Package 'Seurat'

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Title Tools for Single Cell Genomics

Description A toolkit for quality control, analysis, and exploration of single cell RNA sequencing data. 'Seurat' aims to enable users to identify and interpret sources of heterogeneity from single cell transcriptomic measurements, and to integrate diverse types of single cell data. See Satija R, Farrell J, Gennert D, et al (2015) <doi:10.1038/nbt.3192>, Macosko E, Basu A, Satija R, et al (2015) <doi:10.1016/j.cell.2015.05.002>, and Butler A and Satija R (2017) <doi:10.1101/164889> for more details.

URL http://www.satijalab.org/seurat, https://github.com/satijalab/seurat

BugReports https://github.com/satijalab/seurat/issues

Depends R (>= 3.2.0), ggplot2, cowplot, Matrix,

SystemRequirements Java (>= 1.6)

Imports methods, ROCR, stringr, mixtools, lars, ica, tsne, Rtsne, fpc, ape, VGAM, pbapply, igraph, FNN, caret, dplyr, RColorBrewer, MASS, irlba, reshape2, gridExtra, gplots, gdata, Rcpp, tclust, ranger, NMF, dtw, SDMTools, plotly, diffusionMap, Hmisc, tidyr, ggjoy

LinkingTo Rcpp, RcppEigen, RcppProgress

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LazyData true

Collate 'RcppExports.R' 'seurat.R' 'cluster_determination.R' 'cluster_determination_internal.R' 'cluster_validation.R' 'data.R' 'deprecated_functions.R' 'differential_expression.R' 'differential_expression_internal.R' 'dimensional_reduction.R' 'dimensional_reduction_internal.R' 'dimensional_reduction_utilities.R' 'interaction.R' 'jackstraw.R' 'jackstraw_internal.R' 'multi_modal.R' 'plotting.R' 'plotting_internal.R' 'plotting_utilities.R' 'preprocessing.R' 'preprocessing_internal.R'

'printing_utilities.R' 'scoring.R' 'snn.R' 'spatial.R'
'spatial_internal.R' 'tSNE_project.R' 'utilities.R'
'utilities_internal.R'

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Suggests testthat, SummarizedExperiment, MAST, DESeq2

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R topics documented:

AddImputedScore *Calculate imputed expression values*

Description

Uses L1-constrained linear models (LASSO) to impute single cell gene expression values.

Usage

```
AddImputedScore(object, genes.use = NULL, genes.fit = NULL, s.use = 20,
  do.print = FALSE, gram = TRUE)
```

Arguments

object	Seurat object
genes.use	A vector of genes (predictors) that can be used for building the LASSO models.
genes.fit	A vector of genes to impute values for
s.use	Maximum number of steps taken by the algorithm (lower values indicate a greater degree of smoothing)
do.print	Print progress (output the name of each gene after it has been imputed).
gram	The use.gram argument passed to lars

Value

Returns a Seurat object where the imputed values have been added to object@imputed

Examples

```
pbmc_small <- AddImputedScore(object = pbmc_small, genes.fit = "MS4A1")
```

AddMetaData *Add Metadata*

Description

Adds additional data for single cells to the Seurat object. Can be any piece of information associated with a cell (examples include read depth, alignment rate, experimental batch, or subpopulation identity). The advantage of adding it to the Seurat object is so that it can be analyzed/visualized using FetchData, VlnPlot, GenePlot, SubsetData, etc.

Usage

```
AddMetaData(object, metadata, col.name = NULL)
```

Arguments

object	Seurat object
metadata	Data frame where the row names are cell names (note : these must correspond exactly to the items in object@cell.names), and the columns are additional metadata items.
col.name	Name for metadata if passing in single vector of information

Value

Seurat object where the additional metadata has been added as columns in object@meta.data

Examples

```
cluster_letters <- LETTERS[pbmc_small@ident]
pbmc_small <- AddMetaData(
  object = pbmc_small,
  metadata = cluster_letters,
  col.name = 'letter.idents'
)
head(x = pbmc_small@meta.data)
```

AddModuleScore *Calculate module scores for gene expression programs in single cells*

Description

Calculate the average expression levels of each program (cluster) on single cell level, subtracted by the aggregated expression of control gene sets. All analyzed genes are binned based on averaged expression, and the control genes are randomly selected from each bin.

Usage

```
AddModuleScore(object, genes.list = NULL, genes.pool = NULL, n.bin = 25,
  seed.use = 1, ctrl.size = 100, use.k = FALSE, enrich.name = "Cluster")
```

Arguments

object	Seurat object
genes.list	Gene expression programs in list
genes.pool	List of genes to check expression levels agains, defaults to rownames(x = object@data)
n.bin	Number of bins of aggregate expression levels for all analyzed genes
seed.use	Random seed for sampling
ctrl.size	Number of control genes selected from the same bin per analyzed gene
use.k	Use gene clusters returned from DoKMeans()
enrich.name	Name for the expression programs

Value

Returns a Seurat object with module scores added to object@meta.data

References

Tirosh et al, Science (2016)

Examples

```
cd_genes <- list(c(
  'CD79B',
  'CD79A',
  'CD19',
  'CD180',
  'CD200',
  'CD3D',
  'CD2',
  'CD3E',
  'CD7',
  'CD8A',
  'CD14',
  'CD1C',
  'CD68',
  'CD9',
  'CD247'
))
pbmc_small <- AddModuleScore(
  object = pbmc_small,
  genes.list = cd_genes,
  ctrl.size = 5,
  enrich.name = 'CD_Genes'
)
head(x = pbmc_small@meta.data)
```

AddSamples *Add samples into existing Seurat object.*

Description

Add samples into existing Seurat object.

Usage

```
AddSamples(object, new.data, project = NULL, min.cells = 0, min.genes = 0,
  is.expr = 0, do.normalize = TRUE, scale.factor = 10000,
  do.scale = FALSE, do.center = FALSE, names.field = 1,
  names.delim = "_", meta.data = NULL, save.raw = TRUE,
  add.cell.id = NULL)
```

Arguments

object	Seurat object
new.data	Data matrix for samples to be added
project	Project name (string)
min.cells	Include genes with detected expression in at least this many cells
min.genes	Include cells where at least this many genes are detected
is.expr	Expression threshold for 'detected' gene
do.normalize	Normalize the data after merging. Default is TRUE. If set, will perform the same normalization strategy as stored in the object
scale.factor	scale factor in the log normalization
do.scale	In object@scale.data, perform row-scaling (gene-based z-score)
do.center	In object@scale.data, perform row-centering (gene-based centering)
names.field	For the initial identity class for each cell, choose this field from the cell's column name
names.delim	For the initial identity class for each cell, choose this delimiter from the cell's column name
meta.data	Additional metadata to add to the Seurat object. Should be a data frame where the rows are cell names, and the columns are additional metadata fields
save.raw	TRUE by default. If FALSE, do not save the unmodified data in object@raw.data which will save memory downstream for large datasets
add.cell.id	String to be appended to the names of all cells in new.data. E.g. if add.cell.id = "rep1", "cell1" becomes "cell1.rep1"

Examples

```
pbmc1 <- SubsetData(object = pbmc_small, cells.use = pbmc_small@cell.names[1:40])
pbmc1
pbmc2 <- SubsetData(object = pbmc_small, cells.use = pbmc_small@cell.names[41:80])
pbmc2_data <- pbmc2@data
dim(pbmc2_data)
pbmc_added <- AddSamples(object = pbmc1, new.data = pbmc2_data)
pbmc_added
```

AddSmoothedScore *Calculate smoothed expression values*

Description

Smooths expression values across the k-nearest neighbors based on dimensional reduction

Usage

```
AddSmoothedScore(object, genes.fit = NULL, dim.1 = 1, dim.2 = 2,
  reduction.use = "tsne", k = 30, do.log = FALSE, do.print = FALSE)
```

Arguments

object	Seurat object
genes.fit	Genes to calculate smoothed values for
dim.1	Dimension 1 to use for dimensional reduction
dim.2	Dimension 2 to use for dimensional reduction
reduction.use	Dimensional reduction to use
k	k-param for k-nearest neighbor calculation. 30 by default
do.log	Whether to perform smoothing in log space. Default is false.
do.print	Print progress (output the name of each gene after it has been imputed).

Examples

```
pbmc_small <- AddSmoothedScore(object = pbmc_small, genes.fit = "MS4A1", reduction.use = "pca")
```

AlignSubspace *Align subspaces using dynamic time warping (DTW)*

Description

Aligns subspaces so that they line up across grouping variable (only implemented for case with 2 categories in grouping.var)

Usage

```
AlignSubspace(object, reduction.type, grouping.var, dims.align,
  num.genes = 30, show.plots = FALSE)
```

Arguments

object	Seurat object
reduction.type	reduction to align scores for
grouping.var	Name of the grouping variable for which to align the scores
dims.align	Dims to align, default is all
num.genes	Number of genes to use in construction of "metagene"
show.plots	show debugging plots

Value

Returns Seurat object with the dims aligned, stored in object@dr$reduction.type.aligned

Examples

```
## Not run:
pbmc_small
# Requires CCA to have previously been run
# As CCA requires two datasets, we will split our test object into two just for this example
pbmc1 <- SubsetData(pbmc_small,cells.use = pbmc_small@cell.names[1:40])
pbmc2 <- SubsetData(pbmc_small,cells.use = pbmc_small@cell.names[41:80])
pbmc1@meta.data$group <- "group1"
pbmc2@meta.data$group <- "group2"
pbmc_cca <- RunCCA(pbmc1,pbmc2)
pbmc_cca <- AlignSubspace(pbmc_cca,reduction.type = "cca", grouping.var = "group", dims.align = 1:2)

## End(Not run)
```

AssessNodes *Assess Internal Nodes*

Description

Method for automating assessment of tree splits over all internal nodes, or a provided list of internal nodes. Uses AssessSplit() for calculation of Out of Bag error (proxy for confidence in split).

Usage

```
AssessNodes(object, node.list, all.below = FALSE)
```

Arguments

object	Seurat object
node.list	List of internal nodes to assess and return
all.below	If single node provided in node.list, assess all splits below (and including) provided node .

Value

Returns the Out of Bag error for a random forest classifiers trained on each internal node split or each split provided in the node list.

Examples

```
pbmc_small
pbmc_small <- FindClusters(object = pbmc_small, reduction.type = "pca",
                           dims.use = 1:10, resolution = 1.1, save.SNN = TRUE)
pbmc_small <- BuildClusterTree(pbmc_small, reorder.numeric = TRUE, do.reorder = TRUE)
AssessNodes(pbmc_small)
```

AssessSplit *Assess Cluster Split*

Description

Method for determining confidence in specific bifurcations in the cluster tree. Use the Out of Bag (OOB) error of a random forest classifier to judge confidence.

Usage

```
AssessSplit(object, node, cluster1, cluster2, print.output = TRUE, ...)
```

Arguments

object	Seurat object
node	Node in the cluster tree in question
cluster1	First cluster to compare
cluster2	Second cluster to compare
print.output	Print the OOB error for the classifier
...	Arguments passed on to BuildRFClassifier **training.genes** Vector of genes to build the classifier on **training.classes** Vector of classes to build the classifier on **verbose** Additional progress print statements

Value

Returns the Out of Bag error for a random forest classifier trained on the split from the given node

Examples

```
pbmc_small
pbmc_small <- FindClusters(object = pbmc_small, reduction.type = "pca",
                           dims.use = 1:10, resolution = 1.1, save.SNN = TRUE)
pbmc_small <- BuildClusterTree(pbmc_small, reorder.numeric = TRUE, do.reorder = TRUE)
# Assess based on a given node
AssessSplit(pbmc_small, node = 11)
# Asses based on two given clusters (or vectors of clusters)
AssessSplit(pbmc_small, cluster1 = 5, cluster2 = 6)
AssessSplit(pbmc_small, cluster1 = 4, cluster2 = c(5, 6))
```

AverageDetectionRate *Probability of detection by identity class*

Description

For each gene, calculates the probability of detection for each identity class.

Usage

```
AverageDetectionRate(object, thresh.min = 0)
```

Arguments

object	Seurat object
thresh.min	Minimum threshold to define 'detected' (log-scale)

Value

Returns a matrix with genes as rows, identity classes as columns.

Examples

```
head(AverageDetectionRate(object = pbmc_small))
```

AverageExpression *Averaged gene expression by identity class*

Description

Returns gene expression for an 'average' single cell in each identity class

Usage

```
AverageExpression(object, genes.use = NULL, return.seurat = FALSE,
  add.ident = NULL, use.scale = FALSE, use.raw = FALSE,
  show.progress = TRUE, ...)
```

Arguments

object	Seurat object
genes.use	Genes to analyze. Default is all genes.
return.seurat	Whether to return the data as a Seurat object. Default is false.
add.ident	Place an additional label on each cell prior to averaging (very useful if you want to observe cluster averages, separated by replicate, for example).
use.scale	Use scaled values for gene expression
use.raw	Use raw values for gene expression
show.progress	Show progress bar (default is T)
...	Arguments to be passed to methods such as Seurat

Details

Output is in log-space, but averaging is done in non-log space.

Value

Returns a matrix with genes as rows, identity classes as columns.

Examples

```
head(AverageExpression(object = pbmc_small))
```

AveragePCA *Average PCA scores by identity class*

Description

Returns the PCA scores for an 'average' single cell in each identity class

Usage

```
AveragePCA(object)
```

Arguments

object	Seurat object

Value

Returns a matrix with genes as rows, identity classes as columns

Examples

```
head(AveragePCA(object = pbmc_small))
```

BlackAndWhite *A black and white color palette*

Description

A black and white color palette

Usage

```
BlackAndWhite(...)
```

Arguments

...	Extra parameters to CustomPalette

Value

A color palette

See Also

CustomPalette

Examples

```
df <- data.frame(x = rnorm(n = 100, mean = 20, sd = 2), y = rbinom(n = 100, size = 100, prob = 0.2))
plot(df, col = BlackAndWhite())
```

BuildClusterTree *Phylogenetic Analysis of Identity Classes*

Description

Constructs a phylogenetic tree relating the 'average' cell from each identity class. Tree is estimated based on a distance matrix constructed in either gene expression space or PCA space.

