Package 'DOtools'

November 11, 2026

Title Convenient functions to streamline your single cell data analysis workflow

Version 1.2.0

Description This package provides functions for creating various visualizations, convenient wrappers, and quality-of-life utilities for single cell experiment objects. It offers a streamlined approach to visualize results and integrates different tools for easy use.

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BugReports https://github.com/MarianoRuzJurado/DOtools/issues

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Author Mariano Ruz Jurado [aut, cre] (ORCID:
<https://orcid.org/0000-0001-5354-5336>),
David Rodriguez Morales [aut] (ORCID:
<https://orcid.org/0000-0002-1819-6991>),
David John [aut] (ORCID: <https://orcid.org/0000-0003-3217-5449>),
DFG SFB 1366, Project B04 [fnd],
DFG SFB 1531, Project 456687919 [fnd]

Maintainer Mariano Ruz Jurado <ruzjurado@med.uni-frankfurt.de>

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`.annoSegment` *Annotation modifier for plots*

Description

Used for segment the plot for further annotations

Usage

```
.annoSegment(
  object = NULL,
  relSideDist = 0.1,
  aesGroup = FALSE,
  aesGroName = NULL,
  annoPos = "top",
  xPosition = NULL,
  yPosition = NULL,
  pCol = NULL,
  segWidth = 1,
  lty = NULL,
  lwd = 2,
  alpha = NULL,
  lineend = "square",
  annoManual = FALSE,
  mArrow = NULL,
  addBranch = FALSE,
  bArrow = NULL,
  branDirection = 1,
  branRelSegLen = 0.3,
  addText = FALSE,
  textCol = NULL,
  textSize = NULL,
  fontfamily = NULL,
  fontface = NULL,
```

```
  textLabel = NULL,
  textRot = 45,
  textHVjust = 0.2,
  hjust = 0.1,
  vjust = -0.5,
  myFacetGrou = NULL,
  aes_x = NULL,
  aes_y = NULL,
  segment_gap = 0.9
)
```

Arguments

object	ggplot list. Default(NULL).
relSideDist	The relative distance ratio to the y axis range. Default(0.1).
aesGroup	Whether use your group column to add rect annotation. Default("FALSE").
aesGroName	The mapping column name. Default(NULL).
annoPos	The position for the annotation to be added. Default("top").
xPosition	The x axis coordinate for the segment. Default(NULL).
yPosition	The y axis coordinate for the segment. Default(NULL).
pCol	The segment colors. Default(NULL).
segWidth	The relative segment width. Default(1).
lty	The segment line type. Default(NULL).
lwd	The segment line width. Default(NULL).
alpha	The segment color alpha. Default(NULL).
lineend	The segment line end. Default("square").
annoManual	Whether annotate by yourself by supplying with x and y coordinates. Default(FALSE).
mArrow	Whether add segment arrow. Default(FALSE).
addBranch	Whether add segment branch. Default(FALSE).
bArrow	Whether add branch arrow. Default(FALSE).
branDirection	The branch direction. Default(1).
branRelSegLen	The branch relative length to the segment. Default(0.3).
addText	Whether add text label on segment. Default(FALSE).
textCol	The text colors. Default(NULL).
textSize	The text size. Default(NULL).
fontfamily	The text fontfamily. Default(NULL).
fontface	The text fontface. Default(NULL).
textLabel	The text textLabel. Default(NULL).
textRot	The text angle. Default(NULL).
textHVjust	The text distance from the segment. Default(0.2).

hjust	The text hjust. Default(NULL).
vjust	The text vjust. Default(NULL).
myFacetGrou	Your facet group name to be added with annotation when object is a faceted object. Default(NULL).
aes_x	= NULL You should supply the plot X mapping name when annotate a facetd plot. Default(NULL).
aes_y	= NULL You should supply the plot Y mapping name when annotate a facetd plot. Default(NULL).
segment_gap	= 0.9 define the gap between segmentation brackets

Value

ggplot

Author(s)

Mariano Ruz Jurado (edited from: Jun Zhang)

.calculate_density *Estimate weighted kernel density*

Description

Estimate weighted kernel density

Usage

```
.calculate_density(w, x, method, adjust = 1, map = TRUE)
```

Arguments

w	Vector with weights for each observation
x	Matrix with dimensions where to calculate the density from. Only the first two dimensions will be used
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.
adjust	Numeric value to adjust to bandwidth. Default: 1. Not available for ks method
map	Whether to map densities to individual observations

Value

If map is TRUE, a vector with corresponding densities for each observation is returned. Otherwise, a list with the density estimates from the selected method is returned.

Author(s)

Jose Alquicira-Hernandez (modified)

`.DO.BarcodeRanks` *DO.BarcodeRanks*

Description

Given a raw count matrix (e.g. from a CellRanger HDF5 file), estimate the number of expected cells and droplets using the knee and inflection points from barcodeRanks.

Usage

```
.DO.BarcodeRanks(SCE_obj)
```

Arguments

`SCE_obj`	A Single cell experiment object.

Value

A named numeric vector: ‘c(xpc_cells = ..., total_cells = ...)

Author(s)

Mariano Ruz Jurado

`.example_10x` *Download example dataset 10x*

Description

Download example dataset 10x

Usage

```
.example_10x(base_dir = tempfile("dotools_datasets_"))
```

Value

directory path where the data was saved

.kBET_fct *kBET - k-nearest neighbour batch effect test*

Description

kBET runs a chi square test to evaluate the probability of a batch effect.

Usage

```
.kBET_fct(
  df,
  batch,
  k0 = NULL,
  knn = NULL,
  testSize = NULL,
  do.pca = TRUE,
  dim.pca = 50,
  heuristic = TRUE,
  n_repeat = 100,
  alpha = 0.05,
  addTest = FALSE,
  verbose = FALSE,
  plot = TRUE,
  adapt = TRUE
)
```

Arguments

df	dataset (rows: cells, columns: features)
batch	batch id for each cell or a data frame with both condition and replicates
k0	number of nearest neighbours to test on (neighbourhood size)
knn	an n x k matrix of nearest neighbours for each cell (optional)
testSize	number of data points to test, (10 percent sample size default, but at least 25)
do.pca	perform a pca prior to knn search? (defaults to TRUE)
dim.pca	if do.pca=TRUE, choose the number of dimensions to consider (defaults to 50)
heuristic	compute an optimal neighbourhood size k (defaults to TRUE)
n_repeat	to create a statistics on batch estimates, evaluate 'n_repeat' subsets
alpha	significance level
addTest	perform an LRT-approximation to the multinomial test AND a multinomial exact test (if appropriate)
verbose	displays stages of current computation (defaults to FALSE)
plot	if stats > 10, then a boxplot of the resulting rejection rates is created
adapt	In some cases, a number of cells do not contribute to any neighbourhood and this may cause an imbalance in observed and expected batch label frequencies. Frequencies will be adapted if adapt=TRUE (default).

Value

list object

1. `summary` - a rejection rate for the data, an expected rejection rate for random labeling and the significance for the observed result
2. `results` - detailed list for each tested cells; p-values for expected and observed label distribution
3. `average.pval` - significance level over the averaged batch label distribution in all neighbourhoods
4. `stats` - extended test summary for every sample
5. `params` - list of input parameters and adapted parameters, respectively
6. `outsider` - only shown if `adapt=TRUE`. List of samples without mutual nearest neighbour:
   - `index` - index of each outsider sample)
   - `categories` - tabularised labels of outsiders
   - `p.val` - Significance level of outsider batch label distribution vs expected frequencies. If the significance level is lower than `alpha`, expected frequencies will be adapted

If the optimal neighborhood size (k0) is smaller than 10, NA is returned.

Author(s)

Mariano Ruz Jurado (edited from: Maren Buettner)

`.plot_density_` *Plot density estimates*

Description

Plot density estimates

Usage

```
.plot_density_(
  z,
  dens,
  feature,
  cell_embeddings,
  dim_names,
  shape = 16,
  size = 1,
  text_size = 14,
  density_quantile_threshold = 0.5,
  n_bands = 256,
  legend.position = "right",
  legend_title = "Density",
```

```
  pal = "Reds",
  raster = FALSE,
  rev_colours_density = FALSE,
  ...
)
```

Arguments

z	Vector with density values for each cells
dens	density grid retrieved from KDE or wkde2d
feature	Name of the feature being plotted
cell_embeddings	Matrix with cell embeddings
dim_names	Names of the dimensions from the cell embeddings
shape	Geom shape
size	Geom size
text_size	text_size given from parent function
legend.position	legend.position from parent function
legend_title	String used as legend title
pal	String specifying the color palette to use, can be one of hcl.pals
raster	Rasterise plot
...	Further scale arguments passed to scale_color_viridis_c

Value

A ggplot object

Author(s)

Jose Alquicira-Hernandez (modified)

`.QC_Vlnplot` *.QC_Vlnplot*

Description

Generates violin plots for common quality control (QC) metrics of single-cell RNA-seq data from a Seurat object. The function displays three violin plots for the number of detected genes per cell (`nFeature_RNA`), total UMI counts per cell (`nCount_RNA`), and mitochondrial gene content percentage (`pt_mito`). Useful for visual inspection of QC thresholds and outliers.

