.file("extdata/seurat_dataset_example.rds", package = "SCpubr"))</pre>
  # Basic Nebulosa plot.
  p <- SCpubr::do_NebulosaPlot(sample = sample,</pre>
                                features = "EPC1")
  # Compute joint density.
  p <- SCpubr::do_NebulosaPlot(sample = sample,</pre>
                                features = c("EPC1", "TOX2"),
                                joint = TRUE)
} else if (base::isFALSE(value)){
 message("This function can not be used without its suggested packages.")
 message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
}
```

• bold.italic: For text both in itallic and bold.

do\_PathwayActivityPlot

Plot Pathway Activities from decoupleR using Progeny prior knowledge.

## **Description**

Plot Pathway Activities from decoupleR using Progeny prior knowledge.

```
do_PathwayActivityPlot(
  sample,
  activities,
  group.by = NULL,
  split.by = NULL,
  slot = "scale.data",
  statistic = "norm_wmean",
  pt.size = 1,
  border.size = 2,
  na.value = "grey75",
  legend.position = "bottom",
  legend.width = 1,
  legend.length = 20,
  legend.framewidth = 0.5,
  legend.tickwidth = 0.5,
  legend.framecolor = "grey50",
  legend.tickcolor = "white",
  legend.type = "colorbar",
  font.size = 14,
  font.type = "sans",
  axis.text.x.angle = 45,
  enforce_symmetry = TRUE,
 min.cutoff = NA,
 max.cutoff = NA,
  number.breaks = 5,
  diverging.palette = "RdBu",
  diverging.direction = -1,
  use_viridis = FALSE,
  viridis.palette = "G",
  viridis.direction = -1,
  sequential.palette = "YlGnBu",
  sequential.direction = 1,
  flip = FALSE,
  return_object = FALSE,
  grid.color = "white",
  border.color = "black",
  plot.title.face = "bold",
  plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
  legend.title.face = "bold",
  legend.text.face = "plain"
)
```

#### **Arguments**

sample Seurat | A Seurat object, generated by CreateSeuratObject. tibble | Result of running decoupleR method with progeny regulon prior knowlactivities edge. character | Metadata variable to group the output by. Has to be a character of group.by factor column. split.by character | Secondary metadata variable to further group (split) the output by. Has to be a character of factor column. character | Data slot to use. Only one of: counts, data, scale.data. Defaults to slot "data". character | DecoupleR statistic to use. One of: statistic • wmean: For weighted mean. • norm\_wmean: For normalized weighted mean. • corr\_wmean: For corrected weighted mean. numeric | Size of the dots. pt.size border.size numeric | Width of the border of the cells. na.value character | Color value for NA. legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. • none: No legend is displayed. legend.length, legend.width numeric | Length and width of the legend. Will adjust automatically depending on legend side. legend.framewidth, legend.tickwidth numeric | Width of the lines of the box in the legend. legend.framecolor character | Color of the lines of the box in the legend. legend.tickcolor character | Color of the ticks of the box in the legend. legend.type character | Type of legend to display. One of: • normal: Default legend displayed by **ggplot2**. • colorbar: Redefined colorbar legend, using guide\_colorbar. font.size numeric | Overall font size of the plot. All plot elements will have a size relationship with this font size. font.type character | Base font family for the plot. One of: • mono: Mono spaced font. • serif: Serif font family.

• sans: Default font family.

axis.text.x.angle

numeric | Degree to rotate the X labels. One of: 0, 45, 90.

enforce\_symmetry

logical | Return a symmetrical plot axes-wise or continuous color scale-wise, when applicable.

min.cutoff, max.cutoff

numeric | Set the min/max ends of the color scale. Any cell/group with a value lower than min.cutoff will turn into min.cutoff and any cell with a value higher than max.cutoff will turn into max.cutoff. In FeaturePlots, provide as many values as features. Use NAs to skip a feature.

number.breaks numeric | Controls the number of breaks in continuous color scales of ggplot2-based plots.

diverging.palette

character | Type of symmetrical color palette to use. Out of the diverging palettes defined in brewer.pal.

diverging.direction

numeric | Either 1 or -1. Direction of the divering palette. This basically flips the two ends.

use\_viridis logical | Whether to use viridis color scales.

viridis.palette

character | A capital letter from A to H or the scale name as in scale\_fill\_viridis.

viridis.direction

numeric | Either 1 or -1. Controls how the gradient of viridis scale is formed.

sequential.palette

character | Type of sequential color palette to use. Out of the sequential palettes defined in brewer.pal.

sequential.direction

numeric | Direction of the sequential color scale. Either 1 or -1.

flip logical | Whether to invert the axis of the displayed plot.

logical | Returns the Seurat object with the modifications performed in the function. Nomally, this contains a new assay with the data that can then be used for any other visualization desired.

grid.color character | Color of the grid in the plot. In heatmaps, color of the border of the

cells.

border.color character | Color for the border of the heatmap body.

- plain: For normal text.
- italic: For text in itallic.
- bold: For text in bold.
- bold.italic: For text both in itallic and bold.

## Value

A ggplot2 object.

## **Examples**

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_PathwayActivityPlot", passive = TRUE)</pre>
if (isTRUE(value)){
  # Consult the full documentation in https://enblacar.github.io/SCpubr-book/
  # Define your Seurat object.
  sample <- readRDS(system.file("extdata/seurat_dataset_example.rds",</pre>
                                 package = "SCpubr"))
  # Define your activities object.
  progeny_activities <- readRDS(system.file("extdata/progeny_activities_example.rds",</pre>
                                              package = "SCpubr"))
  # General heatmap.
  out <- SCpubr::do_PathwayActivityPlot(sample = sample,</pre>
                                          activities = progeny_activities)
  p <- out$heatmaps$average_scores</pre>
} else if (base::isFALSE(value)){
  message("This function can not be used without its suggested packages.")
  message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
```