Usage

```
BuildClusterTree(object, genes.use = NULL, pcs.use = NULL, SNN.use = NULL,
  do.plot = TRUE, do.reorder = FALSE, reorder.numeric = FALSE)
```

Arguments

object	Seurat object
genes.use	Genes to use for the analysis. Default is the set of variable genes (object@var.genes). Assumes pcs.use=NULL (tree calculated in gene expression space)
pcs.use	If set, tree is calculated in PCA space, using the eigenvalue-WeightedEucleideanDist distance across these PC scores.
SNN.use	If SNN is passed, build tree based on SNN graph connectivity between clusters
do.plot	Plot the resulting phylogenetic tree
do.reorder	Re-order identity classes (factor ordering), according to position on the tree. This groups similar classes together which can be helpful, for example, when drawing violin plots.
reorder.numeric	Re-order identity classes according to position on the tree, assigning a numeric value ('1' is the leftmost node)

Details

Note that the tree is calculated for an 'average' cell, so gene expression or PC scores are averaged across all cells in an identity class before the tree is constructed.

Value

A Seurat object where the cluster tree is stored in object@cluster.tree[[1]]

Examples

```
pbmc_small
pbmc_small <- BuildClusterTree(pbmc_small, do.plot = FALSE)
```

BuildRFClassifier *Build Random Forest Classifier*

Description

Train the random forest classifier

Usage

```
BuildRFClassifier(object, training.genes = NULL, training.classes = NULL,
  verbose = TRUE, ...)
```

Arguments

object	Seurat object on which to train the classifier
training.genes	Vector of genes to build the classifier on
training.classes	Vector of classes to build the classifier on
verbose	Additional progress print statements
...	additional parameters passed to ranger

Value

Returns the random forest classifier

Examples

```
pbmc_small
# Builds the random forest classifier to be used with ClassifyCells
# Useful if you want to use the same classifier with several sets of new data
classifier <- BuildRFClassifier(pbmc_small, training.classes = pbmc_small@ident)
```

BuildSNN *SNN Graph Construction*

Description

Constructs a Shared Nearest Neighbor (SNN) Graph for a given dataset. We first determine the k-nearest neighbors of each cell (defined by k.param * k.scale). We use this knn graph to construct the SNN graph by calculating the neighborhood overlap (Jaccard distance) between every cell and its k.param * k.scale nearest neighbors (defining the neighborhood for each cell as the k.param nearest neighbors).

Usage

```
BuildSNN(object, genes.use = NULL, reduction.type = "pca",
  dims.use = NULL, k.param = 10, k.scale = 10, plot.SNN = FALSE,
  prune.SNN = 1/15, print.output = TRUE, distance.matrix = NULL,
  force.recalc = FALSE)
```

Arguments

object	Seurat object
genes.use	A vector of gene names to use in construction of SNN graph if building directly based on expression data rather than a dimensionally reduced representation (i.e. PCs).
reduction.type	Name of dimensional reduction technique to use in construction of SNN graph. (e.g. "pca", "ica")

dims.use	A vector of the dimensions to use in construction of the SNN graph (e.g. To use the first 10 PCs, pass 1:10)
k.param	Defines k for the k-nearest neighbor algorithm
k.scale	Granularity option for k.param
plot.SNN	Plot the SNN graph
prune.SNN	Sets the cutoff for acceptable Jaccard distances when computing the neighborhood overlap for the SNN construction. Any edges with values less than or equal to this will be set to 0 and removed from the SNN graph. Essentially sets the strigency of pruning (0 — no pruning, 1 — prune everything).
print.output	Whether or not to print output to the console
distance.matrix	Build SNN from distance matrix (experimental)
force.recalc	Force recalculation of SNN.

Value

Returns the object with object@snn filled

Examples

```
pbmc_small
# Compute an SNN on the gene expression level
pbmc_small <- BuildSNN(pbmc_small, genes.use = pbmc_small@var.genes)

# More commonly, we build the SNN on a dimensionally reduced form of the data
# such as the first 10 principle components.

pbmc_small <- BuildSNN(pbmc_small, reduction.type = "pca", dims.use = 1:10)
```

CalcVarExpRatio *Calculate the ratio of variance explained by ICA or PCA to CCA*

Description

Calculate the ratio of variance explained by ICA or PCA to CCA

Usage

```
CalcVarExpRatio(object, reduction.type = "pca", grouping.var, dims.use)
```

Arguments

object	Seurat object
reduction.type	type of dimensional reduction to compare to CCA (pca, pcafast, ica)
grouping.var	variable to group by
dims.use	Vector of dimensions to project onto (default is the 1:number stored for cca)

Value

Returns Seurat object with ratio of variance explained stored in object@meta.data$var.ratio

Examples

```
pbmc_small
# Requires CCA to have previously been run
# As CCA requires two datasets, we will split our test object into two just for this example
pbmc1 <- SubsetData(pbmc_small,cells.use = pbmc_small@cell.names[1:40])
pbmc2 <- SubsetData(pbmc_small,cells.use = pbmc_small@cell.names[41:80])
pbmc1@meta.data$group <- "group1"
pbmc2@meta.data$group <- "group2"
pbmc_cca <- RunCCA(pbmc1,pbmc2)
pbmc_cca <- CalcVarExpRatio(pbmc_cca,reduction.type = "pca", grouping.var = "group", dims.use = 1:5)
```

CaseMatch *Match the case of character vectors*

Description

Match the case of character vectors

Usage

```
CaseMatch(search, match)
```

Arguments

search	A vector of search terms
match	A vector of characters whose case should be matched

Value

Values from search present in match with the case of match

Examples

```
cd_genes <- c('Cd79b', 'Cd19', 'Cd200')
CaseMatch(search = cd_genes, match = rownames(x = pbmc_small@raw.data))
```

cc.genes *Cell cycle genes*

Description

A list of genes used in cell-cycle regression

Usage

```
cc.genes
```

Format

A list of two vectors

s.genes Genes associated with S-phase

g2m.genes Genes associated with G2M-phase

Source

http://science.sciencemag.org/content/352/6282/189

CellCycleScoring *Score cell cycle phases*

Description

Score cell cycle phases

Usage

```
CellCycleScoring(object, g2m.genes, s.genes, set.ident = FALSE)
```

Arguments

object	A Seurat object
g2m.genes	A vector of genes associated with G2M phase
s.genes	A vector of genes associated with S phases
set.ident	If true, sets identity to phase assignments Stashes old identities in 'old.ident'

Value

A Seurat object with the following columns added to object@meta.data: S.Score, G2M.Score, and Phase

See Also

AddModuleScore

Examples

```
## Not run:
# pbmc_small doesn't have any cell-cycle genes
# To run CellCycleScoring, please use a dataset with cell-cycle genes
# An example is available at http://satijalab.org/seurat/cell_cycle_vignette.html
pbmc_small <- CellCycleScoring(
  object = pbmc_small,
  g2m.genes = cc.genes$g2m.genes,
  s.genes = cc.genes$s.genes
)
head(x = pbmc_small@meta.data)

## End(Not run)
```

CellPlot *Cell-cell scatter plot*

Description

Creates a plot of scatter plot of genes across two single cells

Usage

```
CellPlot(object, cell1, cell2, gene.ids = NULL, col.use = "black",
  nrpoints.use = Inf, pch.use = 16, cex.use = 0.5, do.hover = FALSE,
  do.identify = FALSE, ...)
```

Arguments

object	Seurat object
cell1	Cell 1 name (can also be a number, representing the position in object@cell.names)
cell2	Cell 2 name (can also be a number, representing the position in object@cell.names)
gene.ids	Genes to plot (default, all genes)
col.use	Colors to use for the points
nrpoints.use	Parameter for smoothScatter
pch.use	Point symbol to use
cex.use	Point size
do.hover	Enable hovering over points to view information
do.identify	Opens a locator session to identify clusters of cells. points to reveal gene names (hit ESC to stop)
...	Additional arguments to pass to smoothScatter

Value

No return value (plots a scatter plot)

Examples

```
CellPlot(object = pbmc_small, cell1 = 'ATAGGAGAAACAGA', cell2 = 'CATCAGGATGCACA')
```

`ClassifyCells` *Classify New Data*

Description

Classify new data based on the cluster information of the provided object. Random Forests are used as the basis of the classification.

Usage

```
ClassifyCells(object, classifier, training.genes = NULL,
  training.classes = NULL, new.data = NULL, ...)
```

Arguments

`object`	Seurat object on which to train the classifier
`classifier`	Random Forest classifier from BuildRFClassifier. If not provided, it will be built from the training data provided.
`training.genes`	Vector of genes to build the classifier on
`training.classes`	Vector of classes to build the classifier on
`new.data`	New data to classify
`...`	additional parameters passed to ranger

Value

Vector of cluster ids

Examples

```
pbmc_small
# take the first 10 cells as test data and train on the remaining 70 cells
test.pbmc <- SubsetData(object = pbmc_small, cells.use = pbmc_small@cell.names[1:10])
train.pbmc <- SubsetData(object = pbmc_small, cells.use = pbmc_small@cell.names[11:80])
predicted.classes <- ClassifyCells(
  object = train.pbmc,
  training.classes = train.pbmc@ident,
  new.data = test.pbmc@data
)
```

CollapseSpeciesExpressionMatrix *Slim down a multi-species expression matrix, when only one species is primarily of interenst.*

Description

Valuable for CITE-seq analyses, where we typically spike in rare populations of 'negative control' cells from a different species.

Usage

```
CollapseSpeciesExpressionMatrix(data.matrix, prefix.1 = "HUMAN_",
  prefix.controls = "MOUSE_", features.controls.toKeep = 100)
```

Arguments

data.matrix	A UMI count matrix. Should contain rownames that start with the ensuing arguments prefix.1 or prefix.2
prefix.1	The prefix denoting rownames for the species of interest. Default is "HUMAN_". These rownames will have this prefix removed in the returned matrix.
prefix.controls	The prefix denoting rownames for the species of 'negative control' cells. Default is "MOUSE_".
features.controls.toKeep	How many of the most highly expressed (average) negative control features (by default, 100 mouse genes), should be kept? All other rownames starting with prefix.2 are discarded.

Value

A UMI count matrix. Rownames that started with prefix.1 have this prefix discarded. For rownames starting with prefix.2, only the most highly expressed features are kept, and the prefix is kept. All other rows are retained.

Examples

```
## Not run:
cbmc.rna.collapsed <- CollapseSpeciesExpressionMatrix(cbmc.rna)

## End(Not run)
```

`ColorTSNESplit` *Color tSNE Plot Based on Split*

Description

Returns a tSNE plot colored based on whether the cells fall in clusters to the left or to the right of a node split in the cluster tree.

Usage

```
ColorTSNESplit(object, node, color1 = "red", color2 = "blue",
  color3 = "gray", ...)
```

Arguments

`object`	Seurat object
`node`	Node in cluster tree on which to base the split
`color1`	Color for the left side of the split
`color2`	Color for the right side of the split
`color3`	Color for all other cells
`...`	Arguments passed on to `TSNEPlot` **do.label** FALSE by default. If TRUE, plots an alternate view where the center of each cluster is labeled **pt.size** Set the point size **label.size** Set the size of the text labels **cells.use** Vector of cell names to use in the plot. **colors.use** Manually set the color palette to use for the points

Value

Returns a tSNE plot

Examples

```
pbmc_small
PlotClusterTree(pbmc_small)
ColorTSNESplit(pbmc_small, node = 6)
```

`CreateSeuratObject` *Initialize and setup the Seurat object*

Description

Initializes the Seurat object and some optional filtering

Usage

```
CreateSeuratObject(raw.data, project = "SeuratProject", min.cells = 0,
  min.genes = 0, is.expr = 0, normalization.method = NULL,
  scale.factor = 10000, do.scale = FALSE, do.center = FALSE,
  names.field = 1, names.delim = "_", meta.data = NULL, save.raw = TRUE,
  display.progress = TRUE)
```

Arguments

`raw.data`	Raw input data
`project`	Project name (string)
`min.cells`	Include genes with detected expression in at least this many cells. Will subset the raw.data matrix as well. To reintroduce excluded genes, create a new object with a lower cutoff.
`min.genes`	Include cells where at least this many genes are detected.
`is.expr`	Expression threshold for 'detected' gene. For most datasets, particularly UMI datasets, will be set to 0 (default). If not, when initializing, this should be set to a level based on pre-normalized counts (i.e. require at least 5 counts to be treated as expresesd) All values less than this will be set to 0 (though maintained in object@raw.data).
`normalization.method`	Method for cell normalization. Default is no normalization. In this case, run NormalizeData later in the workflow. As a shortcut, you can specify a normalization method (i.e. LogNormalize) here directly.
`scale.factor`	If normalizing on the cell level, this sets the scale factor.
`do.scale`	In object@scale.data, perform row-scaling (gene-based z-score). FALSE by default. In this case, run ScaleData later in the workflow. As a shortcut, you can specify do.scale = TRUE (and do.center = TRUE) here.
`do.center`	In object@scale.data, perform row-centering (gene-based centering)
`names.field`	For the initial identity class for each cell, choose this field from the cell's column name
`names.delim`	For the initial identity class for each cell, choose this delimiter from the cell's column name
`meta.data`	Additional metadata to add to the Seurat object. Should be a data frame where the rows are cell names, and the columns are additional metadata fields

save.raw TRUE by default. If FALSE, do not save the unmodified data in object@raw.data which will save memory downstream for large datasets

display.progress display progress bar for normalization and/or scaling procedure.

Value

Returns a Seurat object with the raw data stored in object@raw.data. object@data, object@meta.data, object@ident, also initialized.

Examples

```
pbmc_raw <- read.table(
  file = system.file('extdata', 'pbmc_raw.txt', package = 'Seurat'),
  as.is = TRUE
)
pbmc_small <- CreateSeuratObject(raw.data = pbmc_raw)
pbmc_small
```

CustomDistance *Run a custom distance function on an input data matrix*

Description

Run a custom distance function on an input data matrix

Usage

```
CustomDistance(my.mat, my.function, ...)
```

Arguments

my.mat	A matrix to calculate distance on
my.function	A function to calculate distance
...	Extra parameters to my.function

Value

A distance matrix

Author(s)

Jean Fan

Examples

```
# Define custom distance matrix
manhattan.distance <- function(x, y) return(sum(abs(x-y)))

input.data <- GetAssayData(pbmc_small, assay.type = "RNA", slot = "scale.data")
cell.manhattan.dist <- CustomDistance(input.data, manhattan.distance)
```

`CustomPalette` *Create a custom color palette*

Description

Creates a custom color palette based on low, middle, and high color values

Usage

```
CustomPalette(low = "white", high = "red", mid = NULL, k = 50)
```

Arguments

`low`	low color
`high`	high color
`mid`	middle color. Optional.
`k`	number of steps (colors levels) to include between low and high values

Value

A color palette for plotting

Examples

```
myPalette <- CustomPalette()
myPalette
```

DarkTheme *Dark Theme*

Description

Add a dark theme to ggplot objects

Usage

```
DarkTheme(...)
```

Arguments

...	Extra parameters to be passed to theme()

Value

A ggplot2 theme object

See Also

theme

Examples

```
df <- data.frame(x = rnorm(n = 100, mean = 20, sd = 2), y = rbinom(n = 100, size = 100, prob = 0.2))
p <- ggplot(data = df, mapping = aes(x = x, y = y)) + geom_point(mapping = aes(color = 'red'))
p + DarkTheme(legend.position = 'none')
```

DBClustDimension *Perform spectral density clustering on single cells*

Description

Find point clounds single cells in a two-dimensional space using density clustering (DBSCAN).