Usage

```
.QC_Vlnplot(
  sce_object,
  id,
  layer = "counts",
  features = c("nFeature_RNA", "nCount_RNA", "pt_mito")
)
```

Arguments

sce_object	A Seurat object containing single-cell RNA-seq data.
layer	A character string specifying the assay layer to use (default is "counts").
features	A character vector of length 3 indicating the feature names to plot. Default is c("nFeature_RNA", "nCount_RNA", "pt_mito").

Value

A ggplot object arranged in a single row showing violin plots for the specified features with overlaid boxplots.

Author(s)

Mariano Ruz Jurado

.run_kbet *kBET function*

Description

kBET quantifies batch mixing in single-cell data by testing whether local neighborhood batch composition deviates from the global distribution, with lower rejection indicating better integration.

Usage

```
.run_kbet(
  sce_object,
  embedding,
  batch,
  cells.use = NULL,
  subsample = NULL,
  min_per_batch = NULL,
  ...
)
```

Arguments

sce_object	Seurat or SCE object.
embedding	Name of the embedding to test
batch	Name of the sample column in meta.data
cells.use	specify a specific set of cells as vector
subsample	set a fraction for stratified subsetting
min_per_batch	should always be higher than or equal k0

Value

list with summary stats of kBET

Author(s)

Mariano Ruz Jurado

.wkde2d *Weighted 2D kernel density estimation*

Description

Weighted 2D kernel density estimation

Usage

```
.wkde2d(x, y, w, h, adjust = 1, n = 100, lims = c(range(x), range(y)))
```

Arguments

x	Dimension 1
y	Dimension 2
w	Weight variable
h	vector of bandwidths for x and y directions. Defaults to normal reference bandwidth (ks::hpi). A scalar value will be taken to apply to both directions.
adjust	Bandwidth adjustment
n	Number of grid points in each direction. Can be scalar or a length-2 integer vector.
lims	The limits of the rectangle covered by the grid as c(xl, xu, yl, yu).

Value

A list of three components.

- x, y The x and y coordinates of the grid points, vectors of length n.
- z A matrix of the weighted estimated density: rows correspond to the value of x, columns to the value of y.

Author(s)

Jose Alquicira-Hernandez (modified)

`DO.Barplot` *SEM Graph with wilcox test on single cell level*

Description

Perform SEM-based graphs with Wilcox test on single-cell level for Seurat and SCE objects. Calculates mean expression values and SEM for the selected feature, and visualizes them. Performs pairwise Wilcox tests comparing conditions, with optional custom control condition and clustering. Optionally returns a summary data frame, statistical test results, and the generated plot.

Usage

```
DO.Barplot(
  sce_object,
  Feature,
  ListTest = NULL,
  returnValues = FALSE,
  ctrl.condition = NULL,
  group.by = "condition",
  test_use = "wilcox",
  correction_method = "fdr",
  p_values = NULL,
  bar_colours = NULL,
  stat_pos_mod = 1.15,
  step_mod = 0.2,
  x_label_rotation = 45,
  plot_raw_pvalue = FALSE,
  y_limits = NULL,
  log1p_nUMI = TRUE,
  random_seed = 42
)
```

Arguments

`sce_object`	combined SCE object or Seurat
`Feature`	name of the feature/gene
`ListTest`	List for which conditions wilcoxon test will be performed, if NULL always CTRL group against everything
`returnValues`	return data frames needed for the plot, containing df.melt, df.melt.sum, df.melt.orig and wilcoxstats
`ctrl.condition`	set your ctrl condition, relevant if running with empty comparison List
`group.by`	select the seurat object slot where your conditions can be found, default conditon

test_use	perform one of c("wilcox", "wilcox_limma", "bimod", "t", "negbinom", "poisson", "LR", "MAST", "DESeq2", "none"). default "wilcox"
correction_method	correction for p-value calculation. One of c("BH", "bonferroni", "holm", "BY", "fdr", "none")
p_values	Manually providing p-values for plotting, be aware of group size and if necessary make your test return the same amount of values
bar_colours	colour vector
stat_pos_mod	Defines the distance to the graphs of the statistic
step_mod	Defines the distance between each statistics bracket
x_label_rotation	Rotation of x-labels
plot_raw_pvalue	plot the non adjusted p-value without correcting for multiple tests
y_limits	set limits for y-axis
log1p_nUMI	If nUMIs should be log1p transformed
random_seed	parameter for random state initialisation

Value

a ggplot or a list with plot and data frame

Author(s)

Mariano Ruz Jurado

Examples

```
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

ListTest <- list()
ListTest[[1]] <- c("healthy", "disease")

DO.Barplot(
    sce_object = sce_data,
    Feature = "NKG7",
    test_use = "wilcox",
    correction_method="fdr",
    ListTest = ListTest,
    ctrl.condition = "healthy",
    group.by = "condition"
)
```

DO.BarplotClustert *SEM Graph with t test on cluster level*

Description

Perform SEM-based graphs with t-test on cluster level for SCE objects. Calculates mean expression values and SEM for selected features and visualizes them. Performs pairwise t-tests comparing conditions, with optional custom control condition and clustering. Optionally returns a summary data frame.

Usage

```
DO.BarplotClustert(
  sce_object,
  Feature,
  ListTest = NULL,
  returnValues = FALSE,
  ctrl.condition = NULL,
  group.by = "condition",
  returnPlot = TRUE,
  bar_colours = NULL,
  stat_pos_mod = 1.15,
  step_mod = 0.2,
  x_label_rotation = 45,
  y_limits = NULL,
  log1p_nUMI = TRUE,
  random_seed = 42
)
```

Arguments

sce_object	Combined SCE object or Seurat
Feature	gene name
ListTest	List with conditions t-test will be performed, if NULL always against provided CTRL
returnValues	return df.melt.sum data frame containing means and SEM for the set group
ctrl.condition	set your ctrl condition, relevant if running with empty comparison List
group.by	select the seurat object slot where your conditions can be found, default conditon
returnPlot	IF TRUE returns ggplot
bar_colours	colour vector
stat_pos_mod	Defines the distance to the graphs of the statistic
step_mod	Defines the distance between each statistics bracket
x_label_rotation	Rotation of x-labels

y_limits	set limits for y-axis
log1p_nUMI	If nUMIs should be log1p transformed
random_seed	parameter for random state initialisation

Value

a ggplot or a dataframe

Author(s)

Mariano Ruz Jurado

Examples

```
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

set.seed(123)
sce_data$orig.ident <-
    sample(rep(c("A", "B", "C"), length.out = ncol(sce_data)))

ListTest <- list()
ListTest[[1]] <- c("healthy", "disease")


DO.BarplotClustert(
    sce_object = sce_data,
    Feature = "NKG7",
    ListTest = ListTest,
    ctrl.condition = "healthy",
    group.by = "condition"
)
```

DO.BoxPlot *Box Graph with wilcox test on single cell level*

Description

Creates a box plot using a pseudo-bulk approach and performs a Wilcoxon test on single-cell level. Allows customization of outlier removal, statistical labels, and color schemes. Supports comparison of conditions with optional second grouping. Useful for visualizing gene expression and statistical differences.

Usage

```
DO.BoxPlot(
  sce_object,
  Feature,
  sample.column = "orig.ident",
  ListTest = NULL,
  group.by = "condition",
  group.by.2 = NULL,
  ctrl.condition = NULL,
  outlier_removal = TRUE,
  plot_sample = TRUE,
 vector_colors = c("#1f77b4", "#ea7e1eff", "royalblue4", "tomato2", "darkgoldenrod",
    "palegreen4", "maroon", "thistle3"),
  test_use = "wilcox",
  correction_method = "fdr",
  p_values = NULL,
  stat_pos_mod = 1.15,
  step_mod = 0,
  hjust_test = 0.5,
  vjust_test = 0.25,
  size_test = 3.33,
  hjust_test_2 = 0.5,
  vjust_test_2 = 0,
  sign_bar = 0.8,
  orderAxis = NULL
)
```

Arguments

sce_object	The SCE object or Seurat
Feature	name of the feature/gene
sample.column	meta data column containing sample IDs
ListTest	List for which conditions wilcox will be performed, if NULL always CTRL group against everything
group.by	group name to look for in meta data
group.by.2	second group name to look for in meta data
ctrl.condition	select condition to compare to
outlier_removal	Outlier calculation
plot_sample	Plot individual sample dot in graph
vector_colors	get the colours for the plot
test_use	perform one of c("wilcox", "wilcox_limma", "bimod", "t", "negbinom", "poisson", "LR", "MAST", "DESeq2", "none"). default "wilcox"
correction_method	correction for p-value calculation. One of c("BH", "bonferroni", "holm", "BY", "fdr", "none"). default "fdr"

p_values	Manually providing p-values for plotting, be aware of group size and if necessary make your test return the same amount of values
stat_pos_mod	modificator for where the p-value is plotted increase for higher
step_mod	value for defining the space between one test and the next one
hjust_test	value for adjusting height of the text
vjust_test	value for vertical of text
size_test	value for size of text of statistical test
hjust_test_2	value for adjusting height of the text, with group.by.2 specified
vjust_test_2	value for vertical of text, with group.by.2 specified
sign_bar	adjusts the sign_bar with group.by.2 specified
orderAxis	vector for xaxis sorting, alphabetically by default

Value

a ggplot

Author(s)

Mariano Ruz Jurado

Examples

```
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

set.seed(123)
sce_data$orig.ident <-
    sample(rep(c("A", "B", "C"), length.out = ncol(sce_data)))

ListTest <- list()
ListTest[[1]] <- c("healthy", "disease")

DO.BoxPlot(
    sce_object = sce_data,
    Feature = "NKG7",
    sample.column = "orig.ident",
    ListTest = ListTest,
    group.by = "condition",
    ctrl.condition = "healthy",
)
```

DO.CellBender *DO.CellBender*

Description

This function wraps a system call to a bash script for running CellBender on CellRanger outputs. It ensures required inputs are available and optionally installs CellBender in a conda env.