do\_RidgePlot

Create ridge plots.

#### **Description**

This function computes ridge plots based on the **ggridges** package.

```
do_RidgePlot(
   sample,
   feature,
   group.by = NULL,
   split.by = NULL,
   assay = "SCT",
   slot = "data",
   continuous_scale = FALSE,
```

```
legend.title = NULL,
  legend.ncol = NULL,
  legend.nrow = NULL,
  legend.byrow = FALSE,
  legend.position = NULL,
  legend.width = 1,
  legend.length = 20,
  legend.framewidth = 0.5,
  legend.tickwidth = 0.5,
  legend.framecolor = "grey50",
  legend.tickcolor = "white",
  legend.type = "colorbar",
  colors.use = NULL,
  font.size = 14,
  font.type = "sans",
  axis.text.x.angle = 45,
  plot.title = NULL,
  plot.subtitle = NULL,
  plot.caption = NULL,
  xlab = NULL,
  ylab = NULL,
  compute_quantiles = FALSE,
  compute_custom_quantiles = FALSE,
  quantiles = c(0.25, 0.5, 0.75),
  compute_distribution_tails = FALSE,
  prob_tails = 0.025,
  color_by_probabilities = FALSE,
  use_viridis = TRUE,
  viridis.palette = "G",
  viridis.direction = 1,
  sequential.palette = "YlGnBu",
  sequential.direction = 1,
  plot.grid = TRUE,
  grid.color = "grey75",
  grid.type = "dashed",
  flip = FALSE,
  number.breaks = 5,
  plot.title.face = "bold",
  plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
  legend.title.face = "bold",
  legend.text.face = "plain"
)
```

## **Arguments**

sample Seurat | A Seurat object, generated by CreateSeuratObject.

feature character | Feature to represent. character | Metadata variable to group the output by. Has to be a character of group.by factor column. split.by character | Secondary metadata variable to further group (split) the output by. Has to be a character of factor column. character | Assay to use. Defaults to the current assay. assay slot character | Data slot to use. Only one of: counts, data, scale.data. Defaults to "data". continuous\_scale logical | Whether to color the ridges depending on a categorical or continuous legend.title character | Title for the legend. legend.ncol numeric | Number of columns in the legend. legend.nrow numeric | Number of rows in the legend. legend.byrow logical | Whether the legend is filled by row or not. legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. • none: No legend is displayed. legend.length, legend.width numeric | Length and width of the legend. Will adjust automatically depending on legend side. legend.framewidth, legend.tickwidth numeric | Width of the lines of the box in the legend. legend.framecolor character | Color of the lines of the box in the legend. legend.tickcolor character | Color of the ticks of the box in the legend. legend.type character | Type of legend to display. One of: • normal: Default legend displayed by **ggplot2**. • colorbar: Redefined colorbar legend, using guide\_colorbar. character | Named vector of colors to use. Has to match the unique values of colors.use group.by or color.by (if used) when scale\_type is set to categorical. font.size numeric | Overall font size of the plot. All plot elements will have a size relationship with this font size. character | Base font family for the plot. One of: font.type • mono: Mono spaced font. • serif: Serif font family. • sans: Default font family.