Usage

```
DBClustDimension(object, dim.1 = 1, dim.2 = 2, reduction.use = "tsne",
  G.use = NULL, set.ident = TRUE, seed.use = 1, ...)
```

Arguments

object	Seurat object
dim.1	First dimension to use
dim.2	second dimension to use
reduction.use	Which dimensional reduction to use (either 'pca' or 'ica')
G.use	Parameter for the density clustering. Lower value to get more fine-scale clustering
set.ident	TRUE by default. Set identity class to the results of the density clustering. Unassigned cells (cells that cannot be assigned a cluster) are placed in cluster 1, if there are any.
seed.use	Random seed for the dbscan function
...	Additional arguments to be passed to the dbscan function

Examples

```
pbmc_small
# Density based clustering on the first two tSNE dimensions
pbmc_small <- DBClustDimension(pbmc_small)
```

DESeq2DETest *Differential expression using DESeq2*

Description

Identifies differentially expressed genes between two groups of cells using DESeq2

Usage

```
DESeq2DETest(object, cells.1, cells.2, min.cells = 3, genes.use = NULL,
  assay.type = "RNA", ...)
```

Arguments

object	Seurat object
cells.1	Group 1 cells
cells.2	Group 2 cells
min.cells	Minimum number of cells expressing the gene in at least one of the two groups
genes.use	Genes to use for test
assay.type	Type of assay to fetch data for (default is RNA)
...	Extra parameters to pass to DESeq2::results

Details

This test does not support pre-filtering of genes based on average difference (or percent detection rate) between cell groups. However, genes may be pre-filtered based on their minimum detection rate (min.pct) across both cell groups. To use this method, please install DESeq2, using the instructions at https://bioconductor.org/packages/release/bioc/html/DESeq2.html

Value

Returns a p-value ranked matrix of putative differentially expressed genes.

References

Love MI, Huber W and Anders S (2014). "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2." Genome Biology. https://bioconductor.org/packages/release/bioc/html/DESeq2.html

Examples

```
## Not run:
  pbmc_small
  DESeq2DETest(pbmc_small, cells.1 = WhichCells(object = pbmc_small, ident = 1),
              cells.2 = WhichCells(object = pbmc_small, ident = 2))

## End(Not run)
```

DiffExpTest *Likelihood ratio test for zero-inflated data*

Description

Identifies differentially expressed genes between two groups of cells using the LRT model proposed in McDavid et al, Bioinformatics, 2013

Usage

```
DiffExpTest(object, cells.1, cells.2, assay.type = "RNA", genes.use = NULL,
  print.bar = TRUE)
```

Arguments

object	Seurat object
cells.1	Group 1 cells
cells.2	Group 2 cells
assay.type	Type of assay to fetch data for (default is RNA)
genes.use	Genes to test. Default is to use all genes
print.bar	Print a progress bar once expression testing begins (uses pbapply to do this)

Value

Returns a p-value ranked matrix of putative differentially expressed genes.

Examples

```
pbmc_small
DiffExpTest(pbmc_small, cells.1 = WhichCells(object = pbmc_small, ident = 1),
            cells.2 = WhichCells(object = pbmc_small, ident = 2))
```

DiffTTest *Differential expression testing using Student's t-test*

Description

Identify differentially expressed genes between two groups of cells using the Student's t-test

Usage

```
DiffTTest(object, cells.1, cells.2, genes.use = NULL, print.bar = TRUE,
  assay.type = "RNA")
```

Arguments

object	Seurat object
cells.1	Group 1 cells
cells.2	Group 2 cells
genes.use	Genes to test. Default is to use all genes
print.bar	Print a progress bar once expression testing begins (uses pbapply to do this)
assay.type	Type of assay to fetch data for (default is RNA)

Value

Returns a p-value ranked matrix of putative differentially expressed genes.

Examples

```
pbmc_small
DiffTTest(pbmc_small, cells.1 = WhichCells(object = pbmc_small, ident = 1),
            cells.2 = WhichCells(object = pbmc_small, ident = 2))
```

DimElbowPlot *Quickly Pick Relevant Dimensions*

Description

Plots the standard deviations (or approximate singular values if running PCAFast) of the principle components for easy identification of an elbow in the graph. This elbow often corresponds well with the significant dims and is much faster to run than Jackstraw

Usage

```
DimElbowPlot(object, reduction.type = "pca", dims.plot = 20, xlab = "",
  ylab = "", title = "")
```

Arguments

object	Seurat object
reduction.type	Type of dimensional reduction to plot data for
dims.plot	Number of dimensions to plot sd for
xlab	X axis label
ylab	Y axis label
title	Plot title

Value

Returns ggplot object

Examples

```
DimElbowPlot(object = pbmc_small)
```

DimHeatmap *Dimensional reduction heatmap*

Description

Draws a heatmap focusing on a principal component. Both cells and genes are sorted by their principal component scores. Allows for nice visualization of sources of heterogeneity in the dataset.

Usage

```
DimHeatmap(object, reduction.type = "pca", dim.use = 1, cells.use = NULL,
  num.genes = 30, use.full = FALSE, disp.min = -2.5, disp.max = 2.5,
  do.return = FALSE, col.use = PurpleAndYellow(), use.scale = TRUE,
  do.balanced = FALSE, remove.key = FALSE, label.columns = NULL,
  check.plot = TRUE, ...)
```

Arguments

object	Seurat object.
reduction.type	Which dimmensional reduction t use
dim.use	Dimensions to plot
cells.use	A list of cells to plot. If numeric, just plots the top cells.
num.genes	NUmber of genes to plot
use.full	Use the full PCA (projected PCA). Default is FALSE
disp.min	Minimum display value (all values below are clipped)
disp.max	Maximum display value (all values above are clipped)
do.return	If TRUE, returns plot object, otherwise plots plot object
col.use	Color to plot.
use.scale	Default is TRUE: plot scaled data. If FALSE, plot raw data on the heatmap.
do.balanced	Plot an equal number of genes with both + and - scores.
remove.key	Removes the color key from the plot.
label.columns	Labels for columns
check.plot	Check that plotting will finish in a reasonable amount of time
...	Extra parameters for heatmap plotting.

Value

If do.return==TRUE, a matrix of scaled values which would be passed to heatmap.2. Otherwise, no return value, only a graphical output

Examples

```
DimHeatmap(object = pbmc_small)
```

DimPlot *Dimensional reduction plot*

Description

Graphs the output of a dimensional reduction technique (PCA by default). Cells are colored by their identity class.

Usage

```
DimPlot(object, reduction.use = "pca", dim.1 = 1, dim.2 = 2,
  cells.use = NULL, pt.size = 1, do.return = FALSE, do.bare = FALSE,
  cols.use = NULL, group.by = "ident", pt.shape = NULL,
  do.hover = FALSE, data.hover = "ident", do.identify = FALSE,
  do.label = FALSE, label.size = 4, no.legend = FALSE, no.axes = FALSE,
  dark.theme = FALSE, ...)
```

Arguments

object	Seurat object
reduction.use	Which dimensionality reduction to use. Default is "pca", can also be "tsne", or "ica", assuming these are precomputed.
dim.1	Dimension for x-axis (default 1)
dim.2	Dimension for y-axis (default 2)
cells.use	Vector of cells to plot (default is all cells)
pt.size	Adjust point size for plotting
do.return	Return a ggplot2 object (default : FALSE)
do.bare	Do only minimal formatting (default : FALSE)
cols.use	Vector of colors, each color corresponds to an identity class. By default, ggplot assigns colors.
group.by	Group (color) cells in different ways (for example, orig.ident)
pt.shape	If NULL, all points are circles (default). You can specify any cell attribute (that can be pulled with FetchData) allowing for both different colors and different shapes on cells.
do.hover	Enable hovering over points to view information
data.hover	Data to add to the hover, pass a character vector of features to add. Defaults to cell name and ident. Pass 'NULL' to clear extra information.
do.identify	Opens a locator session to identify clusters of cells.
do.label	Whether to label the clusters
label.size	Sets size of labels
no.legend	Setting to TRUE will remove the legend
no.axes	Setting to TRUE will remove the axes
dark.theme	Use a dark theme for the plot
...	Extra parameters to FeatureLocator for do.identify = TRUE

Value

If do.return==TRUE, returns a ggplot2 object. Otherwise, only graphical output.

See Also

FeatureLocator

Examples

```
DimPlot(object = pbmc_small)
```

DimTopCells *Find cells with highest scores for a given dimensional reduction technique*

Description

Return a list of genes with the strongest contribution to a set of components

Usage

```
DimTopCells(object, dim.use = 1, reduction.type = "pca", num.cells = NULL,
  do.balanced = FALSE)
```

Arguments

object	Seurat object
dim.use	Components to use
reduction.type	Dimensional reduction to find the highest score for
num.cells	Number of cells to return
do.balanced	Return an equal number of cells with both + and - scores.

Value

Returns a vector of cells

Examples

```
pbmc_small
head(DimTopCells(object = pbmc_small, reduction.type = "pca"))
# Can specify which dimension and how many cells to return
DimTopCells(object = pbmc_small, reduction.type = "pca", dim.use = 2, num.cells = 5)
```

DimTopGenes *Find genes with highest scores for a given dimensional reduction technique*

Description

Return a list of genes with the strongest contribution to a set of components

Usage

```
DimTopGenes(object, dim.use = 1, reduction.type = "pca", num.genes = 30,
  use.full = FALSE, do.balanced = FALSE)
```

Arguments

object	Seurat object
dim.use	Dimension to use
reduction.type	Dimensional reduction to find the highest score for
num.genes	Number of genes to return
use.full	Use the full PCA (projected PCA). Default i s FALSE
do.balanced	Return an equal number of genes with both + and - scores.

Value

Returns a vector of genes

Examples

```
pbmc_small
DimTopGenes(object = pbmc_small, dim.use = 1, reduction.type = "pca")
# After projection:
DimTopGenes(object = pbmc_small, dim.use = 1, reduction.type = "pca", use.full = TRUE)
```

DMEmbed *Diffusion Maps Cell Embeddings Accessor Function*

Description

Pull Diffusion maps cell embedding matrix

Usage

```
DMEmbed(object, dims.use = NULL, cells.use = NULL)
```

Arguments

object	Seurat object
dims.use	Dimensions to include (default is all stored dims)
cells.use	Cells to include (default is all cells)

Value

Diffusion maps embedding matrix for given cells and DMs

Examples

```
pbmc_small
pbmc_small <- RunDiffusion(pbmc_small, genes.use = pbmc_small@var.genes)
head(DMEmbed(object = pbmc_small))
```

DMPlot *Plot Diffusion map*

Description

Graphs the output of a Diffusion map analysis Cells are colored by their identity class.

Usage

```
DMPlot(object, ...)
```

Arguments

object	Seurat object
...	Additional parameters to DimPlot, for example, which dimensions to plot.

Details

This function is a wrapper for DimPlot. See ?DimPlot for a full list of possible arguments which can be passed in here.

Examples

```
pbmc_small <- RunDiffusion(object = pbmc_small)
DMPlot(object = pbmc_small)
```

DoHeatmap *Gene expression heatmap*

Description

Draws a heatmap of single cell gene expression using ggplot2.

Usage

```
DoHeatmap(object, data.use = NULL, use.scaled = TRUE, cells.use = NULL,
  genes.use = NULL, disp.min = -2.5, disp.max = 2.5, group.by = "ident",
  draw.line = TRUE, col.low = "#FF00FF", col.mid = "#000000",
  col.high = "#FFFF00", slim.col.label = FALSE, remove.key = FALSE,
  rotate.key = FALSE, title = NULL, cex.col = 10, cex.row = 10,
  group.label.loc = "bottom", group.label.rot = FALSE, group.cex = 15,
  group.spacing = 0.15, assay.type = "RNA", do.plot = TRUE)
```

Arguments

object	Seurat object
data.use	Option to pass in data to use in the heatmap. Default will pick from either object@data or object@scale.data depending on use.scaled parameter. Should have cells as columns and genes as rows.
use.scaled	Whether to use the data or scaled data if data.use is NULL
cells.use	Cells to include in the heatmap (default is all cells)
genes.use	Genes to include in the heatmap (ordered)
disp.min	Minimum display value (all values below are clipped)
disp.max	Maximum display value (all values above are clipped)
group.by	Groups cells by this variable. Default is object@ident
draw.line	Draw vertical lines delineating different groups
col.low	Color for lowest expression value
col.mid	Color for mid expression value
col.high	Color for highest expression value
slim.col.label	display only the identity class name once for each group
remove.key	Removes the color key from the plot.
rotate.key	Rotate color scale horizantally
title	Title for plot
cex.col	Controls size of column labels (cells)
cex.row	Controls size of row labels (genes)
group.label.loc	Place group labels on bottom or top of plot.

group.label.rot	Whether to rotate the group label.
group.cex	Size of group label text
group.spacing	Controls amount of space between columns.
assay.type	to plot heatmap for (default is RNA)
do.plot	Whether to display the plot.

Value

Returns a ggplot2 plot object

Examples

```
DoHeatmap(object = pbmc_small)
```

DoKMeans *K-Means Clustering*

Description

Perform k=means clustering on both genes and single cells

Usage

```
DoKMeans(object, genes.use = NULL, k.genes = NULL, k.cells = 0,
  k.seed = 1, do.plot = FALSE, data.cut = 2.5,
  k.cols = PurpleAndYellow(), set.ident = TRUE, do.constrained = FALSE,
  assay.type = "RNA", ...)
```

Arguments

object	Seurat object
genes.use	Genes to use for clustering
k.genes	K value to use for clustering genes
k.cells	K value to use for clustering cells (default is NULL, cells are not clustered)
k.seed	Random seed
do.plot	Draw heatmap of clustered genes/cells (default is FALSE).
data.cut	Clip all z-scores to have an absolute value below this. Reduces the effect of huge outliers in the data.
k.cols	Color palette for heatmap
set.ident	If clustering cells (so k.cells>0), set the cell identity class to its K-means cluster (default is TRUE)

do.constrained	FALSE by default. If TRUE, use the constrained K-means function implemented in the tclust package.
assay.type	Type of data to normalize for (default is RNA), but can be changed for multimodal analyses.
...	Additional parameters passed to kmeans (or tkmeans)

Details

K-means and heatmap are calculated on object@scale.data

Value

Seurat object where the k-means results for genes is stored in object@kmeans.obj[[1]], and the k-means results for cells is stored in object@kmeans.col[[1]]. The cluster for each cell is stored in object@meta.data[,"kmeans.ident"] and also object@ident (if set.ident=TRUE)

Examples

```
pbmc_small
# Cluster on genes only
pbmc_small <- DoKMeans(pbmc_small, k.genes = 3)
# Cluster on genes and cell
pbmc_small <- DoKMeans(pbmc_small, k.genes = 3, k.cells = 3)
```

DotPlot *Dot plot visualization*

Description

Intuitive way of visualizing how gene expression changes across different identity classes (clusters). The size of the dot encodes the percentage of cells within a class, while the color encodes the AverageExpression level of 'expressing' cells (blue is high).