Usage

```
DO.CellBender(
  cellranger_path,
  output_path,
  samplenames = NULL,
  cuda = TRUE,
  cpu_threads = 15,
  epochs = 150,
  lr = 1e-05,
  estimator_multiple_cpu = FALSE,
  log = TRUE,
  conda_path = NULL,
  BarcodeRanking = TRUE,
  bash_script = system.file("bash", "_run_CellBender.sh", package = "DOtools")
)
```

Arguments

cellranger_path	Path to folder with CellRanger outputs.
output_path	Output directory for CellBender results.
samplenames	Optional vector of sample names. If NULL, will autodetect folders in cellranger_path.
cuda	Logical, whether to use GPU (CUDA).
cpu_threads	Number of CPU threads to use.
epochs	Number of training epochs.
lr	Learning rate.
estimator_multiple_cpu	Use estimator with multiple CPU threads.
log	Whether to enable logging.
conda_path	Optional path to the conda environment.
BarcodeRanking	Optional Calculation of estimated cells in samples through DropletUtils implementation
bash_script	Path to the bash script that runs CellBender.

Value

None

Examples

```
## Not run:
# Define paths
cellranger_path <- "/mnt/data/cellranger_outputs"
output_path <- "/mnt/data/cellbender_outputs"

# Optional: specify sample names if automatic detection is not desired
samplenames <- c("Sample_1", "Sample_2")

# Run CellBender (uses GPU by default)
DO.CellBender(
    cellranger_path = cellranger_path,
    output_path = output_path,
    samplenames = samplenames,
    cuda = TRUE,
    cpu_threads = 8,
    epochs = 100,
    lr = 0.00001,
    estimator_multiple_cpu = FALSE,
    log = TRUE
)

## End(Not run)
```

DO.CellComposition *DO CellComposition*

Description

Computes and visualizes cell composition changes from a Seurat object using Scanpro. Integrates R and Python via reticulate to convert Seurat to AnnData and run Scanpro. Generates customizable plots with options for transformation, grouping, and bootstrapping. Returns a ggplot object or a list containing plot data and visualisation.

Usage

```
DO.CellComposition(
  sce_object,
  assay_normalized = "RNA",
  cluster_column = "seurat_clusters",
  sample_column = "orig.ident",
  condition_column = "condition",
  transform_method = "logit",
  sort_x = NULL,
```

```
  sub_ident = NULL,
  sort_fill = NULL,
  scanpro_plots = FALSE,
  scanpro_group = NULL,
  outputFolder = NULL,
  return_df = FALSE,
  bar_colors = NULL,
  n_reps = NULL,
  legend.pos.x = 0.48,
  legend.pos.y = 0,
  cowplot_width = 0.9,
  cowlegend_width = 0.9,
  random_seed = 42,
  ...
)
```

Arguments

sce_object	The SCE object or Seurat
assay_normalized	Assay with raw counts
cluster_column	Column in meta data which will be used to segment the bar plot
sample_column	Column in meta data containing individual sample names
condition_column	Column in meta data plotted on the xaxis
transform_method	Method of transformation of proporties, default: "logit"
sort_x	Vector sorting the xaxis
sub_ident	vector to subset the whole plot by
sort_fill	Vector sorting the bar segments
scanpro_plots	Boolean, will create plots provided in scanpro package, default: FALSE
scanpro_group	Defines the clusters showed in scanpro plots
outputFolder	Scanpro plots will be saved in this directory, defaults to current working directory
return_df	Boolean, makes a list with first entry beeaing the dataframe used to create the ggplot and second entry is the plot
bar_colors	(named) vector of colors used for plotting, the names will be sued to factorize the segments
n_reps	Number of replicates generated by scanpro
legend.pos.x	adjusts the position of the legend in horizontal
legend.pos.y	adjusts the position of the legend in vertical
cowplot_width	Changes the width on the plotting device for plot
cowlegend_width	Changes the width on the plotting device for legend, useful for adjusting the legend position in combination with legend.pos.x and y

random_seed	parameter for random state initialisation
...	Further arguments passed to scanpro plotting functions

Value

ggplot object or list

Author(s)

Mariano Ruz Jurado

Examples

```
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

DO.CellComposition(
    sce_object = sce_data,
    cluster_column = "annotation",
    condition_column = "condition",
    scanpro_plots = FALSE,
    n_reps = 5
)
```

DO.CellTypist *DO Celltypist*

Description

Runs the CellTypist model on a Seurat or SCE object to predict cell type labels, storing the results as metadata. If the number of cells is less than the specified threshold, it returns NAs for the labels. Optionally updates the CellTypist models and returns the probability matrix. Useful for annotating cell types in single-cell RNA sequencing datasets.

Usage

```
DO.CellTypist(
  sce_object,
  modelName = "Healthy_Adult_Heart.pkl",
  minCellsToRun = 200,
  runCelltypistUpdate = TRUE,
  over_clustering = "seurat_clusters",
  assay_normalized = "RNA",
  returnAll = FALSE,
  SeuV5 = TRUE
)
```

Arguments

sce_object	The seurat or sce object
modelName	Specify the model you want to use for celltypist
minCellsToRun	If the input seurat or SCE object has fewer than this many cells, NAs will be added for all expected columns and celltypist will not be run.
runCelltypistUpdate	If true, –update-models will be run for celltypist prior to scoring cells.
over_clustering	Column in metadata in object with clustering assignments for cells, default seurat_clusters
assay_normalized	Assay with log1p normalized expressions
returnAll	will additionally return the probability matrix, return will give a list with the first element being the object and second plot and third probability matrix
SeuV5	Specify if the Seurat object is made with Seuratv5

Value

a seurat or sce object

Author(s)

Mariano Ruz Jurado

Examples

```
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))


sce_data <- DO.CellTypist(
    sce_object = sce_data,
    modelName = "Healthy_Adult_Heart.pkl",
    runCelltypistUpdate = TRUE,
    over_clustering = "seurat_clusters",
    minCellsToRun = 5,
    SeuV5 = TRUE
)
```

DO.Correlation *DO Correlation Plot for visualizing similarity between categories*

Description

Generates a correlation heatmap from expression data to visualize similarity across sample groups. Allows customization of plot type, correlation method, and color scaling using the ggcorrplot2 and ggplot2 architectures. Ideal for comparing transcriptional profiles between conditions or clusters.

Usage

```
DO.Correlation(
  sce_object,
  group_by = "orig.ident",
  assay = "RNA",
  features = NULL,
  method = "spearman",
  plotdesign = "square",
  plottype = "full",
  auto_limits = TRUE,
  outline.color = "white",
  colormap = c("royalblue4", "lightsteelblue", "tomato", "firebrick4"),
  lab_size = 10,
  lab = TRUE,
  lab_col = "white",
  axis_size_x = 12,
  axis_size_y = 12,
  ...
)
```

Arguments

sce_object	Seurat or SCE Object
group_by	Column to aggregate the expression over it, default "orig.ident"
assay	Assay in object to use, default "RNA"
features	What genes to include by default all, default "None"
method	Correlation method, default "spearman"
plotdesign	Plot design, default "circle"
plottype	Show the full plot or only half of it, default "full"
auto_limits	Automatically rescales the colour bar based on the values in the correlation matrix, default "TRUE"
outline.color	the outline color of square or circle. Default value is "white".
colormap	Defines the colormap used in the plot, default c("royalblue4", "royalblue2","firebrick","firebrick4")
lab_size	Size to be used for the correlation coefficient labels. used when lab = TRUE.

lab	logical value. If TRUE, add correlation coefficient on the plot.
lab_col	color to be used for the correlation coefficient labels. used when lab = TRUE.
axis_size_x	Controls x labels size
axis_size_y	Controls y labels size
...	Additionally arguments passed to ggcorrplot function

Value

ggplot2

Author(s)

Mariano Ruz Jurado

Examples

```
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

DO.Correlation(
    sce_object = sce_data,
    group_by = "orig.ident",
    assay = "RNA",
    features = NULL,
    method = "spearman",
    plotdesign = "square",
    plottype = "full",
    auto_limits = TRUE,
    outline.color = "white",
    colormap = c("royalblue4", "lightsteelblue", "tomato", "firebrick4"),
    lab_size = 10,
    lab = TRUE,
    lab_col = "white"
)
```

DO.DietSCE *Remove Layers from Seurat or SCE Object by Pattern*

Description

This function removes layers from a Seurat or SCE object's RNA assay based on a specified regular expression pattern. It is supposed to remove no longer needed layers from th object.