axis.text.x.angle

```
numeric | Degree to rotate the X labels. One of: 0, 45, 90.
plot.title, plot.subtitle, plot.caption
                  character | Title, subtitle or caption to use in the plot.
xlab, ylab
                  character | Titles for the X and Y axis.
compute_quantiles
                  logical | Whether to compute quantiles of the distribution and color the ridge
                  plots by them.
compute_custom_quantiles
                  logical | Whether to compute custom quantiles.
                  numeric | Numeric vector of quantiles.
quantiles
compute_distribution_tails
                  logical | Whether to compute distribution tails and color them.
prob_tails
                  numeric | The accumulated probability that the tails should contain.
color_by_probabilities
                  logical | Whether to color the ridges depending on the probability.
                  logical | Whether to use viridis color scales.
use_viridis
viridis.palette
                  character | A capital letter from A to H or the scale name as in scale_fill_viridis.
viridis.direction
                  numeric | Either 1 or -1. Controls how the gradient of viridis scale is formed.
sequential.palette
                  character | Type of sequential color palette to use. Out of the sequential
                  palettes defined in brewer.pal.
sequential.direction
                  numeric | Direction of the sequential color scale. Either 1 or -1.
                  logical | Whether to plot grid lines.
plot.grid
                  character | Color of the grid in the plot. In heatmaps, color of the border of the
grid.color
                  character | One of the possible linetype options:
grid.type
                    • blank.
                    • solid.
                    · dashed.
                    • dotted.
                    • dotdash.
                    • longdash.
                    • twodash.
flip
                  logical | Whether to invert the axis of the displayed plot.
number.breaks
                  numeric | Controls the number of breaks in continuous color scales of ggplot2-
                  based plots.
plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.
                  character | Controls the style of the font for the corresponding theme element.
                  One of:
```

- plain: For normal text.
- italic: For text in itallic.
- bold: For text in bold.
- bold.italic: For text both in itallic and bold.

#### Value

A ggplot2 object.

## **Examples**

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_RidgePlot", passive = TRUE)</pre>
if (isTRUE(value)){
  # Consult the full documentation in https://enblacar.github.io/SCpubr-book/
 # Define your Seurat object.
 sample <- readRDS(system.file("extdata/seurat_dataset_example.rds", package = "SCpubr"))</pre>
  # Compute the most basic ridge plot.
  p <- SCpubr::do_RidgePlot(sample = sample,</pre>
                             feature = "nFeature_RNA")
  # Use continuous color scale.
  p <- SCpubr::do_RidgePlot(sample = sample,</pre>
                             feature = "nFeature_RNA",
                             continuous_scale = TRUE,
                             viridis.direction = 1)
  # Draw quantiles of the distribution.
  p <- SCpubr::do_RidgePlot(sample = sample,</pre>
                             feature = "nFeature_RNA",
                             continuous_scale = TRUE,
                             compute_quantiles = TRUE,
                             compute_custom_quantiles = TRUE)
 р
  # Draw probability tails.
  p <- SCpubr::do_RidgePlot(sample = sample,</pre>
                             feature = "nFeature_RNA",
                             continuous_scale = TRUE,
                             compute_quantiles = TRUE,
                             compute_distribution_tails = TRUE)
  р
  # Draw probability tails.
  p <- SCpubr::do_RidgePlot(sample = sample,</pre>
                             feature = "nFeature_RNA",
```

do\_TermEnrichmentPlot Display the enriched terms for a given list of genes.

# **Description**

Display the enriched terms for a given list of genes.