Usage

```
DotPlot(object, genes.plot, cols.use = c("lightgrey", "blue"),
  col.min = -2.5, col.max = 2.5, dot.min = 0, dot.scale = 6, group.by,
  plot.legend = FALSE, do.return = FALSE, x.lab.rot = FALSE)
```

Arguments

object	Seurat object
genes.plot	Input vector of genes
cols.use	colors to plot
col.min	Minimum scaled average expression threshold (everything smaller will be set to this)

col.max	Maximum scaled average expression threshold (everything larger will be set to this)
dot.min	The fraction of cells at which to draw the smallest dot (default is 0.05). All cell groups with less than this expressing the given gene will have no dot drawn.
dot.scale	Scale the size of the points, similar to cex
group.by	Factor to group the cells by
plot.legend	plots the legends
do.return	Return ggplot2 object
x.lab.rot	Rotate x-axis labels

Value

default, no return, only graphical output. If do.return=TRUE, returns a ggplot2 object

Examples

```
cd_genes <- c("CD247", "CD3E", "CD9")
DotPlot(object = pbmc_small, genes.plot = cd_genes)
```

DotPlotOld *Old Dot plot visualization (pre-ggplot implementation) Intuitive way of visualizing how gene expression changes across different identity classes (clusters). The size of the dot encodes the percentage of cells within a class, while the color encodes the AverageExpression level of 'expressing' cells (green is high).*

Description

Old Dot plot visualization (pre-ggplot implementation) Intuitive way of visualizing how gene expression changes across different identity classes (clusters). The size of the dot encodes the percentage of cells within a class, while the color encodes the AverageExpression level of 'expressing' cells (green is high).

Usage

```
DotPlotOld(object, genes.plot, cex.use = 2, cols.use = NULL,
  thresh.col = 2.5, dot.min = 0.05, group.by = NULL)
```

Arguments

object	Seurat object
genes.plot	Input vector of genes
cex.use	Scaling factor for the dots (scales all dot sizes)
cols.use	colors to plot

thresh.col	The raw data value which corresponds to a red dot (lowest expression)
dot.min	The fraction of cells at which to draw the smallest dot (default is 0.05)
group.by	Factor to group the cells by

Value

Only graphical output

Examples

```
cd_genes <- c("CD247", "CD3E", "CD9")
DotPlotOld(object = pbmc_small, genes.plot = cd_genes)
```

ExpMean *Calculate the mean of logged values*

Description

Calculate mean of logged values in non-log space (return answer in log-space)

Usage

```
ExpMean(x)
```

Arguments

x	A vector of values

Value

Returns the mean in log-space

Examples

```
ExpMean(x = c(1, 2, 3))
```

ExpSD *Calculate the standard deviation of logged values*

Description

Calculate SD of logged values in non-log space (return answer in log-space)

Usage

```
ExpSD(x)
```

Arguments

x	A vector of values

Value

Returns the standard deviation in log-space

Examples

```
ExpSD(x = c(1, 2, 3))
```

ExpVar *Calculate the variance of logged values*

Description

Calculate variance of logged values in non-log space (return answer in log-space)

Usage

```
ExpVar(x)
```

Arguments

x	A vector of values

Value

Returns the variance in log-space

Examples

```
ExpVar(x = c(1, 2, 3))
```

`ExtractField` *Extract delimiter information from a string.*

Description

Parses a string (usually a cell name) and extracts fields based on a delimiter

Usage

```
ExtractField(string, field = 1, delim = "_")
```

Arguments

`string`	String to parse.
`field`	Integer(s) indicating which field(s) to extract. Can be a vector multiple numbers.
`delim`	Delimiter to use, set to underscore by default.

Value

A new string, that parses out the requested fields, and (if multiple), rejoins them with the same delimiter

Examples

```
ExtractField(string = 'Hello World', field = 1, delim = '_')
```

`FastWhichCells` *FastWhichCells Identify cells matching certain criteria (limited to character values)*

Description

FastWhichCells Identify cells matching certain criteria (limited to character values)

Usage

```
FastWhichCells(object, group.by, subset.value, invert = FALSE)
```

Arguments

`object`	Seurat object
`group.by`	Group cells in different ways (for example, orig.ident). Should be a column name in object@meta.data
`subset.value`	Return cells matching this value
`invert`	invert cells to return.FALSE by default

Examples

```
FastWhichCells(object = pbmc_small, group.by = 'res.1', subset.value = 1)
```

`FeatureHeatmap` *Vizualization of multiple features*

Description

Similar to FeaturePlot, however, also splits the plot by visualizing each identity class separately.

Usage

```
FeatureHeatmap(object, features.plot, dim.1 = 1, dim.2 = 2,
  idents.use = NULL, pt.size = 2, cols.use = c("grey", "red"),
  pch.use = 16, reduction.use = "tsne", group.by = NULL,
  sep.scale = FALSE, do.return = FALSE, min.exp = -Inf, max.exp = Inf,
  rotate.key = FALSE, plot.horiz = FALSE, key.position = "right")
```

Arguments

`object`	Seurat object
`features.plot`	Vector of features to plot
`dim.1`	Dimension for x-axis (default 1)
`dim.2`	Dimension for y-axis (default 2)
`idents.use`	Which identity classes to display (default is all identity classes)
`pt.size`	Adjust point size for plotting
`cols.use`	Ordered vector of colors to use for plotting. Default is heat.colors(10).
`pch.use`	Pch for plotting
`reduction.use`	Which dimensionality reduction to use. Default is "tsne", can also be "pca", or "ica", assuming these are precomputed.
`group.by`	Group cells in different ways (for example, orig.ident)
`sep.scale`	Scale each group separately. Default is FALSE.
`do.return`	Return the ggplot2 object
`min.exp`	Min cutoff for scaled expression value, supports quantiles in the form of 'q##' (see FeaturePlot)
`max.exp`	Max cutoff for scaled expression value, supports quantiles in the form of 'q##' (see FeaturePlot)
`rotate.key`	rotate the legend
`plot.horiz`	rotate the plot such that the features are columns, groups are the rows
`key.position`	position of the legend ("top", "right", "bottom", "left")

Details

Particularly useful for seeing if the same groups of cells co-exhibit a common feature (i.e. co-express a gene), even within an identity class. Best understood by example.

Value

No return value, only a graphical output

See Also

FeaturePlot

Examples

```
pbmc_small
FeatureHeatmap(object = pbmc_small, features.plot = "PC1")
```

FeatureLocator *Feature Locator*

Description

Select points on a scatterplot and get information about them

Usage

```
FeatureLocator(plot, data.plot, ...)
```

Arguments

plot	A ggplot2 plot
data.plot	The oridinal data that went into the ggplot2 plot
...	Extra parameters, such as dark.theme, recolor, or smooth for using a dark theme, recoloring based on selected cells, or using a smooth scatterplot, respectively

Value

The names of the points selected

See Also

locator

ggplot2::ggplot_build

Examples

```
## Not run:
df <- data.frame(x = rnorm(n = 100, mean = 20, sd = 2), y = rbinom(n = 100, size = 100, prob = 0.2))
p <- ggplot(data = df, mapping = aes(x = x, y = y)) + geom_point(mapping = aes(color = 'red'))
FeatureLocator(plot = p, data.plot = df)

## End(Not run)
```

FeaturePlot *Visualize 'features' on a dimensional reduction plot*

Description

Colors single cells on a dimensional reduction plot according to a 'feature' (i.e. gene expression, PC scores, number of genes detected, etc.)

Usage

```
FeaturePlot(object, features.plot, min.cutoff = NA, max.cutoff = NA,
  dim.1 = 1, dim.2 = 2, cells.use = NULL, pt.size = 1,
  cols.use = c("yellow", "red"), pch.use = 16, overlay = FALSE,
  do.hover = FALSE, data.hover = "ident", do.identify = FALSE,
  reduction.use = "tsne", use.imputed = FALSE, nCol = NULL,
  no.axes = FALSE, no.legend = TRUE, dark.theme = FALSE,
  do.return = FALSE)
```

Arguments

object	Seurat object
features.plot	Vector of features to plot
min.cutoff	Vector of minimum cutoff values for each feature, may specify quantile in the form of 'q##' where '##' is the quantile (eg, 1, 10)
max.cutoff	Vector of maximum cutoff values for each feature, may specify quantile in the form of 'q##' where '##' is the quantile (eg, 1, 10)
dim.1	Dimension for x-axis (default 1)
dim.2	Dimension for y-axis (default 2)
cells.use	Vector of cells to plot (default is all cells)
pt.size	Adjust point size for plotting
cols.use	The two colors to form the gradient over. Provide as string vector with the first color corresponding to low values, the second to high. Also accepts a Brewer color scale or vector of colors. Note: this will bin the data into number of colors provided.
pch.use	Pch for plotting

overlay	Plot two features overlayed one on top of the other
do.hover	Enable hovering over points to view information
data.hover	Data to add to the hover, pass a character vector of features to add. Defaults to cell name and identity. Pass 'NULL' to remove extra data.
do.identify	Opens a locator session to identify clusters of cells
reduction.use	Which dimensionality reduction to use. Default is "tsne", can also be "pca", or "ica", assuming these are precomputed.
use.imputed	Use imputed values for gene expression (default is FALSE)
nCol	Number of columns to use when plotting multiple features.
no.axes	Remove axis labels
no.legend	Remove legend from the graph. Default is TRUE.
dark.theme	Plot in a dark theme
do.return	return the ggplot2 object

Value

No return value, only a graphical output

Examples

```
FeaturePlot(object = pbmc_small, features.plot = 'PC1')
```

FetchData *Access cellular data*

Description

Retreives data (gene expression, PCA scores, etc, metrics, etc.) for a set of cells in a Seurat object

Usage

```
FetchData(object, vars.all = NULL, cells.use = NULL, use.imputed = FALSE,
  use.scaled = FALSE, use.raw = FALSE)
```

Arguments

object	Seurat object
vars.all	List of all variables to fetch
cells.use	Cells to collect data for (default is all cells)
use.imputed	For gene expression, use imputed values. Default is FALSE
use.scaled	For gene expression, use scaled values. Default is FALSE
use.raw	For gene expression, use raw values. Default is FALSE

Value

A data frame with cells as rows and cellular data as columns

Examples

```
pc1 <- FetchData(object = pbmc_small, vars.all = 'PC1')
head(x = pc1)
```

`FilterCells` *Return a subset of the Seurat object*

Description

Creates a Seurat object containing only a subset of the cells in the original object. Takes either a list of cells to use as a subset, or a parameter (for example, a gene), to subset on.

Usage

```
FilterCells(object, subset.names, low.thresholds, high.thresholds,
  cells.use = NULL)
```

Arguments

`object`	Seurat object
`subset.names`	Parameters to subset on. Eg, the name of a gene, PC1, a column name in object@meta.data, etc. Any argument that can be retreived using FetchData
`low.thresholds`	Low cutoffs for the parameters (default is -Inf)
`high.thresholds`	High cutoffs for the parameters (default is Inf)
`cells.use`	A vector of cell names to use as a subset

Value

Returns a Seurat object containing only the relevant subset of cells

Examples

```
head(x = FetchData(object = pbmc_small, vars.all = 'LTB'))
pbmc_filtered <- FilterCells(
  object = pbmc_small,
  subset.names = 'LTB',
  high.thresholds = 6
)
head(x = FetchData(object = pbmc_filtered, vars.all = 'LTB'))
```

`FindAllMarkers` *Gene expression markers for all identity classes*

Description

Finds markers (differentially expressed genes) for each of the identity classes in a dataset

Usage

```
FindAllMarkers(object, genes.use = NULL, logfc.threshold = 0.25,
  test.use = "bimod", min.pct = 0.1, min.diff.pct = -Inf,
  print.bar = TRUE, only.pos = FALSE, max.cells.per.ident = Inf,
  return.thresh = 0.01, do.print = FALSE, random.seed = 1,
  min.cells = 3, latent.vars = "nUMI", assay.type = "RNA", ...)
```

Arguments

`object`	Seurat object
`genes.use`	Genes to test. Default is to use all genes
`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 0.25 Increasing logfc.threshold speeds up the function, but can miss weaker signals.
`test.use`	Denotes which test to use. Available options are: • "wilcox" : Wilcoxon rank sum test (default) • "bimod" : Likelihood-ratio test for single cell gene expression, (McDavid et al., Bioinformatics, 2013) • "roc" : Standard AUC classifier • "t" : Student's t-test • "tobit" : Tobit-test for differential gene expression (Trapnell et al., Nature Biotech, 2014) • "poisson" : Likelihood ratio test assuming an underlying poisson distribution. Use only for UMI-based datasets • "negbinom" : Likelihood ratio test assuming an underlying negative binomial distribution. Use only for UMI-based datasets • "MAST : GLM-framework that treates cellular detection rate as a covariate (Finak et al, Genome Biology, 2015) • "DESeq2 : DE based on a model using the negative binomial distribution (Love et al, Genome Biology, 2014)
`min.pct`	only test genes that are detected in a minimum fraction of min.pct cells in either of the two populations. Meant to speed up the function by not testing genes that are very infrequently expressed. Default is 0.1
`min.diff.pct`	only test genes that show a minimum difference in the fraction of detection between the two groups. Set to -Inf by default

print.bar	Print a progress bar once expression testing begins (uses pbapply to do this)
only.pos	Only return positive markers (FALSE by default)
max.cells.per.ident	Down sample each identity class to a max number. Default is no downsampling.
return.thresh	Only return markers that have a p-value < return.thresh, or a power > return.thresh (if the test is ROC)
do.print	FALSE by default. If TRUE, outputs updates on progress.
random.seed	Random seed for downsampling
min.cells	Minimum number of cells expressing the gene in at least one of the two groups
latent.vars	remove the effects of these variables
assay.type	Type of assay to perform DE for (default is RNA)
...	Additional parameters to pass to specific DE functions

Value

Matrix containing a ranked list of putative markers, and associated statistics (p-values, ROC score, etc.)