Usage

```
DO.DietSCE(sce_object, assay = "RNA", pattern = "^scale\\.data\\.")
```

Arguments

sce_object	Seurat or SCE object.
assay	Name of the assay from where to remove layers from
pattern	regular expression pattern to match layer names. Default "^scale\.data\."

Value

Seurat or SCE object with specified layers removed.

Author(s)

Mariano Ruz Jurado

Examples

```
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

sce_data <- DO.DietSCE(sce_data, pattern = "data")
```

DO.Dotplot *DO Dot plot*

Description

This function generates a dot plot for multiple genes, comparing expression levels across one or two specified groups. It supports both individual and pseudobulk expression calculations. Highly variable customization options allow control over dot size, color scaling, annotations, and axis orientation. The function integrates seamlessly with SCE objects for single-cell RNA-seq analysis.

Usage

```
DO.Dotplot(
  sce_object,
  Feature,
  group.by.x = NULL,
  group.by.y = NULL,
  group.by.y2 = NULL,
  across.group.by.x = FALSE,
  across.group.by.y = FALSE,
  sort_x = NULL,
  sort_y = NULL,
  dot.size = c(1, 6),
  plot.margin = c(1, 1, 1, 1),
  midpoint = 0.5,
  scale_gene = FALSE,
```

```
  returnValue = FALSE,
  log1p_nUMI = TRUE,
  hide_zero = TRUE,
  annotation_x = FALSE,
  annotation_x_position = 0.25,
  annotation_x_rev = FALSE,
  point_stroke = 0.2,
  limits_colorscale = NULL,
  coord_flip = FALSE,
  stats_x = FALSE,
  stats_y = TRUE,
  sig_size = 6,
  nudge_x = 0.3,
  nudge_y = 0.2,
  ...
)
```

Arguments

sce_object	The SCE object or Seurat
Feature	Genes or DF of interest, Data frame should have columns with gene and annotation information, e.g. output of FindAllMarkers
group.by.x	group name to plot on x-axis
group.by.y	group name to look for in meta data
group.by.y2	second group name to look for in meta data
across.group.by.x	calculate a pseudobulk expression approach for the x-axis categories
across.group.by.y	calculate a pseudobulk expression approach for the y-axis categories
sort_x	Vector sorting the xaxis
sort_y	Vector to sort the yaxis
dot.size	Vector of dot size
plot.margin	= plot margins
midpoint	midpoint in color gradient
scale_gene	If True calculates the Z-score of the average expression per gene
returnValue	return the dataframe behind the plot
log1p_nUMI	log1p the plotted values, boolean
hide_zero	Removes dots for genes with 0 expression
annotation_x	Adds annotation on top of x axis instead on y axis
annotation_x_position	specifies the position for the annotation
annotation_x_rev	reverses the annotations label order

point_stroke	Defines the thickness of the black stroke on the dots
limits_colorscale	Set manually colorscale limits
coord_flip	flips the coordinates of the plot with each other
stats_x	Perform statistical test over categories on the xaxis
stats_y	Perform statistical test over categories on the yaxis
sig_size	Control the size of the significance stars in the plot
nudge_x	Control the position of the star on x axis
nudge_y	Control the position of the star on y axis
...	Further arguments passed to annoSegment function if annotation_x == TRUE

Value

a ggplot

Author(s)

Mariano Ruz Jurado

Examples

```
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

DO.Dotplot(
    sce_object = sce_data,
    Feature = c("NKG7", "IL6", "MALAT1"),
    group.by.x = "condition"
)
```

DO.enrichR *DO.enrichR*

Description

Performs Gene Ontology enrichment analysis on differentially expressed genes using the EnrichR API. Separately analyzes upregulated and downregulated genes and returns results.

Usage

```
DO.enrichR(
  df_DGE,
  gene_column,
  pval_column,
  log2fc_column,
```

```
  pval_cutoff = 0.05,
  log2fc_cutoff = 0.25,
  path = NULL,
  filename = "",
  species = "Human",
  go_catgs = c("GO_Molecular_Function_2023", "GO_Cellular_Component_2023",
    "GO_Biological_Process_2023")
)
```

Arguments

df_DGE	data.frame containing differential gene expression results.
gene_column	column name in df with gene symbols.
pval_column	column name in df with p-values.
log2fc_column	column name in df with log2 fold changes.
pval_cutoff	adjusted p-value threshold for significance (default = 0.05).
log2fc_cutoff	log2 fold change threshold for up/down regulation (default = 0.25).
path	folder path where the output Excel file will be saved. A subfolder "GSA_Tables" will be created.
filename	suffix used in the Excel filename (e.g., "GSA_CellType_MyAnalysis.xlsx").
species	species name for enrichment analysis. Options include "Human", "Mouse", "Yeast", etc. (default = "Mouse").
go_catgs	GO databases to use. Defaults to c(GO_Biological_Process_2023").

Value

data.frame with GO enrichment results if path is NULL, otherwise writes an Excel file.

Examples

```
library(enrichR)

sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))
DGE_result <- DO.MultiDGE(sce_data,
    sample_col = "orig.ident",
    method_sc = "wilcox",
    annotation_col = "annotation",
    ident_ctrl = "healthy"
)

DGE_result <- DGE_result[DGE_result$celltype == "CD4_T_cells", ]

result_GO <- DO.enrichR(
    df_DGE = DGE_result,
    gene_column = "gene",
    pval_column = "p_val_SC_wilcox",
    log2fc_column = "avg_log2FC_SC_wilcox",
```

```
    pval_cutoff = 0.05,
    log2fc_cutoff = 0.25,
    path = NULL,
    filename = "",
    species = "Human",
    go_catgs = "GO_Biological_Process_2023"
)
```

`DO.EvalIntegration` *Do batch correction metrics for integration*

Description

This function calculates different metrics to evaluate the integration of scRNA expression matrices in a new dimension. Its a wrapper function around scib batch correction metrics

Usage

```
DO.EvalIntegration(
  sce_object,
  label_key = "annotation",
  batch_key = "orig.ident",
  type_ = "embed",
  pcr_covariate = "orig.ident",
  pcr_n_comps = 30,
  scale = TRUE,
  verbose = FALSE,
  n_cores = 10,
  assay = "RNA",
  integration = "INTEGRATED.CCA",
  kBET = TRUE,
  cells.use = NULL,
  subsample = NULL,
  min_per_batch = NULL,
  all_scores_silhouette = FALSE,
  ...
)
```

Arguments

`sce_object`	Seurat or SCE object.
`label_key`	character, Annotation column
`batch_key`	character, Sample column
`type_`	character, default: "embed"
`pcr_covariate`	character, covariate column for pcr

pcr_n_comps	integer, number of components for pcr
scale	boolean, default: TRUE
verbose	boolean, defult: FALSE
n_cores	integer, Number of cores used for calculations
assay	character, Name of the assay the integration is saved in
integration	character, Name of the integration to evaluate
kBET	boolean, if kBET should be run
cells.use	vector, named cells to use for kBET subsetting
subsample	float, for starified subsampling,
min_per_batch	integer, minimum number of cells per batch
all_scores_silhouette	boolean, define if all scores of silhouette return
...	Additionally arguments for kBET

Value

DataFrame with score for the given integration

Author(s)

Mariano Ruz Jurado

Examples

```
## Not run:
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

DO.EvalIntegration(
    sce_object = sce_data,
    label_key = "annotation",
    batch_key = "orig.ident",
    type_ = "embed",
    pcr_covariate = "orig.ident",
    pcr_n_comps = 30,
    scale = TRUE,
    verbose = FALSE,
    n_cores = 10,
    assay = "RNA",
    integration = "INTEGRATED.CCA",
    kBET = TRUE,
    cells.use = NULL,
    subsample = NULL,
    min_per_batch = NULL,
    all_scores_silhouette = FALSE
)

## End(Not run)
```

DO.FullRecluster *DO.FullRecluster*

Description

Performs iterative reclustering on each major cluster found by FindClusters in a Seurat or SCE object. It refines the clusters using the FindSubCluster function for better resolution and fine-tuned annotation. The new clustering results are stored in a metadata column called `annotation_recluster`. Suitable for improving cluster precision and granularity after initial clustering.