```
do_TermEnrichmentPlot(
  enriched_terms,
  nchar_wrap = 20,
  nterms = 10,
  font.size = 14,
  font.type = "sans",
  plot.title = NULL,
  plot.subtitle = NULL,
  plot.caption = NULL,
  legend.position = "bottom",
  legend.type = "colorbar",
  colors.use = NULL,
  text_labels_size = 4,
  legend.length = 30,
  legend.width = 1,
  legend.framewidth = 0.5,
  legend.tickwidth = 0.5,
  legend.framecolor = "grey50",
  legend.tickcolor = "white",
  plot.title.face = "bold",
  plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
  legend.title.face = "bold",
  legend.text.face = "plain"
)
```

#### **Arguments**

enriched\_terms list | List containing the output(s) of running Enrichr. numeric | Number of characters to use as a limit to wrap the term names. The nchar\_wrap higher this value, the longer the lines would be for each term in the plots. Defaults to 60. nterms numeric | Number of terms to report for each database. Terms are arranged by adjusted p-value and selected from lowest to highest. Defaults to 5. • Enrichr. • FlyEnrichr. • WormEnrichr. • YeastEnrichr. • FishEnrichr. font.size numeric | Overall font size of the plot. All plot elements will have a size relationship with this font size. font.type character | Base font family for the plot. One of: • mono: Mono spaced font. • serif: Serif font family. • sans: Default font family. plot.title, plot.subtitle, plot.caption character | Title, subtitle or caption to use in the plot. legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. • none: No legend is displayed. legend.type character | Type of legend to display. One of: • normal: Default legend displayed by **ggplot2**. • colorbar: Redefined colorbar legend, using guide\_colorbar. colors.use character | Character vector of 2 colors (low and high ends of the color scale) to generate the gradient. text\_labels\_size numeric | Controls how big or small labels are in the plot. legend.length, legend.width numeric | Length and width of the legend. Will adjust automatically depending on legend side. legend.framewidth, legend.tickwidth numeric | Width of the lines of the box in the legend. legend.framecolor character | Color of the lines of the box in the legend.

```
legend.tickcolor
```

character | Color of the ticks of the box in the legend.

plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.character | Controls the style of the font for the corresponding theme element.

One of:

- plain: For normal text.
- italic: For text in itallic.
- bold: For text in bold.
- bold.italic: For text both in itallic and bold.

#### Value

A ggplot2 object with enriched terms.

## **Examples**

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_TermEnrichmentPlot", passive = TRUE)

if (isTRUE(value)){
    # Consult the full documentation in https://enblacar.github.io/SCpubr-book/

# Define your enriched terms.
enriched_terms <- readRDS(system.file("extdata/enriched_terms_example.rds", package = "SCpubr"))
    enriched_terms$GO_Cellular_Component_2021 <- NULL
    enriched_terms$Azimuth_Cell_Types_2021 <- NULL

# Default plot.
p <- SCpubr::do_TermEnrichmentPlot(enriched_terms = enriched_terms)
p
} else if (base::isFALSE(value)){
    message("This function can not be used without its suggested packages.")
    message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
}</pre>
```

do\_TFActivityPlot

Plot TF Activities from decoupleR using Dorothea prior knowledge.

## **Description**

Plot TF Activities from decoupleR using Dorothea prior knowledge.

```
do_TFActivityPlot(
  sample,
  activities,
  n_{tfs} = 25,
  slot = "scale.data",
  statistic = "norm_wmean",
  tfs.use = NULL,
  group.by = NULL,
  split.by = NULL,
  na.value = "grey75",
  legend.position = "bottom",
  legend.width = 1,
  legend.length = 20,
  legend.framewidth = 0.5,
  legend.tickwidth = 0.5,
  legend.framecolor = "grey50",
  legend.tickcolor = "white",
  legend.type = "colorbar",
  font.size = 14,
  font.type = "sans",
  axis.text.x.angle = 45,
  enforce_symmetry = TRUE,
  diverging.palette = "RdBu",
  diverging.direction = -1,
  use_viridis = FALSE,
  viridis.palette = "G",
  viridis.direction = -1,
  sequential.palette = "YlGnBu",
  sequential.direction = 1,
 min.cutoff = NA,
 max.cutoff = NA,
  number.breaks = 5,
  flip = FALSE,
  return_object = FALSE,
  grid.color = "white",
  border.color = "black",
  plot.title.face = "bold",
  plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
  legend.title.face = "bold",
  legend.text.face = "plain"
)
```