Examples

```
all_markers <- FindAllMarkers(object = pbmc_small)
head(x = all_markers)
```

FindAllMarkersNode *Find all markers for a node*

Description

This function finds markers for all splits at or below the specified node

Usage

```
FindAllMarkersNode(object, node = NULL, genes.use = NULL,
  logfc.threshold = 0.25, test.use = "bimod", min.pct = 0.1,
  min.diff.pct = 0.05, print.bar = TRUE, only.pos = FALSE,
  max.cells.per.ident = Inf, return.thresh = 0.01, do.print = FALSE,
  random.seed = 1, min.cells = 3, assay.type = "RNA", ...)
```

Arguments

object	Seurat object. Must have object@cluster.tree slot filled. Use BuildClusterTree() if not.
node	Node from which to start identifying split markers, default is top node.
genes.use	Genes to test. Default is to use all genes
logfc.threshold	Limit testing to genes which show, on average, at least X-fold difference (log-scale) between the two groups of cells.
test.use	Denotes which test to use. Seurat currently implements "bimod" (likelihood-ratio test for single cell gene expression, McDavid et al., Bioinformatics, 2013, default), "roc" (standard AUC classifier), "t" (Students t-test), and "tobit" (Tobit-test for differential gene expression, as in Trapnell et al., Nature Biotech, 2014), 'poisson', and 'negbinom'. The latter two options should only be used on UMI datasets, and assume an underlying poisson or negative-binomial distribution.
min.pct	- only test genes that are detected in a minimum fraction of min.pct cells in either of the two populations. Meant to speed up the function by not testing genes that are very infrequently expression
min.diff.pct	- only test genes that show a minimum difference in the fraction of detection between the two groups. Set to -Inf by default
print.bar	Print a progress bar once expression testing begins (uses pbapply to do this)
only.pos	Only return positive markers (FALSE by default)
max.cells.per.ident	Down sample each identity class to a max number. Default is no downsampling.
return.thresh	Only return markers that have a p-value < return.thresh, or a power > return.thresh (if the test is ROC)
do.print	Print status updates
random.seed	Random seed for downsampling
min.cells	Minimum number of cells expressing the gene in at least one of the two groups
assay.type	Type of assay to fetch data for (default is RNA)
...	Additional parameters to pass to specific DE functions

Value

Returns a dataframe with a ranked list of putative markers for each node and associated statistics

Examples

```
pbmc_small

FindAllMarkersNode(pbmc_small)
```

`FindClusters` *Cluster Determination*

Description

Identify clusters of cells by a shared nearest neighbor (SNN) modularity optimization based clustering algorithm. First calculate k-nearest neighbors and construct the SNN graph. Then optimize the modularity function to determine clusters. For a full description of the algorithms, see Waltman and van Eck (2013) *The European Physical Journal B*.

Usage

```
FindClusters(object, genes.use = NULL, reduction.type = "pca",
  dims.use = NULL, k.param = 30, k.scale = 25, plot.SNN = FALSE,
  prune.SNN = 1/15, print.output = TRUE, distance.matrix = NULL,
  save.SNN = FALSE, reuse.SNN = FALSE, force.recalc = FALSE,
  modularity.fxn = 1, resolution = 0.8, algorithm = 1, n.start = 100,
  n.iter = 10, random.seed = 0, temp.file.location = NULL)
```

Arguments

`object`	Seurat object
`genes.use`	A vector of gene names to use in construction of SNN graph if building directly based on expression data rather than a dimensionally reduced representation (i.e. PCs).
`reduction.type`	Name of dimensional reduction technique to use in construction of SNN graph. (e.g. "pca", "ica")
`dims.use`	A vector of the dimensions to use in construction of the SNN graph (e.g. To use the first 10 PCs, pass 1:10)
`k.param`	Defines k for the k-nearest neighbor algorithm
`k.scale`	Granularity option for k.param
`plot.SNN`	Plot the SNN graph
`prune.SNN`	Sets the cutoff for acceptable Jaccard distances when computing the neighborhood overlap for the SNN construction. Any edges with values less than or equal to this will be set to 0 and removed from the SNN graph. Essentially sets the strigency of pruning (0 — no pruning, 1 — prune everything).
`print.output`	Whether or not to print output to the console
`distance.matrix`	Build SNN from distance matrix (experimental)
`save.SNN`	Saves the SNN matrix associated with the calculation in object@snn
`reuse.SNN`	Force utilization of stored SNN. If none store, this will throw an error.
`force.recalc`	Force recalculation of SNN.
`modularity.fxn`	Modularity function (1 = standard; 2 = alternative).

resolution	Value of the resolution parameter, use a value above (below) 1.0 if you want to obtain a larger (smaller) number of communities.
algorithm	Algorithm for modularity optimization (1 = original Louvain algorithm; 2 = Louvain algorithm with multilevel refinement; 3 = SLM algorithm).
n.start	Number of random starts.
n.iter	Maximal number of iterations per random start.
random.seed	Seed of the random number generator.
temp.file.location	Directory where intermediate files will be written. Specify the ABSOLUTE path.

Value

Returns a Seurat object and optionally the SNN matrix, object@ident has been updated with new cluster info

Examples

```
## Not run:
pbmc_small
pmbc_small <- FindClusters(
  object = pbmc_small,
  reduction.type = "pca",
  dims.use = 1:10,
  save.SNN = TRUE
)
# To explore a range of clustering options, pass a vector of values to the resolution parameter
pbmc_small <- FindClusters(
  object = pbmc_small,
  reduction.type = "pca",
  resolution = c(0.4, 0.8, 1.2),
  dims.use = 1:10,
  save.SNN = TRUE
)

## End(Not run)
```

FindConservedMarkers *Finds markers that are conserved between the two groups*

Description

Finds markers that are conserved between the two groups

Usage

```
FindConservedMarkers(object, ident.1, ident.2 = NULL, grouping.var,
  assay.type = "RNA", ...)
```

Arguments

object	Seurat object
ident.1	Identity class to define markers for
ident.2	A second identity class for comparison. If NULL (default) - use all other cells for comparison.
grouping.var	grouping variable
assay.type	Type of assay to fetch data for (default is RNA)
...	parameters to pass to FindMarkers

Value

Matrix containing a ranked list of putative conserved markers, and associated statistics (p-values within each group and a combined p-value (fisher_pval), percentage of cells expressing the marker, average differences)

Examples

```
pbmc_small
# Create a simulated grouping variable
pbmc_small@meta.data$groups <- sample(
  x = c("g1", "g2"),
  size = length(x = pbmc_small@cell.names),
  replace = TRUE
)
FindConservedMarkers(pbmc_small, ident.1 = 1, ident.2 = 2, grouping.var = "groups")
```

FindMarkers *Gene expression markers of identity classes*

Description

Finds markers (differentially expressed genes) for identity classes

Usage

```
FindMarkers(object, ident.1, ident.2 = NULL, genes.use = NULL,
  logfc.threshold = 0.25, test.use = "wilcox", min.pct = 0.1,
  min.diff.pct = -Inf, print.bar = TRUE, only.pos = FALSE,
  max.cells.per.ident = Inf, random.seed = 1, latent.vars = "nUMI",
  min.cells = 3, pseudocount.use = 1, assay.type = "RNA", ...)
```

Arguments

object	Seurat object
ident.1	Identity class to define markers for
ident.2	A second identity class for comparison. If NULL (default) - use all other cells for comparison.
genes.use	Genes to test. Default is to use all genes
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 0.25 Increasing logfc.threshold speeds up the function, but can miss weaker signals.
test.use	Denotes which test to use. Available options are: • "wilcox" : Wilcoxon rank sum test (default) • "bimod" : Likelihood-ratio test for single cell gene expression, (McDavid et al., Bioinformatics, 2013) • "roc" : Standard AUC classifier • "t" : Student's t-test • "tobit" : Tobit-test for differential gene expression (Trapnell et al., Nature Biotech, 2014) • "poisson" : Likelihood ratio test assuming an underlying poisson distribution. Use only for UMI-based datasets • "negbinom" : Likelihood ratio test assuming an underlying negative binomial distribution. Use only for UMI-based datasets • "MAST : GLM-framework that treates cellular detection rate as a covariate (Finak et al, Genome Biology, 2015) • "DESeq2 : DE based on a model using the negative binomial distribution (Love et al, Genome Biology, 2014)
min.pct	only test genes that are detected in a minimum fraction of min.pct cells in either of the two populations. Meant to speed up the function by not testing genes that are very infrequently expressed. Default is 0.1
min.diff.pct	only test genes that show a minimum difference in the fraction of detection between the two groups. Set to -Inf by default
print.bar	Print a progress bar once expression testing begins (uses pbapply to do this)
only.pos	Only return positive markers (FALSE by default)
max.cells.per.ident	Down sample each identity class to a max number. Default is no downsampling. Not activated by default (set to Inf)
random.seed	Random seed for downsampling
latent.vars	Variables to test
min.cells	Minimum number of cells expressing the gene in at least one of the two groups
pseudocount.use	Pseudocount to add to averaged expression values when calculating logFC. 1 by default.
assay.type	Type of assay to fetch data for (default is RNA)
...	Additional parameters to pass to specific DE functions

Details

p-value adjustment is performed using bonferroni correction based on the total number of genes in the dataset. Other correction methods are not recommended, as Seurat pre-filters genes using the arguments above, reducing the number of tests performed. Lastly, as Aaron Lun has pointed out, p-values should be interpreted cautiously, as the genes used for clustering are the same genes tested for differential expression.

Value

Matrix containing a ranked list of putative markers, and associated statistics (p-values, ROC score, etc.)

See Also

MASTDETest, and DESeq2DETest for more information on these methods

Examples

```
markers <- FindMarkers(object = pbmc_small, ident.1 = 3)
head(markers)
```

FindMarkersNode *Gene expression markers of identity classes defined by a phylogenetic clade*

Description

Finds markers (differentially expressed genes) based on a branching point (node) in the phylogenetic tree. Markers that define clusters in the left branch are positive markers. Markers that define the right branch are negative markers.

Usage

```
FindMarkersNode(object, node, tree.use = NULL, genes.use = NULL,
  logfc.threshold = 0.25, test.use = "bimod", assay.type = "RNA", ...)
```

Arguments

object	Seurat object
node	The node in the phylogenetic tree to use as a branch point
tree.use	Can optionally pass the tree to be used. Default uses the tree in object@cluster.tree
genes.use	Genes to test. Default is to use all genes
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 0.25 Increasing logfc.threshold speeds up the function, but can miss weaker signals.

test.use	Denotes which test to use. Available options are: • "wilcox" : Wilcoxon rank sum test (default) • "bimod" : Likelihood-ratio test for single cell gene expression, (McDavid et al., Bioinformatics, 2013) • "roc" : Standard AUC classifier • "t" : Student's t-test • "tobit" : Tobit-test for differential gene expression (Trapnell et al., Nature Biotech, 2014) • "poisson" : Likelihood ratio test assuming an underlying poisson distribution. Use only for UMI-based datasets • "negbinom" : Likelihood ratio test assuming an underlying negative binomial distribution. Use only for UMI-based datasets • "MAST : GLM-framework that treates cellular detection rate as a covariate (Finak et al, Genome Biology, 2015) • "DESeq2 : DE based on a model using the negative binomial distribution (Love et al, Genome Biology, 2014)
assay.type	Type of assay to fetch data for (default is RNA)
...	Additional arguments passed to FindMarkers

Value

Matrix containing a ranked list of putative markers, and associated statistics (p-values, ROC score, etc.)

Examples

```
FindMarkersNode(pbmc_small, 5)
```

FindVariableGenes *Identify variable genes*

Description

Identifies genes that are outliers on a 'mean variability plot'. First, uses a function to calculate average expression (mean.function) and dispersion (dispersion.function) for each gene. Next, divides genes into num.bin (deafult 20) bins based on their average expression, and calculates z-scores for dispersion within each bin. The purpose of this is to identify variable genes while controlling for the strong relationship between variability and average expression.

Usage

```
FindVariableGenes(object, mean.function = ExpMean,
  dispersion.function = LogVMR, do.plot = TRUE, set.var.genes = TRUE,
  x.low.cutoff = 0.1, x.high.cutoff = 8, y.cutoff = 1,
  y.high.cutoff = Inf, num.bin = 20, do.recalc = TRUE,
  sort.results = TRUE, do.cpp = TRUE, display.progress = TRUE, ...)
```

Arguments

object	Seurat object
mean.function	Function to compute x-axis value (average expression). Default is to take the mean of the detected (i.e. non-zero) values
dispersion.function	Function to compute y-axis value (dispersion). Default is to take the standard deviation of all values/
do.plot	Plot the average/dispersion relationship
set.var.genes	Set object@var.genes to the identified variable genes (default is TRUE)
x.low.cutoff	Bottom cutoff on x-axis for identifying variable genes
x.high.cutoff	Top cutoff on x-axis for identifying variable genes
y.cutoff	Bottom cutoff on y-axis for identifying variable genes
y.high.cutoff	Top cutoff on y-axis for identifying variable genes
num.bin	Total number of bins to use in the scaled analysis (default is 20)
do.recalc	TRUE by default. If FALSE, plots and selects variable genes without recalculating statistics for each gene.
sort.results	If TRUE (by default), sort results in object@hvg.info in decreasing order of dispersion
do.cpp	Run c++ version of mean.function and dispersion.function if they exist.
display.progress	show progress bar for calculations
...	Extra parameters to VariableGenePlot

Details

Exact parameter settings may vary empirically from dataset to dataset, and based on visual inspection of the plot. Setting the y.cutoff parameter to 2 identifies genes that are more than two standard deviations away from the average dispersion within a bin. The default X-axis function is the mean expression level, and for Y-axis it is the log(Variance/mean). All mean/variance calculations are not performed in log-space, but the results are reported in log-space - see relevant functions for exact details.