Usage

```
DO.FullRecluster(
  sce_object,
  over_clustering = "seurat_clusters",
  res = 0.5,
  algorithm = 4,
  graph.name = "RNA_snn",
  random_seed = 42
)
```

Arguments

`sce_object`	The seurat or SCE object
`over_clustering`	Column in metadata in object with clustering assignments for cells, default seurat_clusters
`res`	Resolution for the new clusters, default 0.5
`algorithm`	Set one of the available algorithms found in FindSubCLuster function, default = 4: leiden
`graph.name`	A builded neirest neighbor graph
`random_seed`	parameter for random state initialisation

Value

a Seurat or SCE Object with new clustering named annotation_recluster

Author(s)

Mariano Ruz Jurado

Examples

```
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

sce_data <- DO.FullRecluster(
    sce_object = sce_data
)
```

DO.Heatmap *DO Heatmap of the mean expression of genes across a groups*

Description

Wrapper around heatmap.py, which generates a heatmap of showing the average nUMI for a set of genes in different groups. Addiional an argumnt can be made to show foldchanges between two conditions. Differential gene expression analysis between the different groups can be performed.

Usage

```
DO.Heatmap(
  sce_object,
  features,
  assay_normalized = "RNA",
  group_by = "seurat_clusters",
  groups_order = NULL,
  value_plot = "expr",
  group_fc = "condition",
  group_fc_ident_1 = NULL,
  group_fc_ident_2 = NULL,
  clip_value = FALSE,
  max_fc = 5,
  z_score = NULL,
  path = NULL,
  filename = "Heatmap.svg",
  swap_axes = TRUE,
  cmap = "Reds",
  title = NULL,
  title_fontprop = NULL,
  clustering_method = "complete",
  clustering_metric = "euclidean",
  cluster_x_axis = FALSE,
  cluster_y_axis = FALSE,
  axs = NULL,
  figsize = c(5, 6),
  linewidth = 0.1,
  ticks_fontdict = NULL,
```

```
  xticks_rotation = NULL,
  yticks_rotation = NULL,
  vmin = 0,
  vcenter = NULL,
  vmax = NULL,
  legend_title = "LogMean(nUMI)\nin group",
  add_stats = TRUE,
  df_pvals = NULL,
  stats_x_size = NULL,
  square_x_size = NULL,
  test = "wilcox",
  pval_cutoff = 0.05,
  log2fc_cutoff = 0,
  only_pos = TRUE,
  square = TRUE,
  showP = TRUE,
  logcounts = TRUE
)
```

Arguments

sce_object	SCE object or Seurat with meta.data
features	gene names or continuous value in meta data
assay_normalized	Assay with raw counts
group_by	meta data column name with categorical values
groups_order	order for the categories in the group_by
value_plot	plotted values correspond to expression values or foldchanges
group_fc	if foldchanges specified than the groups must be specified that will be compared
group_fc_ident_1	Defines the first group in the test
group_fc_ident_2	Defines the second group in the test
clip_value	Clips the colourscale to the 99th percentile, useful if one gene is driving the colourscale
max_fc	Clips super high foldchanges to this value, so changes can still be appreciated
z_score	apply z-score transformation, "group" or "var"
path	path to save the plot
filename	name of the file
swap_axes	whether to swap the axes or not
cmap	color map
title	title for the main plot
title_fontprop	font properties for the title (e.g., 'weight' and 'size')

clustering_method	clustering method to use when hierarchically clustering the x and y-axis
clustering_metric	metric to use when hierarchically clustering the x- and y-axis
cluster_x_axis	hierarchically clustering the x-axis
cluster_y_axis	hierarchically clustering the y-axis
axs	matplotlib axis
figsize	figure size
linewidth	line width for the border of cells
ticks_fontdict	font properties for the x and y ticks (e.g., 'weight' and 'size')
xticks_rotation	rotation of the x-ticks
yticks_rotation	rotations of the y-ticks
vmin	minimum value
vcenter	center value
vmax	maximum value
legend_title	title for the color bar
add_stats	add statistical annotation, will add a square with an '*' in the center if the expression is significantly different in a group with respect to the others
df_pvals	dataframe with the p-values, should be gene x group or group x gene in case of swap_axes is False
stats_x_size	size of the asterisk
square_x_size	size and thickness of the square percentual, vector
test	test to use for test for significance
pval_cutoff	cutoff for the p-value
log2fc_cutoff	minimum cutoff for the log2FC
only_pos	if set to TRUE, only use positive genes in the condition
square	whether to make the cell square or not
showP	if set to false return a dictionary with the axis
logcounts	whether the input is logcounts or not

Value

Depending on showP, returns the plot if set to TRUE or a dictionary with the axes.

Author(s)

Mariano Ruz Jurado & David Rodriguez Morales

Examples

```
sce_data <-
  readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

DO.Heatmap(
sce_object = sce_data,
assay_normalized = "RNA",
group_by="seurat_clusters",
features = rownames(sce_data)[1:10],
z_score = NULL,
path = NULL,
filename = "Heatmap.svg",
swap_axes = TRUE,
cmap = "Reds",
title = NULL,
title_fontprop = NULL,
clustering_method = "complete",
clustering_metric = "euclidean",
cluster_x_axis = FALSE,
cluster_y_axis = FALSE,
axs = NULL,
figsize = c(5, 6),
linewidth = 0.1,
ticks_fontdict = NULL,
xticks_rotation = 45,
yticks_rotation = NULL,
vmin = 0.0,
vcenter = NULL,
vmax = NULL,
legend_title = "LogMean(nUMI)\nin group",
add_stats = TRUE,
df_pvals = NULL,
stats_x_size = NULL,
square_x_size = NULL,
test = "wilcox",
pval_cutoff = 0.05,
log2fc_cutoff = 0,
only_pos = TRUE,
square = TRUE,
showP = FALSE,
logcounts = TRUE
)
```

DO.HeatmapFC *DO Heatmap of the mean expression of genes across a groups*

Description

Wrapper around heatmap_foldchange, which generates a heatmap of showing the foldchange for a set of gene expressions between specified groups. Differential gene expression analysis between the different groups can be performed.

Usage

```
DO.HeatmapFC(
  sce_object,
  features,
  reference = NULL,
  assay_normalized = "RNA",
  group_by = "seurat_clusters",
  condition_key = "condition",
  groups_order = NULL,
  conditions_order = NULL,
  layer = NULL,
  figsize = c(5, 6),
  ax = NULL,
  swap_axes = TRUE,
  title = NULL,
  title_fontproperties = list(size = NULL, weight = NULL),
  palette = "RdBu_r",
  palette_conditions = "tab10",
  ticks_fontproperties = list(size = NULL, weight = NULL),
  xticks_rotation = NULL,
  yticks_rotation = NULL,
  vmin = NULL,
  vcenter = NULL,
  vmax = NULL,
  colorbar_legend_title = "Log2FC",
  groups_legend_title = "Comparison",
  group_legend_ncols = 1,
  path = NULL,
  filename = "Heatmap.svg",
  showP = TRUE,
  add_stats = TRUE,
  test = c("wilcox"),
  correction_method = c("fdr"),
  df_pvals = NULL,
  stats_x_size = NULL,
  square_x_size = NULL,
  pval_cutoff = 0.05,
  log2fc_cutoff = 0,
  linewidth = 0.1,
  color_axis_ratio = 0.15
)
```

Arguments

sce_object	A SingleCellExperiment or Seurat object containing expression data and metadata.
features	Character vector of gene names or metadata column names to be visualized.
reference	Reference condition used for fold-change calculation.
assay_normalized	Name of the assay containing normalized expression values (default: "RNA").
group_by	Metadata column defining the primary grouping variable (e.g. clusters).
condition_key	Metadata column defining the condition or comparison variable.
groups_order	Optional character vector specifying the order of groups in group_by.
conditions_order	Optional character vector specifying the order of conditions.
layer	Optional layer name to extract expression values from.
figsize	Numeric vector of length two specifying figure width and height.
ax	Optional matplotlib axis object (for Python backend usage).
swap_axes	Logical; whether to swap x- and y-axes.
title	Optional title for the heatmap.
title_fontproperties	Named list specifying font properties for the title (e.g. size, weight).
palette	Color palette used for the heatmap.
palette_conditions	Color palette used for condition annotations.
ticks_fontproperties	Named list specifying font properties for axis tick labels.
xticks_rotation	Rotation angle for x-axis tick labels.
yticks_rotation	Rotation angle for y-axis tick labels.
vmin	Minimum value for the color scale.
vcenter	Center value for the color scale.
vmax	Maximum value for the color scale.
colorbar_legend_title	Title for the color bar.
groups_legend_title	Title for the group legend.
group_legend_ncols	Number of columns in the group legend.
path	Optional path to save the output figure.
filename	Name of the output file.
showP	Logical; whether to display the plot.
add_stats	Logical; whether to add statistical annotations.

test	Statistical test to use (currently "wilcox").
correction_method	Multiple-testing correction method (currently "fdr").
df_pvals	Optional data frame containing precomputed p-values (groups x genes or genes x groups depending on axis orientation).
stats_x_size	Size of the statistical annotation symbol.
square_x_size	Size of the square annotation.
pval_cutoff	P-value significance threshold.
log2fc_cutoff	Minimum absolute log2 fold-change cutoff.
linewidth	Line width of heatmap cell borders.
color_axis_ratio	Relative size of the color bar axis.