#### **Arguments**

sample Seurat | A Seurat object, generated by CreateSeuratObject. tibble | Result of running decoupleR method with dorothea regulon prior knowlactivities edge. numeric | Number of top regulons to consider for downstream analysis.  $n_tfs$ slot character | Data slot to use. Only one of: counts, data, scale.data. Defaults to "data". statistic character | DecoupleR statistic to use. One of: • wmean: For weighted mean. • norm\_wmean: For normalized weighted mean. • corr\_wmean: For corrected weighted mean. tfs.use character | Restrict the analysis to given regulons. character | Metadata variable to group the output by. Has to be a character of group.by factor column. split.by character | Secondary metadata variable to further group (split) the output by. Has to be a character of factor column. character | Color value for NA. na.value legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. • none: No legend is displayed. legend.length, legend.width numeric | Length and width of the legend. Will adjust automatically depending on legend side. legend.framewidth, legend.tickwidth numeric | Width of the lines of the box in the legend. legend.framecolor character | Color of the lines of the box in the legend. legend.tickcolor character | Color of the ticks of the box in the legend. legend.type character | Type of legend to display. One of: • normal: Default legend displayed by **ggplot2**. • colorbar: Redefined colorbar legend, using guide\_colorbar. font.size numeric | Overall font size of the plot. All plot elements will have a size relationship with this font size. font.type character | Base font family for the plot. One of: • mono: Mono spaced font. • serif: Serif font family.

• sans: Default font family.

axis.text.x.angle

numeric | Degree to rotate the X labels. One of: 0, 45, 90.

enforce\_symmetry

logical | Whether the geyser and feature plot has a symmetrical color scale.

diverging.palette

character | Type of symmetrical color palette to use. Out of the diverging palettes defined in brewer.pal.

diverging.direction

numeric | Either 1 or -1. Direction of the divering palette. This basically flips the two ends.

use\_viridis logical | Whether to use viridis color scales.

viridis.palette

character | A capital letter from A to H or the scale name as in scale\_fill\_viridis.

viridis.direction

numeric | Either 1 or -1. Controls how the gradient of viridis scale is formed.

sequential.palette

character | Type of sequential color palette to use. Out of the sequential palettes defined in brewer.pal.

sequential.direction

numeric | Direction of the sequential color scale. Either 1 or -1.

min.cutoff, max.cutoff

numeric | Set the min/max ends of the color scale. Any cell/group with a value lower than min.cutoff will turn into min.cutoff and any cell with a value higher than max.cutoff will turn into max.cutoff. In FeaturePlots, provide as many values as features. Use NAs to skip a feature.

number.breaks

numeric | Controls the number of breaks in continuous color scales of ggplot2-based plots.

flip logical | Whether to invert the axis of the displayed plot.

return\_object

logical | Returns the Seurat object with the modifications performed in the function. Nomally, this contains a new assay with the data that can then be used for any other visualization desired.

grid.color

character | Color of the grid in the plot. In heatmaps, color of the border of the cells.

border.color character | Color for the border of the heatmap body.

plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.character | Controls the style of the font for the corresponding theme element.

One of:

- plain: For normal text.
- italic: For text in itallic.
- bold: For text in bold.
- bold.italic: For text both in itallic and bold.

#### Value

A ggplot2 object.

## **Examples**

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_TFActivityPlot", passive = TRUE)</pre>
if (isTRUE(value)){
  # Consult the full documentation in https://enblacar.github.io/SCpubr-book/
  # Define your Seurat object.
  sample <- readRDS(system.file("extdata/seurat_dataset_example.rds",</pre>
                                 package = "SCpubr"))
  # Define your activities object.
 dorothea_activities <- readRDS(system.file("extdata/dorothea_activities_example.rds",
                                               package = "SCpubr"))
  # General heatmap.
 out <- SCpubr::do_TFActivityPlot(sample = sample,</pre>
                                    activities = dorothea_activities)
 p <- out$heatmaps$average_scores</pre>
} else if (base::isFALSE(value)){
 message("This function can not be used without its suggested packages.")
 message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
}
```

do\_ViolinPlot

Wrapper for VlnPlot.

## **Description**

Wrapper for VlnPlot.