Value

Returns a Seurat object, placing variable genes in object@var.genes. The result of all analysis is stored in object@hvg.info

See Also

VariableGenePlot

Examples

```
pbmc_small <- FindVariableGenes(object = pbmc_small, do.plot = FALSE)
pbmc_small@var.genes
```

FitGeneK *Build mixture models of gene expression*

Description

Models the imputed gene expression values as a mixture of gaussian distributions. For a two-state model, estimates the probability that a given cell is in the 'on' or 'off' state for any gene. Followed by a greedy k-means step where cells are allowed to flip states based on the overall structure of the data (see Manuscript for details)

Usage

```
FitGeneK(object, gene, do.k = 2, num.iter = 1, do.plot = FALSE,
  genes.use = NULL, start.pct = NULL)
```

Arguments

object	Seurat object
gene	Gene to fit
do.k	Number of modes for the mixture model (default is 2)
num.iter	Number of 'greedy k-means' iterations (default is 1)
do.plot	Plot mixture model results
genes.use	Genes to use in the greedy k-means step (See manuscript for details)
start.pct	Initial estimates of the percentage of cells in the 'on' state (usually estimated from the in situ map)

Value

A Seurat object, where the posterior of each cell being in the 'on' or 'off' state for each gene is stored in object@spatial@mix.probs

Examples

```
## Not run:
# Note that the PBMC test example object does not contain spatially restricted
# examples below are only demonstrate code
pmbc_small <- FitGeneK(object = pbmc_small, gene = "MS4A1")

## End(Not run)
```

GenePlot *Scatter plot of single cell data*

Description

Creates a scatter plot of two features (typically gene expression), across a set of single cells. Cells are colored by their identity class.

Usage

```
GenePlot(object, gene1, gene2, cell.ids = NULL, col.use = NULL,
  pch.use = 16, cex.use = 1.5, use.imputed = FALSE, use.scaled = FALSE,
  use.raw = FALSE, do.hover = FALSE, data.hover = "ident",
  do.identify = FALSE, dark.theme = FALSE, do.spline = FALSE,
  spline.span = 0.75, ...)
```

Arguments

object	Seurat object
gene1	First feature to plot. Typically gene expression but can also be metrics, PC scores, etc. - anything that can be retreived with FetchData
gene2	Second feature to plot.
cell.ids	Cells to include on the scatter plot.
col.use	Colors to use for identity class plotting.
pch.use	Pch argument for plotting
cex.use	Cex argument for plotting
use.imputed	Use imputed values for gene expression (Default is FALSE)
use.scaled	Use scaled data
use.raw	Use raw data
do.hover	Enable hovering over points to view information
data.hover	Data to add to the hover, pass a character vector of features to add. Defaults to cell name and ident. Pass 'NULL' to clear extra information.
do.identify	Opens a locator session to identify clusters of cells.
dark.theme	Use a dark theme for the plot
do.spline	Add a spline (currently hardwired to df=4, to be improved)
spline.span	spline span in loess function call
...	Additional arguments to be passed to plot.

Value

No return, only graphical output

Examples

```
GenePlot(object = pbmc_small, gene1 = 'CD9', gene2 = 'CD3E')
```

GenesInCluster *GenesInCluster*

Description

After k-means analysis, previously run with DoKMeans, returns a set of genes associated with each cluster

Usage

```
GenesInCluster(object, cluster.num, max.genes = 1e+06)
```

Arguments

object	Seurat object. Assumes DoKMeans has already been run
cluster.num	K-means cluster(s) to return genes for
max.genes	max number of genes to return

Value

A vector of genes who are members in the cluster.num k-means cluster(s)

Examples

```
pbmc_small
# Cluster on genes only
pbmc_small <- DoKMeans(object = pbmc_small, k.genes = 3)
pbmc_small <- GenesInCluster(object = pbmc_small, cluster.num = 1)
```

GetAssayData *Accessor function for multimodal data*

Description

Pull information for specified stored dimensional reduction analysis

Usage

```
GetAssayData(object, assay.type = "RNA", slot = "data")
```

Arguments

object	Seurat object
assay.type	Type of assay to fetch data for (default is RNA)
slot	Specific information to pull (i.e. raw.data, data, scale.data,...). Default is data

Value

Returns assay data

Examples

```
# Simulate CITE-Seq results
df <- t(x = data.frame(
  x = round(x = rnorm(n = 80, mean = 20, sd = 2)),
  y = round(x = rbinom(n = 80, size = 100, prob = 0.2))
))
pbmc_small <- SetAssayData(
  object = pbmc_small,
  assay.type = 'CITE',
  new.data = df,
  slot = 'raw.data'
)
GetAssayData(object = pbmc_small, assay.type = 'CITE', slot = 'raw.data')
```

GetCellEmbeddings *Dimensional Reduction Cell Embeddings Accessor Function*

Description

Pull cell embeddings matrix for specified stored dimensional reduction analysis

Usage

```
GetCellEmbeddings(object, reduction.type = "pca", dims.use = NULL,
  cells.use = NULL)
```

Arguments

object	Seurat object
reduction.type	Type of dimensional reduction to fetch (default is PCA)
dims.use	Dimensions to include (default is all stored dims)
cells.use	Cells to include (default is all cells)

Value

Cell embedding matrix for given reduction, cells, and dimensions

Examples

```
pbmc_small
# Examine the head of the first 5 PC cell embeddings
head(GetCellEmbeddings(object = pbmc_small, reduction.type = "pca", dims.use = 1:5))
```

`GetCentroids` *Get cell centroids*

Description

Calculate the spatial mapping centroids for each cell, based on previously calculated mapping probabilities for each bin.

Usage

```
GetCentroids(object, cells.use = NULL, get.exact = TRUE)
```

Arguments

`object`	Seurat object
`cells.use`	Cells to calculate centroids for (default is all cells)
`get.exact`	Get exact centroid (Default is TRUE). If FALSE, identify the single closest bin.

Details

Currently, Seurat assumes that the tissue of interest has an 8x8 bin structure. This will be broadened in a future release.

Value

Data frame containing the x and y coordinates for each cell centroid.

Examples

```
## Not run:
# Note that the PBMC test example object does not contain spatially restricted
# examples below are only demonstrate code
pmbc_small <- GetCentroids(pbmc_small, cells.use=pbmc_small@cell.names)

## End(Not run)
```

GetClusters *Get Cluster Assignments*

Description

Retrieve cluster IDs as a dataframe. First column will be the cell name, second column will be the current cluster identity (pulled from object@ident).

Usage

```
GetClusters(object)
```

Arguments

object	Seurat object with cluster assignments

Value

Returns a dataframe with cell names and cluster assignments

Examples

```
pbmc_small
clusters <- GetClusters(object = pbmc_small)
head(clusters)
```

GetDimReduction *Dimensional Reduction Accessor Function*

Description

General accessor function for dimensional reduction objects. Pulls slot contents for specified stored dimensional reduction analysis.

Usage

```
GetDimReduction(object, reduction.type = "pca", slot = "gene.loadings")
```

Arguments

object	Seurat object
reduction.type	Type of dimensional reduction to fetch (default is PCA)
slot	Specific information to pull (must be one of the following: "cell.embeddings", "gene.loadings", "gene.loadings.full", "sdev", "key", "misc")

Value

Returns specified slot results from given reduction technique

Examples

```
pbmc_small
# Get the PCA cell embeddings and print the top left corner
GetDimReduction(object = pbmc_small, reduction.type = "pca",
                slot = "cell.embeddings")[1:5, 1:5]
# Get the standard deviation of each PC
GetDimReduction(object = pbmc_small, reduction.type = "pca", slot = "sdev")
```

GetGeneLoadings *Dimensional Reduction Gene Loadings Accessor Function*

Description

Pull gene loadings matrix for specified stored dimensional reduction analysis.

Usage

```
GetGeneLoadings(object, reduction.type = "pca", dims.use = NULL,
  genes.use = NULL, use.full = FALSE)
```

Arguments

`object`	Seurat object
`reduction.type`	Type of dimensional reduction to fetch (default is PCA)
`dims.use`	Dimensions to include (default is all stored dims)
`genes.use`	Genes to include (default is all genes)
`use.full`	Return projected gene loadings (default is FALSE)

Value

Gene loading matrix for given reduction, cells, and genes

Examples

```
pbmc_small
# Examine the head of the first 5 PC gene loadings
head(GetGeneLoadings(object = pbmc_small, reduction.type = "pca", dims.use = 1:5))
```

HoverLocator *Hover Locator*

Description

Get quick information from a scatterplot by hovering over points

Usage

```
HoverLocator(plot, data.plot, features.info = NULL, dark.theme = FALSE, ...)
```

Arguments

plot	A ggplot2 plot
data.plot	The oridinal data that went into the ggplot2 plot
features.info	An optional dataframe or matrix of extra information to be displayed on hover
dark.theme	Plot using a dark theme?
...	Extra parameters to be passed to plotly::layout

See Also

plotly::layout

ggplot2::ggplot_build

Examples

```
## Not run:
df <- data.frame(x = rnorm(n = 100, mean = 20, sd = 2), y = rbinom(n = 100, size = 100, prob = 0.2))
p <- ggplot(data = df, mapping = aes(x = x, y = y)) + geom_point(mapping = aes(color = 'red'))
HoverLocator(plot = p, data.plot = df)

## End(Not run)
```

ICAEmbed *ICA Cell Embeddings Accessor Function*

Description

Pull ICA cell embeddings matrix

Usage

```
ICAEmbed(object, dims.use = NULL, cells.use = NULL)
```

Arguments

object	Seurat object
dims.use	Dimensions to include (default is all stored dims)
cells.use	Cells to include (default is all cells)

Value

ICA cell embeddings matrix for given cells and ICs

Examples

```
pbmc_small
pbmc_small <- RunICA(pbmc_small, ics.compute = 10, ics.print = 0)
head(ICAEmbed(pbmc_small))
# Optionally, you can specify subsets of dims or cells to use
ICAEmbed(pbmc_small, dims.use = 1:5, cells.use = pbmc_small@cell.names[1:5])
```

ICALoad *ICA Gene Loadings Accessor Function*

Description

Pull the ICA gene loadings matrix

Usage

```
ICALoad(object, dims.use = NULL, genes.use = NULL, use.full = FALSE)
```

Arguments

object	Seurat object
dims.use	Dimensions to include (default is all stored dims)
genes.use	Genes to include (default is all)
use.full	Return projected gene loadings (default is FALSE)

Value

ICA gene loading matrix for given genes and ICs

Examples

```
pbmc_small
pbmc_small <- RunICA(pbmc_small, ics.compute = 10, ics.print = 0)
head(ICALoad(pbmc_small))
# Optionally, you can specify subsets of dims or cells to use
ICALoad(pbmc_small, dims.use = 1:5, genes.use = pbmc_small@var.genes[1:5])
```

ICAPlot *Plot ICA map*

Description

Graphs the output of a ICA analysis Cells are colored by their identity class.

Usage

```
ICAPlot(object, ...)
```

Arguments

object	Seurat object
...	Additional parameters to DimPlot, for example, which dimensions to plot.

Details

This function is a wrapper for DimPlot. See ?DimPlot for a full list of possible arguments which can be passed in here.

Examples

```
pbmc_small <- RunICA(object = pbmc_small, ics.compute = 25, print.results = FALSE)
ICAPlot(object = pbmc_small)
```

ICHeatmap *Independent component heatmap*

Description

Draws a heatmap focusing on a principal component. Both cells and genes are sorted by their principal component scores. Allows for nice visualization of sources of heterogeneity in the dataset."()

Usage

```
ICHeatmap(object, ic.use = 1, cells.use = NULL, num.genes = 30,
  disp.min = -2.5, disp.max = 2.5, do.return = FALSE,
  col.use = PurpleAndYellow(), use.scale = TRUE, do.balanced = FALSE,
  remove.key = FALSE, label.columns = NULL, ...)
```

Arguments

object	Seurat object
ic.use	Components to use
cells.use	A list of cells to plot. If numeric, just plots the top cells.
num.genes	NUmber of genes to plot
disp.min	Minimum display value (all values below are clipped)
disp.max	Maximum display value (all values above are clipped)
do.return	If TRUE, returns plot object, otherwise plots plot object
col.use	Colors to plot.
use.scale	Default is TRUE: plot scaled data. If FALSE, plot raw data on the heatmap.
do.balanced	Plot an equal number of genes with both + and - scores.
remove.key	Removes the color key from the plot.
label.columns	Labels for columns
...	Extra parameters passed to DimHeatmap

Value

If do.return==TRUE, a matrix of scaled values which would be passed to heatmap.2. Otherwise, no return value, only a graphical output

Examples

```
pbmc_small <- RunICA(object = pbmc_small, ics.compute = 25, print.results = FALSE)
ICHeatmap(object = pbmc_small)
```

ICTopCells *Find cells with highest ICA scores*

Description

Return a list of genes with the strongest contribution to a set of principal components

Usage

```
ICTopCells(object, ic.use = 1, num.cells = NULL, do.balanced = FALSE)
```

Arguments

object	Seurat object
ic.use	Independent component to use
num.cells	Number of cells to return
do.balanced	Return an equal number of cells with both + and - PC scores.

Value

Returns a vector of cells

Examples

```
pbmc_small
pbmc_small <- RunICA(object = pbmc_small, ics.compute = 10, ics.print = 0)
pbmc_small <- ProjectDim(object = pbmc_small, reduction.type = "ica", do.print = FALSE)
ICTopCells(object = pbmc_small)
# Can specify which dimension and how many cells to return
ICTopCells(object = pbmc_small, ic.use = 2, num.cells = 5)
```

ICTopGenes *Find genes with highest ICA scores*

Description

Return a list of genes with the strongest contribution to a set of indepdendent components

Usage

```
ICTopGenes(object, ic.use = 1, num.genes = 30, use.full = FALSE,
  do.balanced = FALSE)
```

Arguments

object	Seurat object
ic.use	Independent components to use
num.genes	Number of genes to return
use.full	Use the full ICA (projected ICA), default is FALSE
do.balanced	Return an equal number of genes with both + and - IC scores.

Value

Returns a vector of genes

Examples

```
pbmc_small
pbmc_small <- RunICA(object = pbmc_small, ics.compute = 10, ics.print = 0)
pbmc_small <- ProjectDim(object = pbmc_small, reduction.type = "ica", do.print = FALSE)
ICTopGenes(object = pbmc_small, ic.use = 1)
# After projection:
ICTopGenes(object = pbmc_small, ic.use = 1, use.full = TRUE)
```

InitialMapping *Infer spatial origins for single cells*

Description

Probabilistically maps single cells based on (imputed) gene expression estimates, a set of mixture models, and an in situ spatial reference map.

Usage

```
InitialMapping(object, cells.use = NULL)
```

Arguments

object	Seurat object
cells.use	Which cells to map

Value

Seurat object, where mapping probabilities for each bin are stored in object@final.prob

Examples

```
## Not run:
# Note that the PBMC test example object does not contain spatially restricted
# examples below are only demonstrate code
pmbc_small <- InitialMapping(pbmc_small)

## End(Not run)
```

JackStraw *Determine statistical significance of PCA scores.*

Description

Randomly permutes a subset of data, and calculates projected PCA scores for these 'random' genes. Then compares the PCA scores for the 'random' genes with the observed PCA scores to determine statistical signifance. End result is a p-value for each gene's association with each principal component.