Value

Depending on showP, returns the plot if set to TRUE or a dictionary with the axes.

Author(s)

Mariano Ruz Jurado & David Rodriguez Morales

Examples

```
sce_data <-
  readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

DO.HeatmapFC(
sce_object = sce_data,
features = c("HES4", "ISG15", "TNFRSF18", "TNFRSF4", "MMP23B"),
reference = NULL,
assay_normalized = "RNA",
group_by = "seurat_clusters",
condition_key = "condition",
groups_order = c("1", "2", "3", "4", "5", "6", "7", "8"),
conditions_order = NULL,
layer = NULL,

# Figure parameters
figsize = c(5, 6),
ax = NULL,
swap_axes = TRUE,
title = NULL,
title_fontproperties = list(size = NULL, weight = NULL),
palette = "RdBu_r",
palette_conditions = "tab10",
ticks_fontproperties = list(size = NULL, weight = NULL),
xticks_rotation = 45,
yticks_rotation = NULL,
vmin = NULL,
```

```
vcenter = NULL,
vmax = NULL,
colorbar_legend_title = "Log2FC",
groups_legend_title = "Comparison",
group_legend_ncols = 1,

# IO
path = NULL,
filename = "Heatmap.svg",
showP = FALSE,

#   # Statistics
add_stats = TRUE,
test = c("wilcox"),
correction_method = c("bonferroni"),
df_pvals = NULL,
stats_x_size = NULL,
square_x_size = NULL,
pval_cutoff = 0.05,
log2fc_cutoff = 0.0,

# Fx specific
linewidth = 0.1,
color_axis_ratio = 0.15
)
```

DO.Import *DO.Import*

Description

Imports and processes single-cell RNA-seq data from various formats (10x Genomics, CellBender, or CSV), performs quality control (QC), filtering , normalization, variable gene selection, and optionally detects doublets. Returns a merged and processed Seurat or SCE object ready for downstream analysis.

Usage

```
DO.Import(
  pathways,
  ids,
  minCellGenes = 5,
  FilterCells = TRUE,
  cut_mt = 0.05,
  min_counts = NULL,
  max_counts = NULL,
  min_genes = NULL,
  max_genes = NULL,
```

```
  low_quantile = NULL,
  high_quantile = NULL,
  DeleteDoublets = TRUE,
  include_rbs = TRUE,
  Seurat = TRUE,
  ...
)
```

Arguments

pathways	A character vector of paths to directories or files containing raw expression matrices.
ids	A character vector of sample identifiers, matching the order of pathways.
minCellGenes	Integer. Minimum number of cells a gene must be expressed in to be retained. Default is 5.
FilterCells	Logical. If TRUE, applies QC filtering on cells based on mitochondrial content, counts, and feature thresholds. Default is TRUE.
cut_mt	Numeric. Maximum allowed mitochondrial gene proportion per cell. Default is 0.05.
min_counts	Numeric. Minimum UMI count threshold (optional, used only if low_quantile is NULL).
max_counts	Numeric. Maximum UMI count threshold (optional, used only if high_quantile is NULL).
min_genes	Numeric. Minimum number of genes detected per cell to retain. Optional.
max_genes	Numeric. Maximum number of genes detected per cell to retain. Optional.
low_quantile	Numeric. Quantile threshold (0 to 1) to filter low UMI cells (used if min_counts is NULL).
high_quantile	Numeric. Quantile threshold (0 to 1) to filter high UMI cells (used if max_counts is NULL).
DeleteDoublets	Logical. If TRUE, doublets are detected and removed using scDblFinder. Default is TRUE.
include_rbs	Logical. If TRUE, calculates ribosomal gene content in addition to mitochondrial content. Default is TRUE.
Seurat	Logical. If TRUE, returns Seurat object otherwise SCE object.
...	Additional arguments passed to RunPCA().

Value

A merged Seurat or SCE object containing all samples, with normalization, QC, scaling, PCA, and optional doublet removal applied.

Author(s)

Mariano Ruz Jurado & David John

Examples

```
## Not run:
merged_obj <- DO.Import(
    pathways = c("path/to/sample1", "path/to/sample2"),
    ids = c("sample1", "sample2"),
    TenX = TRUE,
    CellBender = FALSE,
    minCellGenes = 5,
    FilterCells = TRUE,
    cut_mt = 0.05,
    min_counts = 1000,
    max_counts = 20000,
    min_genes = 200,
    max_genes = 6000,
    DeleteDoublets = TRUE
)

## End(Not run)
```

`DO.Integration` *DO.Integration SCE object integration directly*

Description

Integrates single-cell RNA-seq data directly from SingleCellExperiment or Seurat objects. Supports detection of variable genes , scaling, PCA, neighbor graph construction, clustering, and UMAP embedding, with multiple integration methods.

Usage

```
DO.Integration(
  sce_object,
  split_key = "orig.ident",
  HVG = FALSE,
  scale = FALSE,
  pca = FALSE,
  neighbors = TRUE,
  neighbors_dim = seq_len(50),
  clusters = TRUE,
  clusters_res = 0.3,
  clusters_algorithm = 4,
  umap = TRUE,
  umap_key = "UMAP",
  umap_dim = seq_len(50),
  integration_method = "CCAIntegration",
  selection_method = "vst",
  loess_span = 0.3,
```

```
  clip_max = "auto",
  num_bin = 20,
  binning_method = "equal_width",
  scale_max = 10,
  pca_key = "PCA",
  integration_key = "INTEGRATED.CCA",
  npcs = 50,
  verbose = FALSE,
  random_seed = 42
)
```

Arguments

sce_object	Seurat or SCE Object
split_key	Character. Column in meta data to split the samples by, default orig.ident
HVG	Logical. Perform detection of highly variable genes
scale	Logical. Perform scaling of the expression data
pca	Logical. Perform principal component analysis
neighbors	Logical. Perform Nearest-neighbor graph after integration
neighbors_dim	Numeric range. Dimensions of reduction to use as input
clusters	Logical. Perform clustering of cells
clusters_res	Numeric. Value of the resolution parameter, use a value above (below) 1.0 if you want to obtain a larger (smaller) number of communities.
clusters_algorithm	Numeric. Define the algorithm for clustering, default 4 for "Leiden"
umap	Logical. Runs the Uniform Manifold Approximation and Projection
umap_key	Character name for
umap_dim	Numeric range. Which dimensions to use as input features
integration_method	Character. Define the integration method, please check what versions are supported in Seurat::IntegrateLayers function
selection_method	Character. Default "vst". Options: "mean.var.plot", "dispersion"
loess_span	Numeric. Loess span parameter used when fitting the variance-mean relationship
clip_max	Character. After standardization values larger than clip.max will be set to clip.max; default is 'auto' which sets this value to the square root of the number of cells
num_bin	Numeric. Total number of bins to use in the scaled analysis (default is 20)
binning_method	Character. "equal_width": each bin is of equal width along the x-axis (default). Options: "equal_frequency":
scale_max	Numeric. Max value to return for scaled data. The default is 10.
pca_key	Character. Key name to save the pca result in

integration_key
: Character. Key name to save the integration result in

npcs
: Numeric. Total Number of PCs to compute and store (50 by default)

verbose
: Logical. Verbosity for all functions

random_seed
: parameter for random state initialisation

Value

integrated sce/seurat object

Author(s)

Mariano Ruz Jurado

Examples

```
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

DO.Integration(
    sce_object = sce_data,
    split_key = "orig.ident",
    HVG = TRUE,
    scale = TRUE,
    pca = TRUE,
    integration_method = "CCAIntegration"
)
```

DO.MultiDGE *DO.MultiDGE*

Description

Performs differential gene expression analysis using both single-cell and pseudo-bulk approaches across all annotated cell types. The single-cell method uses Seurat's `FindMarkers`, while pseudo-bulk testing uses `DESeq2` on aggregated expression profiles. Outputs a merged data frame with DGE statistics from both methods per condition and cell type.