```
do_ViolinPlot(
   sample,
   features,
   assay = NULL,
   slot = NULL,
   group.by = NULL,
   split.by = NULL,
   colors.use = NULL,
```

```
pt.size = 0,
  line_width = 0.5,
 y_cut = rep(NA, length(features)),
  plot_boxplot = TRUE,
  boxplot_width = 0.2,
  legend.position = "bottom",
  plot.title = NULL,
  plot.subtitle = NULL,
  plot.caption = NULL,
  xlab = rep(NA, length(features)),
 ylab = rep(NA, length(features)),
  font.size = 14,
  font.type = "sans",
  axis.text.x.angle = 45,
  plot.grid = TRUE,
  grid.color = "grey75",
  grid.type = "dashed",
  flip = FALSE,
  ncol = NULL,
  share.y.lims = FALSE,
  legend.title = NULL,
  legend.title.position = "top",
  legend.ncol = NULL,
  legend.nrow = NULL,
  legend.byrow = FALSE,
  plot.title.face = "bold",
 plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
  legend.title.face = "bold",
  legend.text.face = "plain"
)
```

## **Arguments**

sample	Seurat   A Seurat object, generated by CreateSeuratObject.
features	character   Features to represent.
assay	character   Assay to use. Defaults to the current assay.
slot	character   Data slot to use. Only one of: counts, data, scale.data. Defaults to "data".
group.by	character   Metadata variable to group the output by. Has to be a character of factor column.
split.by	character   Secondary metadata variable to further group (split) the output by. Has to be a character of factor column.
colors.use	named_vector   Named vector of valid color representations (either name of HEX codes) with as many named colors as unique values of group.by. If group.by

is not provided, defaults to the unique values of Idents. If not provided, a color scale will be set by default. numeric | Size of points in the Violin plot. pt.size numeric | Width of the lines drawn in the plot. Defaults to 1. line\_width numeric | Vector with the values in which the Violins should be cut. Only works y\_cut for one feature. plot\_boxplot logical | Whether to plot a Box plot inside the violin or not. boxplot\_width numeric | Width of the boxplots. Defaults to 0.2. legend.position character | Position of the legend in the plot. One of: • top: Top of the figure. • bottom: Bottom of the figure. • left: Left of the figure. • right: Right of the figure. • none: No legend is displayed. plot.title, plot.subtitle, plot.caption character | Title, subtitle or caption to use in the plot. xlab, ylab character | Titles for the X and Y axis. font.size numeric | Overall font size of the plot. All plot elements will have a size relationship with this font size. font.type character | Base font family for the plot. One of: • mono: Mono spaced font. • serif: Serif font family. • sans: Default font family. axis.text.x.angle numeric | Degree to rotate the X labels. One of: 0, 45, 90. plot.grid logical | Whether to plot grid lines. character | Color of the grid in the plot. In heatmaps, color of the border of the grid.color grid.type character | One of the possible linetype options: • blank. • solid. · dashed. • dotted. • dotdash. • longdash. • twodash. flip logical | Whether to invert the axis of the displayed plot. ncol numeric | Number of columns used in the arrangement of the output plot using "split.by" parameter.

```
logical | When querying multiple features, force the Y axis of all of them
share.y.lims
                  to be on the same range of values (this being the max and min of all features
                  combined).
legend.title
                  character | Title for the legend.
legend.title.position
                  character | Position for the title of the legend. One of:
                    • top: Top of the legend.
                    • bottom: Bottom of the legend.
                    • left: Left of the legend.
                    • right: Right of the legend.
legend.ncol
                  numeric | Number of columns in the legend.
legend.nrow
                  numeric | Number of rows in the legend.
                  logical | Whether the legend is filled by row or not.
legend.byrow
plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.
                  character | Controls the style of the font for the corresponding theme element.
                  One of:
                    • plain: For normal text.
                    • italic: For text in itallic.
                    • bold: For text in bold.
```

#### Value

A ggplot2 object containing a Violin Plot.

## **Examples**

• bold.italic: For text both in itallic and bold.

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```
} else if (base::isFALSE(value)){
  message("This function can not be used without its suggested packages.")
  message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
}
```

do\_VolcanoPlot

Compute a Volcano plot out of DE genes.