Usage

```
JackStraw(object, num.pc = 20, num.replicate = 100, prop.freq = 0.01,
  do.print = FALSE)
```

Arguments

object	Seurat object
num.pc	Number of PCs to compute significance for
num.replicate	Number of replicate samplings to perform
prop.freq	Proportion of the data to randomly permute for each replicate
do.print	Print the number of replicates that have been processed.

Value

Returns a Seurat object where object@dr$pca@jackstraw@emperical.p.value represents p-values for each gene in the PCA analysis. If ProjectPCA is subsequently run, object@dr$pca@jackstraw@emperical.p.value.full then represents p-values for all genes.

References

Inspired by Chung et al, Bioinformatics (2014)

Examples

```
pbmc_small = suppressWarnings(JackStraw(pbmc_small))
head(pbmc_small@dr$pca@jackstraw@emperical.p.value)
```

JackStrawPlot *JackStraw Plot*

Description

Plots the results of the JackStraw analysis for PCA significance. For each PC, plots a QQ-plot comparing the distribution of p-values for all genes across each PC, compared with a uniform distribution. Also determines a p-value for the overall significance of each PC (see Details).

Usage

```
JackStrawPlot(object, PCs = 1:5, nCol = 3, score.thresh = 1e-05,
  plot.x.lim = 0.1, plot.y.lim = 0.3)
```

Arguments

object	Seurat plot
PCs	Which PCs to examine
nCol	Number of columns
score.thresh	Threshold to use for the proportion test of PC significance (see Details)
plot.x.lim	X-axis maximum on each QQ plot.
plot.y.lim	Y-axis maximum on each QQ plot.

Details

Significant PCs should show a p-value distribution (black curve) that is strongly skewed to the left compared to the null distribution (dashed line) The p-value for each PC is based on a proportion test comparing the number of genes with a p-value below a particular threshold (score.thresh), compared with the proportion of genes expected under a uniform distribution of p-values.

Value

A ggplot object

Author(s)

Thanks to Omri Wurtzel for integrating with ggplot

Examples

```
JackStrawPlot(object = pbmc_small)
```

JoyPlot *Single cell joy plot*

Description

Draws a joy plot of single cell data (gene expression, metrics, PC scores, etc.)

Usage

```
JoyPlot(object, features.plot, ident.include = NULL, nCol = NULL,
  do.sort = FALSE, y.max = NULL, same.y.lims = FALSE, size.x.use = 16,
  size.y.use = 16, size.title.use = 20, cols.use = NULL,
  group.by = NULL, y.log = FALSE, x.lab.rot = FALSE, y.lab.rot = FALSE,
  legend.position = "right", single.legend = TRUE, remove.legend = FALSE,
  do.return = FALSE, return.plotlist = FALSE, ...)
```

Arguments

object	Seurat object
features.plot	Features to plot (gene expression, metrics, PC scores, anything that can be retreived by FetchData)
ident.include	Which classes to include in the plot (default is all)
nCol	Number of columns if multiple plots are displayed
do.sort	Sort identity classes (on the x-axis) by the average expression of the attribute being potted
y.max	Maximum y axis value
same.y.lims	Set all the y-axis limits to the same values

size.x.use	X axis title font size
size.y.use	Y axis title font size
size.title.use	Main title font size
cols.use	Colors to use for plotting
group.by	Group (color) cells in different ways (for example, orig.ident)
y.log	plot Y axis on log scale
x.lab.rot	Rotate x-axis labels
y.lab.rot	Rotate y-axis labels
legend.position	Position the legend for the plot
single.legend	Consolidate legend the legend for all plots
remove.legend	Remove the legend from the plot
do.return	Return a ggplot2 object (default : FALSE)
return.plotlist	Return the list of individual plots instead of compiled plot.
...	additional parameters to pass to FetchData (for example, use.imputed, use.scaled, use.raw)

Value

By default, no return, only graphical output. If do.return=TRUE, returns a list of ggplot objects.

Examples

```
JoyPlot(object = pbmc_small, features.plot = 'PC1')
```

KClustDimension *Perform spectral k-means clustering on single cells*

Description

Find point clounds single cells in a low-dimensional space using k-means clustering. Can be useful for smaller datasets, where graph-based clustering can perform poorly

Usage

```
KClustDimension(object, dims.use = c(1, 2), reduction.use = "tsne",
  k.use = 5, set.ident = TRUE, seed.use = 1)
```

Arguments

object	A Seurat object
dims.use	Dimensions to use for clustering
reduction.use	Dimmensional Reduction to use for k-means clustering
k.use	Number of clusters
set.ident	Set identity of Seurat object
seed.use	Random seed to use

Value

Object with clustering information

Examples

```
pbmc_small
# K-means clustering on the first two tSNE dimensions
pbmc_small <- KClustDimension(pbmc_small)
```

KMeansHeatmap *Plot k-means clusters*

Description

Plot k-means clusters

Usage

```
KMeansHeatmap(object, cells.use = object@cell.names, genes.cluster = NULL,
  max.genes = 1e+06, slim.col.label = TRUE, remove.key = TRUE,
  row.lines = TRUE, ...)
```

Arguments

object	A Seurat object
cells.use	Cells to include in the heatmap
genes.cluster	Clusters to include in heatmap
max.genes	Maximum number of genes to include in the heatmap
slim.col.label	Instead of displaying every cell name on the heatmap, display only the identity class name once for each group
remove.key	Removes teh color key from the plot
row.lines	Color separations of clusters
...	Extra parameters to DoHeatmap

See Also

DoHeatmap

Examples

```
pbmc_small <- DoKMeans(object = pbmc_small, k.genes = 3)
KMeansHeatmap(object = pbmc_small)
```

LogNormalize *Normalize raw data*

Description

Normalize count data per cell and transform to log scale

Usage

```
LogNormalize(data, scale.factor = 10000, display.progress = TRUE)
```

Arguments

data	Matrix with the raw count data
scale.factor	Scale the data. Default is 1e4
display.progress	Print progress

Value

Returns a matrix with the normalize and log transformed data

Examples

```
mat <- matrix(data = rbinom(n = 25, size = 5, prob = 0.2), nrow = 5)
mat
mat_norm <- LogNormalize(data = mat)
mat_norm
```

LogVMR *Calculate the variance to mean ratio of logged values*

Description

Calculate the variance to mean ratio (VMR) in non-logspace (return answer in log-space)

Usage

```
LogVMR(x)
```

Arguments

x	A vector of values

Value

Returns the VMR in log-space

Examples

```
LogVMR(x = c(1, 2, 3))
```

MakeSparse *Make object sparse*

Description

Converts stored data matrices to sparse matrices to save space. Converts object@raw.data and object@data to sparse matrices.

Usage

```
MakeSparse(object)
```

Arguments

object	Seurat object

Value

Returns a seurat object with data converted to sparse matrices.

Examples

```
pbmc_raw <- read.table(
  file = system.file('extdata', 'pbmc_raw.txt', package = 'Seurat'),
  as.is = TRUE
)
pbmc_small <- CreateSeuratObject(raw.data = pbmc_raw)
class(x = pbmc_small@raw.data)
pbmc_small <- MakeSparse(object = pbmc_small)
class(x = pbmc_small@raw.data)
```

MarkerTest *ROC-based marker discovery*

Description

Identifies 'markers' of gene expression using ROC analysis. For each gene, evaluates (using AUC) a classifier built on that gene alone, to classify between two groups of cells.

Usage

```
MarkerTest(object, cells.1, cells.2, genes.use = NULL, print.bar = TRUE,
  assay.type = "RNA")
```

Arguments

object	Seurat object
cells.1	Group 1 cells
cells.2	Group 2 cells
genes.use	Genes to test. Default is to use all genes
print.bar	Print a progress bar once expression testing begins (uses pbapply to do this)
assay.type	Type of assay to fetch data for (default is RNA)

Details

An AUC value of 1 means that expression values for this gene alone can perfectly classify the two groupings (i.e. Each of the cells in cells.1 exhibit a higher level than each of the cells in cells.2). An AUC value of 0 also means there is perfect classification, but in the other direction. A value of 0.5 implies that the gene has no predictive power to classify the two groups.

Value

Returns a 'predictive power' (abs(AUC-0.5)) ranked matrix of putative differentially expressed genes.

Examples

```
pbmc_small
MarkerTest(pbmc_small, cells.1 = WhichCells(object = pbmc_small, ident = 1),
            cells.2 = WhichCells(object = pbmc_small, ident = 2))
```

MASTDETest *Differential expression using MAST*

Description

Identifies differentially expressed genes between two groups of cells using a hurdle model tailored to scRNA-seq data. Utilizes the MAST package to run the DE testing.

Usage

```
MASTDETest(object, cells.1, cells.2, min.cells = 3, genes.use = NULL,
  latent.vars = NULL, assay.type = "RNA", ...)
```

Arguments

object	Seurat object
cells.1	Group 1 cells
cells.2	Group 2 cells
min.cells	Minimum number of cells expressing the gene in at least one of the two groups
genes.use	Genes to use for test
latent.vars	Confounding variables to adjust for in DE test. Default is "nUMI", which adjusts for cellular depth (i.e. cellular detection rate). For non-UMI based data, set to nGene instead.
assay.type	Type of assay to fetch data for (default is RNA)
...	Additional parameters to zero-inflated regression (zlm) function in MAST

Details

To use this method, please install MAST, using instructions at https://github.com/RGLab/MAST/

Value

Returns a p-value ranked matrix of putative differentially expressed genes.

References

Andrew McDavid, Greg Finak and Masanao Yajima (2017). MAST: Model-based Analysis of Single Cell Transcriptomics. R package version 1.2.1. https://github.com/RGLab/MAST/

Examples

```
## Not run:
  pbmc_small
  MASTDETest(pbmc_small, cells.1 = WhichCells(object = pbmc_small, ident = 1),
              cells.2 = WhichCells(object = pbmc_small, ident = 2))

## End(Not run)
```

MatrixRowShuffle *Independently shuffle values within each row of a matrix*

Description

Creates a matrix where correlation structure has been removed, but overall values are the same

Usage

```
MatrixRowShuffle(x)
```

Arguments

x	Matrix to shuffle

Value

Returns a scrambled matrix, where each row is shuffled independently

Examples

```
mat <- matrix(data = rbinom(n = 25, size = 20, prob = 0.2 ), nrow = 5)
mat
MatrixRowShuffle(x = mat)
```

MergeNode *Merge childen of a node*

Description

Merge the childen of a node into a single identity class

Usage

```
MergeNode(object, node.use = NULL, rebuild.tree = FALSE, ...)
```

Arguments

object	Seurat object
node.use	Merge children of this node
rebuild.tree	Rebuild cluster tree after the merge?
...	Extra parameters to BuildClusterTree, used only if rebuild.tree = TRUE

See Also

BuildClusterTree

Examples

```
PlotClusterTree(object = pbmc_small)
pbmc_small <- MergeNode(object = pbmc_small, node.use = 7, rebuild.tree = TRUE)
PlotClusterTree(object = pbmc_small)
```

MergeSeurat *Merge Seurat Objects*

Description

Merge two Seurat objects

Usage

```
MergeSeurat(object1, object2, project = NULL, min.cells = 0,
  min.genes = 0, is.expr = 0, do.normalize = TRUE, scale.factor = 10000,
  do.scale = FALSE, do.center = FALSE, names.field = 1,
  names.delim = "_", save.raw = TRUE, add.cell.id1 = NULL,
  add.cell.id2 = NULL)
```

Arguments

object1	First Seurat object to merge
object2	Second Seurat object to merge
project	Project name (string)
min.cells	Include genes with detected expression in at least this many cells
min.genes	Include cells where at least this many genes are detected
is.expr	Expression threshold for 'detected' gene
do.normalize	Normalize the data after merging. Default is TRUE. If set, will perform the same normalization strategy as stored for the first object
scale.factor	If normalizing on the cell level, this sets the scale factor.

do.scale	In object@scale.data, perform row-scaling (gene-based z-score). FALSE by default, so run ScaleData after merging.
do.center	In object@scale.data, perform row-centering (gene-based centering). FALSE by default
names.field	For the initial identity class for each cell, choose this field from the cell's column name
names.delim	For the initial identity class for each cell, choose this delimiter from the cell's column name
save.raw	TRUE by default. If FALSE, do not save the unmodified data in object@raw.data which will save memory downstream for large datasets
add.cell.id1	String to be appended to the names of all cells in object1
add.cell.id2	String to be appended to the names of all cells in object2

Value

Merged Seurat object

Examples

```
# Split pbmc_small for this example
pbmc1 <- SubsetData(object = pbmc_small, cells.use = pbmc_small@cell.names[1:40])
pbmc1
pbmc2 <- SubsetData(object = pbmc_small, cells.use = pbmc_small@cell.names[41:80])
pbmc2
# Merge pbmc1 and pbmc2 into one Seurat object
pbmc_merged <- MergeSeurat(object1 = pbmc1, object2 = pbmc2)
pbmc_merged
```

MinMax *Apply a ceiling and floor to all values in a matrix*

Description

Apply a ceiling and floor to all values in a matrix

Usage

```
MinMax(data, min, max)
```

Arguments

data	Matrix or data frame
min	all values below this min value will be replaced with min
max	all values above this max value will be replaced with max

Value

Returns matrix after performing these floor and ceil operations

Examples

```
mat <- matrix(data = rbinom(n = 25, size = 20, prob = 0.2 ), nrow = 5)
mat
MinMax(data = mat, min = 4, max = 5)
```

NegBinomDETest *Negative binomial test for UMI-count based data*

Description

Identifies differentially expressed genes between two groups of cells using a negative binomial generalized linear model

Usage

```
NegBinomDETest(object, cells.1, cells.2, genes.use = NULL,
  latent.vars = NULL, print.bar = TRUE, min.cells = 3,
  assay.type = "RNA")
```

Arguments

object	Seurat object
cells.1	Group 1 cells
cells.2	Group 2 cells
genes.use	Genes to use for test
latent.vars	Latent variables to test
print.bar	Print progress bar
min.cells	Minimum number of cells threshold
assay.type	Type of assay to fetch data for (default is RNA)

Value

Returns a p-value ranked matrix of putative differentially expressed genes.