Usage

```
DO.MultiDGE(
  sce_object,
  assay = "RNA",
  method_sc = "wilcox",
  group_by = "condition",
  annotation_col = "annotation",
  sample_col = "orig.ident",
```

```
  ident_ctrl = "ctrl",
  min_pct = 0,
  logfc_threshold = 0,
  only_pos = FALSE,
  min_cells_group = 3,
  ...
)
```

Arguments

sce_object	The seurat or SCE object
assay	Specified assay in Seurat or SCE object, default "RNA"
method_sc	method to use for single cell DEG analysis, see FindMarkers from Seurat for options, default "wilcox"
group_by	Column in meta data containing groups used for testing, default "condition"
annotation_col	Column in meta data containing information of cell type annotation
sample_col	Column in meta data containing information of sample annotation, default "orig.ident"
ident_ctrl	Name of the condition in group_by to test against as ctrl, default "ctrl"
min_pct	only test genes that are detected in a minimum fraction of min.pct cells in either of the two populations, default is 0
logfc_threshold	Limit testing to genes which show, on average, at least X-fold difference (log-scale) between the two groups of cells, default is 0.
only_pos	Only return positive markers, default FALSE
min_cells_group	Minimum number of cells in one of the groups, default 3
...	Additional arguments passed to FindMarkers function

Value

Dataframe containing statistics for each gene from the single cell and the Pseudobulk DGE approach.

Author(s)

Mariano Ruz Jurado

Examples

```
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))
DGE_result <- DO.MultiDGE(sce_data,
    sample_col = "orig.ident",
    method_sc = "wilcox",
    annotation_col = "annotation",
    ident_ctrl = "healthy"
)
```

DO.PyEnv *DO.PyEnv*

Description

Sets up or connects to a conda Python environment for use with DOtools. If no environment path is provided, it will create one at `~/.venv/DOtools` and install required Python packages: `scvi-tools`, `celltypist`, and `scanpro`.

Usage

```
DO.PyEnv(conda_path = NULL)
```

Arguments

`conda_path`	character string specifying the path to an existing or new conda environment.

Value

None

Examples

```
# Creates DOtools environment at ~/.venv/DOtools if it doesn't exist
DO.PyEnv()

# Use an existing conda environment at a custom location
# DO.PyEnv(conda_path = "~/miniconda3/envs/my_dotools_env")
```

DO.scVI *DO.scVI*

Description

This function will run the scVI Integration from the scVI python package. It includes all parameters from the actual python package and runs it by using an internal python script. The usage of a gpu is incorporated and highly recommended.

Usage

```
DO.scVI(
  sce_object,
  batch_key,
  layer_counts = "counts",
  layer_logcounts = "logcounts",
  categorical_covariates = NULL,
  continuos_covariates = NULL,
  n_hidden = 128,
  n_latent = 30,
  n_layers = 3,
  dispersion = "gene-batch",
  gene_likelihood = "zinb",
  get_model = FALSE
)
```

Arguments

sce_object	Seurat or SCE object with annotation in meta.data
batch_key	meta data column with batch information.
layer_counts	layer with counts. Raw counts are required.
layer_logcounts	layer with log-counts. Log-counts required for calculation of HVG.
categorical_covariates	list of meta data column names with categorical covariates for scVI inference.
continuos_covariates	list of meta data column names with continuous covariates for scVI inference.
n_hidden	number of hidden layers.
n_latent	dimensions of the latent space.
n_layers	number of layers.
dispersion	dispersion mode for scVI.
gene_likelihood	gene likelihood.
get_model	return the trained model.

Value

Seurat or SCE Object with dimensionality reduction from scVI

Examples

```
## Not run:
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

# Run scVI using the 'orig.ident' column as the batch key
```

```
sce_data <- DO.scVI(sce_data, batch_key = "orig.ident")

## End(Not run)
```

DO.SplitBarGSEA *DO Bar plot for GSEA df result*

Description

This function generates a split barplot. This is a plot where the top 10 Go terms are shown, sorted based on a column ('col_split'). Two conditions are shown at the same time. One condition is shown in the positive axis, while the other in the negative one. The condition to be shown as positive is set with 'pos_col'.

The GO terms will be shown inside the bars, if the term is too long, using 'cutoff', you can control the maximum number of characters per line.

Pre-filter of the dataframe to contain significant Terms is recommended

Usage

```
DO.SplitBarGSEA(
  df_GSEA,
  term_col,
  col_split,
  cond_col,
  pos_cond,
  cutoff = 40,
  log10_transform = TRUE,
  figsize = c(12, 8),
  topN = 10,
  colors_pairs = c("sandybrown", "royalblue"),
  alpha_colors = 0.3,
  path = NULL,
  spacing = 5,
  txt_size = 12,
  filename = "SplitBar.svg",
  title = "Top 10 GO Terms in each Condition: ",
  showP = FALSE,
  celltype = "all"
)
```

Arguments

df_GSEA	dataframe with the results of a gene set enrichment analysis
term_col	column in the dataframe that contains the terms
col_split	column in the dataframe that will be used to sort and split the plot

cond_col	column in the dataframe that contains the condition information
pos_cond	condition that will be shown in the positive side of the plot
cutoff	maximum number of characters per line
log10_transform	if col_split contains values between 0 and 1, assume they are pvals and apply a -log10 transformation
figsize	figure size
topN	how many terms are shown
colors_pairs	colors for each condition (1st color –> negative axis; 2nd color –> positive axis)
alpha_colors	alpha value for the colors of the bars
path	path to save the plot
spacing	space to add between bars and origin. It is a percentage value , indicating that the bars start at 5 % of the maximum X axis value.
txt_size	size of the go terms text
filename	filename for the plot
title	title of the plot
showP	if False, the axis is return
celltype	vector with cell types you want to subset for, use "all" for all celltypes contained in the dataframe column "celltype"

Value

: None or the axis

Author(s)

Mariano Ruz Jurado

Examples

```
library(enrichR)

sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))
DGE_result <- DO.MultiDGE(sce_data,
    sample_col = "orig.ident",
    method_sc = "wilcox",
    annotation_col = "annotation",
    ident_ctrl = "healthy"
)
DGE_result <- DGE_result[DGE_result$celltype == "CD4_T_cells", ]

result_GO <- DO.enrichR(
    df_DGE = DGE_result,
    gene_column = "gene",
    pval_column = "p_val_SC_wilcox",
```

```
    log2fc_column = "avg_log2FC_SC_wilcox",
    pval_cutoff = 0.05,
    log2fc_cutoff = 0.25,
    path = NULL,
    filename = "",
    species = "Human",
    go_catgs = "GO_Biological_Process_2023"
)

result_GO$celltype <- "CM1"

# Run SplitBarGSEA visualisation
DO.SplitBarGSEA(
    df_GSEA = result_GO,
    term_col = "Term",
    col_split = "Combined.Score",
    cond_col = "State",
    pos_cond = "enriched",
    cutoff = 40,
    log10_transform = TRUE,
    figsize = c(12, 8),
    topN = 10,
    colors_pairs = c("sandybrown", "royalblue"),
    alpha_colors = 0.3,
    path = NULL,
    spacing = 5,
    txt_size = 12,
    filename = "SplitBar.svg",
    title = "Top 10 GO Terms in each Condition: ",
    showP = FALSE,
    celltype = "all"
)
```

`DO.Subset` *DO.Subset*

Description

Creates a subset of a Seurat or SCE object based on either categorical or numeric thresholds in metadata. Allows for subsetting by specifying the ident column, group name, or threshold criteria. Ideal for extracting specific cell populations or clusters based on custom conditions. Returns a new Seurat or SCE object containing only the subsetted cells and does not come with the Seuratv5 subset issue. Please be aware that right now, after using this function the subset might be treated with Seuv5=False in other functions.

Usage

```
DO.Subset(
  sce_object,
```

```
  assay = "RNA",
  ident,
  ident_name = NULL,
  ident_thresh = NULL
)
```

Arguments

sce_object	The seurat or SCE object
assay	assay to subset by
ident	meta data column to subset for
ident_name	name of group of barcodes in ident of subset for
ident_thresh	numeric thresholds as character, e.g ">5" or c(">5", "<200"), to subset barcodes in ident

Value

a subsetted Seurat or SCE object

Author(s)

Mariano Ruz Jurado

Examples

```
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

sce_data_sub <- DO.Subset(
    sce_object = sce_data,
    ident = "condition",
    ident_name = "healthy"
)
```

DO.TransferLabel *DO.TransferLabel*

Description

Transfers cell-type annotations from a re-annotated subset of a Seurat or SCE object back to the full Seurat or SCE object. This is useful when clusters have been refined or re-labeled in a subset and need to be reflected in the original object.

Usage

```
DO.TransferLabel(sce_object, Subset_obj, annotation_column, subset_annotation)
```

Arguments

sce_object	Seurat or SCE object with annotation in meta.data
Subset_obj	subsetted Seurat or SCE object with re-annotated clusters
annotation_column	column name in meta.data with annotation
subset_annotation	column name in meta.data with annotation in the subsetted object

Value

Seurat or SCE Object with transfered labels

Examples

```
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

sce_data <- DO.TransferLabel(sce_data,
    sce_data,
    annotation_column = "annotation",
    subset_annotation = "annotation"
)
```

DO.UMAP *DO.UMAP*

Description

Creates a polished UMAP plot using Seurat's DimPlot or FeaturePlot functions. In addition a density plot can be made in a similar way to nebulosa R package. It allows customization of colors, labels, and other plot elements for better visualisation. The function handles both cluster-based visualisations and gene-based visualisations in a UMAP plot. Ideal for refining UMAP outputs with added flexibility and enhanced presentation.