## **Description**

Compute a Volcano plot out of DE genes.

```
do_VolcanoPlot(
  sample,
  de_genes,
  pval_cutoff = 0.05,
  FC_{cutoff} = 2,
  pt.size = 2,
  border.size = 1.5,
  border.color = "black",
  font.size = 14,
  font.type = "sans",
  plot.title = NULL,
  plot.subtitle = NULL,
  plot.caption = NULL,
  plot_lines = TRUE,
  line_color = "grey75",
  line_size = 0.5,
  add_gene_tags = TRUE,
  order_tags_by = "both",
  n_{genes} = 5,
  use_labels = FALSE,
  colors.use = "steelblue",
  plot.title.face = "bold",
 plot.subtitle.face = "plain",
  plot.caption.face = "italic",
  axis.title.face = "bold",
  axis.text.face = "plain",
  legend.title.face = "bold",
  legend.text.face = "plain"
)
```

do\_VolcanoPlot 97

#### **Arguments**

```
sample
                  Seurat | A Seurat object, generated by CreateSeuratObject.
                  tibble | Output of Seurat::FindMarkers().
de_genes
pval_cutoff
                  numeric | Cutoff for the p-value.
FC_cutoff
                  numeric | Cutoff for the avg_log2FC.
                  numeric | Size of the dots.
pt.size
border.size
                  numeric | Width of the border of the cells.
border.color
                  character | Color for the border of the heatmap body.
font.size
                  numeric | Overall font size of the plot. All plot elements will have a size rela-
                  tionship with this font size.
font.type
                  character | Base font family for the plot. One of:
                     • mono: Mono spaced font.
                     • serif: Serif font family.
                     • sans: Default font family.
plot.title, plot.subtitle, plot.caption
                  character | Title, subtitle or caption to use in the plot.
plot_lines
                  logical | Whether to plot the division lines.
line_color
                  character | Color for the lines.
line_size
                  numeric | Size of the lines in the plot.
                  logical | Whether to plot the top genes.
add_gene_tags
order_tags_by
                  character | Either "both", "pvalue" or "logfc".
n_genes
                  numeric | Number of top genes in each side to plot.
use labels
                  logical | Whether to use labels instead of text for the tags.
colors.use
                  character | Color to generate a tetradic color scale with.
plot.title.face, plot.subtitle.face, plot.caption.face, axis.title.face, axis.text.face, legend.title.
                  character | Controls the style of the font for the corresponding theme element.
                  One of:
                     • plain: For normal text.
```

- italic: For text in itallic.
- bold: For text in bold.
- bold.italic: For text both in itallic and bold.

# Value

A volcano plot as a ggplot2 object.

98 human\_chr\_locations

#### **Examples**

```
# Check Suggests.
value <- SCpubr:::check_suggests(function_name = "do_VolcanoPlot", passive = TRUE)</pre>
if (isTRUE(value)){
  # Consult the full documentation in https://enblacar.github.io/SCpubr-book/
  # Define your Seurat object.
sample <- readRDS(system.file("extdata/seurat_dataset_example.rds", package = "SCpubr"))</pre>
  # Retrieve DE genes.
 de_genes <- readRDS(system.file("extdata/de_genes_example.rds", package = "SCpubr"))</pre>
  # Generate a volcano plot.
 p <- SCpubr::do_VolcanoPlot(sample = sample,</pre>
                               de_genes = de_genes)
  р
} else if (base::isFALSE(value)){
  message("This function can not be used without its suggested packages.")
  message("Check out which ones are needed using `SCpubr::state_dependencies()`.")
}
```

human\_chr\_locations

Chromosome arm locations for human genome GRCh38.

# Description

A tibble containing the chromosome, arm and start and end coordinates.

#### Usage

```
data(human_chr_locations)
```

#### **Format**

A tibble with 48 rows and 4 columns:

chr Chromosome.

arm Chromosome arm.

start Start coordinates.

end End coordinates.

package\_report 99

package_report	Generate a status report of SCpubr and its dependencies.	

# Description

This function generates a summary report of the installation status of SCpubr, which packages are still missing and which functions can or can not currently be used.

#### Usage

```
package_report(startup = FALSE, extended = FALSE)
```

# Arguments

startup logical | Whether the message should be displayed at startup, therefore, also

containing welcoming messages and tips. If FALSE, only the report itself will be

printed.

extended logical | Whether the message should also include installed packages, current

and available version, and which SCpubr functions can be used with the cur-

rently installed packages.

#### Value

None

# Examples

```
# Print a package report.
SCpubr::package_report(startup = FALSE, extended = FALSE)
```

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