Usage

```
DO.UMAP(
  sce_object,
  features = NULL,
  group.by = "seurat_clusters",
  FeaturePlot = FALSE,
  DensityPlot = FALSE,
  reduction = NULL,
  dims = c(1, 2),
  layer = NULL,
  umap_colors = NULL,
```

```
  text_size = 14,
  label = TRUE,
  order = TRUE,
  plot.title = TRUE,
  legend.position = "none",
  method = c("ks", "wkde"),
  ...
)
```

Arguments

sce_object	The seurat or SCE object
features	features for Featureplot
group.by	grouping of plot in DImplot and defines in featureplot the labels
FeaturePlot	Is it going to be a FeaturePlot?
DensityPlot	Is it going to be a DensityPlot?
reduction	reduction to use
dims	Dimensions to plot, must be a two-length numeric vector specifying x- and y-dimensions description
layer	Layer to use for DensityPlot, default data
umap_colors	what colors to use for UMAP, specify as vector
text_size	Size of text
label	label the clusters on the plot by group.by column
order	Boolean determining whether to plot cells in order of expression.
plot.title	title for UMAP
legend.position	specify legend position
method	Kernel density estimation method, can be "ks" or "wkde"
...	Further arguments passed to DimPlot, FeaturePlot or DensityPlot functions

Value

Plot with Refined colors and axes

Author(s)

Mariano Ruz Jurado

Examples

```
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

DO.UMAP(
    sce_object = sce_data,
```

```
    group.by = "seurat_clusters"
)

DO.UMAP(
    sce_object = sce_data,
    FeaturePlot = TRUE,
    features = c("BAG2", "CD74")
)

DO.UMAP(
    sce_object = sce_data,
    DensityPlot = TRUE,
    features = c("CD74")
)
```

DO.VlnPlot *Violin Graph with wilcox test on single cell level*

Description

Creates a violin plot to compare gene expression across different conditions or groups within a Seurat object. It incorporates different tests to evaluate statistical differences between conditions. The plot can be customized with options for data transformation, jitter display, and significance annotations. The function also supports multiple conditions and allows for visualisation of statistical results from different test.

Usage

```
DO.VlnPlot(
  sce_object,
  Feature,
  ListTest = NULL,
  returnValues = FALSE,
  ctrl.condition = NULL,
  group.by = "condition",
  group.by.2 = NULL,
  geom_jitter_args = c(0.2, 0.25, 0.25),
  geom_jitter_args_group_by2 = c(0.1, 0.1, 1),
 vector_colors = c("#1f77b4", "#ea7e1eff", "royalblue4", "tomato2", "darkgoldenrod",
    "palegreen4", "maroon", "thistle3"),
  test_use = "wilcox",
  correction_method = "fdr",
  p_values = NULL,
  y_title = "log(nUMI)",
  stat_pos_mod = 1.15,
  hjust_test = 0.8,
  vjust_test = 2,
```

```
  size_test = 3.33,
  step_mod = 0,
  hjust_test_2 = 0.5,
  vjust_test_2 = 0,
  sign_bar = 0.8,
  random_seed = 42
)
```

Arguments

sce_object	combined SCE object or Seurat
Feature	name of the feature
ListTest	List for which conditions wilcox will be performed, if NULL always CTRL group against everything
returnValues	return df.melt.sum data frame containing means and SEM for the set group
ctrl.condition	set your ctrl condition, relevant if running with empty comparison List
group.by	select the seurat sce_object slot where your conditions can be found, default conditon
group.by.2	relevant for multiple group testing, e.g. for each cell type the test between each of them in two conditions provided
geom_jitter_args	vector for dots visualisation in vlnplot: size, width, alpha value
geom_jitter_args_group_by2	controls the jittering of points if group.by.2 is specified
vector_colors	specify a minimum number of colours as you have entries in your condition, default 2
test_use	perform one of c("wilcox", "wilcox_limma", "bimod", "t", "negbinom", "poisson", "LR", "MAST", "DESeq2", "none"). default "wilcox"
correction_method	correction for p-value calculation. One of c("BH", "bonferroni", "holm", "BY", "fdr", "none"). default "fdr"
p_values	Manually providing p-values for plotting, be aware of group size and if necessary make your test return the same amount of values
y_title	specify title on the y axis. default "log(nUMI)"
stat_pos_mod	value for modifiyng statistics height
hjust_test	value for adjusting height of the text
vjust_test	value for vertical of text
size_test	value for size of text of statistical test
step_mod	value for defining the space between one test and the next one
hjust_test_2	value for adjusting height of the text, with group.by.2 specified
vjust_test_2	value for vertical of text, with group.by.2 specified
sign_bar	adjusts the sign_bar with group.by.2 specified
random_seed	parameter for random state initialisation

Value

a ggplot or a list used data frames

Author(s)

Mariano Ruz Jurado

Examples

```
sce_data <-
    readRDS(system.file("extdata", "sce_data.rds", package = "DOtools"))

ListTest <- list()
ListTest[[1]] <- c("healthy", "disease")

DO.VlnPlot(
    sce_object = sce_data,
    Feature = "NKG7",
    ListTest = ListTest,
    ctrl.condition = "healthy",
    group.by = "condition"
)
```

DOtools *DOtools: A Toolkit for scRNA Data Analysis*

Description

The 'DOtools' package provides a set of functions for advanced data processing, visualisation, and statistical analysis in Seurat objects. It includes functions for cell-type prediction, reclustering, creating polished UMAP plots, subsetting Seurat objects, and various statistical analyses like Wilcoxon tests and SEM graphs.

Details

This package includes the following functions:

- DO.BoxPlot: A function for creating box plots with Wilcoxon test results.
- DO.CellTypist: A function for running CellTypist on Seurat and SCE objects to predict cell types.
- DO.DietSCE: A function for diet-based analysis of Seurat and SCE objects.
- DO.Dotplot: A function for creating dot plots for visualizing gene expression.
- DO.FullRecluster: A function for fine-grained reclustering of Seurat and SCE objects.
- DO.BarplotClustert: A function for generating mean and SEM graphs for cluster-based analysis with t-tests.

- DO.Barplot: A function for generating mean and SEM graphs with a statistical test indicating significance.
- DO.Subset: A function for subsetting Seurat and SCE objects based on metadata.
- DO.UMAP: A function for creating polished UMAP plots using either DimPlot or FeaturePlot.
- DO.VlnPlot: A function for generating violin plots with Wilcoxon test results.
- DO.CellComposition: A function for visualizing and statistically analyzing cell-type composition changes across conditions using the Scanpro Python package, with support for bootstrapping, proportion plots, and customizable output.
- DO.Import: A function for building a merged Seurat and SCE object from 10x software output, or directly from provided tables.
- DO.Integration: A function for integrating SCE objects and Seurat objects with the provided method.
- DO.CellBender: A function for running CellBender in a virtual conda env with provided raw count h5 files.
- DO.SplitBarGSEA: A function for viusalizing GSEA result from a provided df from e.g. metascape
- DO.scVI: A function for running the scVI Integration implemented in scvi-tools.
- DO.TransferLabel: A function for transfering annotation from a subseted object to the original seurat and SCE object.
- DO.PyEnv: A function for creating a conda envrionment holding all python packages needed for some functions.
- DO.Correlation: A function for creating a correlation plot between provided samples in the category specified.
- DO.Heatmap: A function for generating Heat maps on gene expression data.
- DO.HeatmapFC: A function for generating Heat maps showing foldchanges in expression between specified conditions.
- DO.MultiDGE: A function for calculating DEGs on a single cell and speudo bulk level.
- DO.EvalIntegration: A function for calculating sciB metrics on integration embeddings
- dot-Do.BarcodeRanks: A function for estimating the number of expected cells and droplets.
- dot-QC.Vlnplot: A function for estimating the number of expected cells and droplets.
- dot-run_kbet: A self-contained call for running the kBET function.
- dot-kBet_fct: A self-contained version of the kBET algorithm.

Value

This is a package-level documentation file and does not return a value.

Author(s)

Mariano Ruz Jurado, David Rodriguez Morales

See Also

DO.BoxPlot, DO.CellTypist, DO.DietSCE, DO.Dotplot, DO.FullRecluster, DO.BarplotClustert, DO.Barplot, DO.Subset, DO.UMAP, DO.VlnPlot, DO.Import,DO.Integration, DO.CellBender, DO.SplitBarGSEA, DO.scVI, DO.TransferLabel,DO.Heatmap,DO.HeatmapFC, DO.PyEnv, DO.Correlation, DO.MultiDGE,DO.EvalIntegration, DO.TransferLabel, dot-Do.BarcodeRanks, dot-QC.Vlnplot, dot-run_kbet, dot-kBet_fct

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