Examples

```
pbmc_small
# Note, not recommended for particularly small datasets - expect warnings
NegBinomDETest(pbmc_small, cells.1 = WhichCells(object = pbmc_small, ident = 1),
            cells.2 = WhichCells(object = pbmc_small, ident = 2))
```

NegBinomRegDETest *Negative binomial test for UMI-count based data (regularized version)*

Description

Identifies differentially expressed genes between two groups of cells using a likelihood ratio test of negative binomial generalized linear models where the overdispersion parameter theta is determined by pooling information across genes.

Usage

```
NegBinomRegDETest(object, cells.1, cells.2, genes.use = NULL,
  latent.vars = NULL, print.bar = TRUE, min.cells = 3,
  assay.type = "RNA")
```

Arguments

object	Seurat object
cells.1	Group 1 cells
cells.2	Group 2 cells
genes.use	Genes to use for test
latent.vars	Latent variables to test
print.bar	Print progress bar
min.cells	Minimum number of cells threshold
assay.type	Type of assay to fetch data for (default is RNA)

Value

Returns a p-value ranked data frame of test results.

Examples

```
# Note, not recommended for particularly small datasets - expect warnings
NegBinomDETest(
  object = pbmc_small,
  cells.1 = WhichCells(object = pbmc_small, ident = 1),
  cells.2 = WhichCells(object = pbmc_small, ident = 2)
)
```

NormalizeData *Normalize Assay Data*

Description

Normalize data for a given assay

Usage

```
NormalizeData(object, assay.type = "RNA",
  normalization.method = "LogNormalize", scale.factor = 10000,
  display.progress = TRUE)
```

Arguments

object	Seurat object
assay.type	Type of assay to normalize for (default is RNA), but can be changed for multimodal analyses.
normalization.method	Method for normalization. Default is log-normalization (LogNormalize). More methods to be added very shortly.
scale.factor	Sets the scale factor for cell-level normalization
display.progress	display progress bar for scaling procedure.

Value

Returns object after normalization. Normalized data is stored in data or scale.data slot, depending on the method

Examples

```
pbmc_small
pmbc_small <- NormalizeData(object = pbmc_small)
```

NumberClusters *Convert the cluster labels to a numeric representation*

Description

Convert the cluster labels to a numeric representation

Usage

```
NumberClusters(object)
```

Arguments

object	Seurat object

Value

Returns a Seurat object with the identities relabeled numerically starting from 1.

Examples

```
# Append "Cluster_" to cluster IDs to demonstrate numerical conversion
new.cluster.labels <- paste0("Cluster_", pbmc_small@ident)
pbmc_small <- SetIdent(
  object = pbmc_small,
  cells.use = pbmc_small@cell.names,
  ident.use = new.cluster.labels
)
unique(pbmc_small@ident)
# Now relabel the IDs numerically starting from 1
pbmc_small <- NumberClusters(pbmc_small)
unique(pbmc_small@ident)
```

OldDoHeatmap *Gene expression heatmap*

Description

Draws a heatmap of single cell gene expression using the heatmap.2 function. Has been replaced by the ggplot2 version (now in DoHeatmap), but kept for legacy

Usage

```
OldDoHeatmap(object, cells.use = NULL, genes.use = NULL, disp.min = NULL,
  disp.max = NULL, draw.line = TRUE, do.return = FALSE,
  order.by.ident = TRUE, col.use = PurpleAndYellow(),
  slim.col.label = FALSE, group.by = NULL, remove.key = FALSE,
  cex.col = NULL, do.scale = TRUE, ...)
```

Arguments

object	Seurat object
cells.use	Cells to include in the heatmap (default is all cells)
genes.use	Genes to include in the heatmap (ordered)
disp.min	Minimum display value (all values below are clipped)
disp.max	Maximum display value (all values above are clipped)
draw.line	Draw vertical lines delineating cells in different identity classes.

do.return	Default is FALSE. If TRUE, return a matrix of scaled values which would be passed to heatmap.2
order.by.ident	Order cells in the heatmap by identity class (default is TRUE). If FALSE, cells are ordered based on their order in cells.use
col.use	Color palette to use
slim.col.label	if (order.by.ident==TRUE) then instead of displaying every cell name on the heatmap, display only the identity class name once for each group
group.by	If (order.by.ident==TRUE) default, you can group cells in different ways (for example, orig.ident)
remove.key	Removes the color key from the plot.
cex.col	positive numbers, used as cex.axis in for the column axis labeling. The defaults currently only use number of columns
do.scale	whether to use the data or scaled data
...	Additional parameters to heatmap.2. Common examples are cexRow and cexCol, which set row and column text sizes

Value

If do.return==TRUE, a matrix of scaled values which would be passed to heatmap.2. Otherwise, no return value, only a graphical output

Examples

```
pbmc_small
OldDoHeatmap(object = pbmc_small, genes.use = pbmc_small@var.genes)
```

---

pbmc_small *A small example version of the PBMC dataset*

Description

A subsetted version of 10X Genomics' 3k PBMC dataset

Usage

```
pbmc_small
```

Format

A Seurat object with the following slots filled

raw.data Raw expression data

data Normalized expression data

scale.data Scaled expression data

var.genes Variable genes

dr Dimmensional reductions: currently PCA and tSNE

hvg.info Information about highly variable genes

cluster.tree Cluster tree

calc.params Parameters for calculations done thus far

Source

https://support.10xgenomics.com/single-cell-gene-expression/datasets/1.1.0/pbmc3k

---

`PCAEmbed` *PCA Cell Embeddings Accessor Function*

Description

Pull PCA cell embedding matrix

Usage

```
PCAEmbed(object, dims.use = NULL, cells.use = NULL)
```

Arguments

`object`	Seurat object
`dims.use`	Dimensions to include (default is all stored dims)
`cells.use`	Cells to include (default is all cells)

Value

PCA cell embedding matrix for given cells and PCs

Examples

```
pbmc_small
head(PCAEmbed(pbmc_small))
# Optionally, you can specify subsets of dims or cells to use
PCAEmbed(pbmc_small, dims.use = 1:5, cells.use = pbmc_small@cell.names[1:5])
```

PCALoad *PCA Gene Loadings Accessor Function*

Description

Pull the PCA gene loadings matrix

Usage

```
PCALoad(object, dims.use = NULL, genes.use = NULL, use.full = FALSE)
```

Arguments

object	Seurat object
dims.use	Dimensions to include (default is all stored dims)
genes.use	Genes to include (default is all genes)
use.full	Return projected gene loadings (default is FALSE)

Value

PCA gene loading matrix for given genes and PCs

Examples

```
pbmc_small
head(PCALoad(pbmc_small))
# Optionally, you can specify subsets of dims or genes to use
PCALoad(pbmc_small, dims.use = 1:5, genes.use = pbmc_small@var.genes[1:5])
```

PCAPlot *Plot PCA map*

Description

Graphs the output of a PCA analysis Cells are colored by their identity class.

Usage

```
PCAPlot(object, ...)
```

Arguments

object	Seurat object
...	Additional parameters to DimPlot, for example, which dimensions to plot.

Details

This function is a wrapper for DimPlot. See ?DimPlot for a full list of possible arguments which can be passed in here.

Examples

```
PCAPlot(object = pbmc_small)
```

PCASigGenes *Significant genes from a PCA*

Description

Returns a set of genes, based on the JackStraw analysis, that have statistically significant associations with a set of PCs.

Usage

```
PCASigGenes(object, pcs.use, pval.cut = 0.1, use.full = FALSE,
  max.per.pc = NULL)
```

Arguments

object	Seurat object
pcs.use	PCS to use.
pval.cut	P-value cutoff
use.full	Use the full list of genes (from the projected PCA). Assumes that ProjectPCA has been run. Currently, must be set to FALSE.
max.per.pc	Maximum number of genes to return per PC. Used to avoid genes from one PC dominating the entire analysis.

Value

A vector of genes whose p-values are statistically significant for at least one of the given PCs.

Examples

```
PCASigGenes(pbmc_small, pcs.use = 1:2)
```

PCElbowPlot *Quickly Pick Relevant PCs*

Description

Plots the standard deviations (or approximate singular values if running PCAFast) of the principle components for easy identification of an elbow in the graph. This elbow often corresponds well with the significant PCs and is much faster to run.

Usage

```
PCElbowPlot(object, num.pc = 20)
```

Arguments

object	Seurat object
num.pc	Number of PCs to plot

Value

Returns ggplot object

Examples

```
PCElbowPlot(object = pbmc_small)
```

PCHeatmap *Principal component heatmap*

Description

Draws a heatmap focusing on a principal component. Both cells and genes are sorted by their principal component scores. Allows for nice visualization of sources of heterogeneity in the dataset.

Usage

```
PCHeatmap(object, pc.use = 1, cells.use = NULL, num.genes = 30,
  use.full = FALSE, disp.min = -2.5, disp.max = 2.5, do.return = FALSE,
  col.use = PurpleAndYellow(), use.scale = TRUE, do.balanced = FALSE,
  remove.key = FALSE, label.columns = NULL, ...)
```

Arguments

object	Seurat object.
pc.use	PCs to plot
cells.use	A list of cells to plot. If numeric, just plots the top cells.
num.genes	Number of genes to plot
use.full	Use the full PCA (projected PCA). Default is FALSE
disp.min	Minimum display value (all values below are clipped)
disp.max	Maximum display value (all values above are clipped)
do.return	If TRUE, returns plot object, otherwise plots plot object
col.use	Color to plot.
use.scale	Default is TRUE: plot scaled data. If FALSE, plot raw data on the heatmap.
do.balanced	Plot an equal number of genes with both + and - scores.
remove.key	Removes the color key from the plot.
label.columns	Whether to label the columns. Default is TRUE for 1 PC, FALSE for > 1 PC
...	Extra parameters for DimHeatmap

Value

If do.return==TRUE, a matrix of scaled values which would be passed to heatmap.2. Otherwise, no return value, only a graphical output

Examples

```
PCHeatmap(object = pbmc_small)
```

PCTopCells *Find cells with highest PCA scores*

Description

Return a list of genes with the strongest contribution to a set of principal components

Usage

```
PCTopCells(object, pc.use = 1, num.cells = NULL, do.balanced = FALSE)
```

Arguments

object	Seurat object
pc.use	Principal component to use
num.cells	Number of cells to return
do.balanced	Return an equal number of cells with both + and - PC scores.

Value

Returns a vector of cells

Examples

```
pbmc_small
head(PCTopCells(object = pbmc_small))
# Can specify which dimension and how many cells to return
DimTopCells(object = pbmc_small, dim.use = 2, num.cells = 5)
```

`PCTopGenes` *Find genes with highest PCA scores*

Description

Return a list of genes with the strongest contribution to a set of principal components

Usage

```
PCTopGenes(object, pc.use = 1, num.genes = 30, use.full = FALSE,
  do.balanced = FALSE)
```

Arguments

`object`	Seurat object
`pc.use`	Principal components to use
`num.genes`	Number of genes to return
`use.full`	Use the full PCA (projected PCA). Default is FALSE
`do.balanced`	Return an equal number of genes with both + and - PC scores.

Value

Returns a vector of genes

Examples

```
pbmc_small
PCTopGenes(object = pbmc_small, pc.use = 1)
# After projection:
PCTopGenes(object = pbmc_small, pc.use = 1, use.full = TRUE)
```

PlotClusterTree *Plot phylogenetic tree*

Description

Plots previously computed phylogenetic tree (from BuildClusterTree)

Usage

```
PlotClusterTree(object, ...)
```

Arguments

object	Seurat object
...	Additional arguments for plotting the phylogeny

Value

Plots dendogram (must be precomputed using BuildClusterTree), returns no value

Examples

```
PlotClusterTree(object = pbmc_small)
```

PoissonDETest *Poisson test for UMI-count based data*

Description

Identifies differentially expressed genes between two groups of cells using a poisson generalized linear model

Usage

```
PoissonDETest(object, cells.1, cells.2, min.cells = 3, genes.use = NULL,
  latent.vars = NULL, print.bar = TRUE, assay.type = "RNA")
```

Arguments

object	Seurat object
cells.1	Group 1 cells
cells.2	Group 2 cells
min.cells	Minimum number of cells expressing the gene in at least one of the two groups
genes.use	Genes to use for test
latent.vars	Latent variables to test
print.bar	Print progress bar
assay.type	Type of assay to fetch data for (default is RNA)

Value

Returns a p-value ranked matrix of putative differentially expressed genes.

Examples

```
pbmc_small
# Note, expect warnings with example dataset due to min.cells threshold.
PoissonDETest(pbmc_small, cells.1 = WhichCells(object = pbmc_small, ident = 1),
            cells.2 = WhichCells(object = pbmc_small, ident = 2))
```

PrintAlignSubspaceParams

Print AlignSubspace Calculation Parameters

Description

Print the parameters chosen for the latest AlignSubspace calculation for each stored aligned subspace.

Usage

```
PrintAlignSubspaceParams(object, raw = FALSE)
```

Arguments

object	Seurat object
raw	Print the entire contents of the calculation settings slot (calc.params) for the AlignSubspace calculation. Default (FALSE) will print a nicely formatted summary.

Value

No return value. Only prints to console.

Examples

```
## Not run:
# Requires CCA to have previously been run
# As CCA requires two datasets, we will split our test object into two just for this example
pbmc1 <- SubsetData(pbmc_small,cells.use = pbmc_small@cell.names[1:40])
pbmc2 <- SubsetData(pbmc_small,cells.use = pbmc_small@cell.names[41:80])
pbmc1@meta.data$group <- "group1"
pbmc2@meta.data$group <- "group2"
pbmc_cca <- RunCCA(pbmc1,pbmc2)
pbmc_cca <- AlignSubspace(pbmc_cca,reduction.type = "cca", grouping.var = "group", dims.align = 1:2)
PrintAlignSubspaceParams(object = pbmc_small)

## End(Not run)
```

PrintCalcParams *Print the calculation*

Description

Print entire contents of calculation settings slot (calc.params) for given calculation.

Usage

```
PrintCalcParams(object, calculation, raw = FALSE, return.list = FALSE)
```

Arguments

object	Seurat object
calculation	Name of calculation (function name) to check parameters for
raw	Print the entire contents of the calculation settings slot (calc.params) for the RunPCA calculation.
return.list	Return the calculation parameters as a list

Value

Prints the calculation settings and optionally returns them as a list

Examples

```
PrintCalcParams(object = pbmc_small, calculation = 'RunPCA')
PrintCalcParams(object = pbmc_small, calculation = 'RunPCA', raw = TRUE)
```

PrintCalcVarExpRatioParams *Print Parameters Associated with CalcVarExpRatio*

Description

Print the parameters chosen for CalcVarExpRatio.

Usage

```
PrintCalcVarExpRatioParams(object, raw = FALSE)
```

Arguments

object	Seurat object
raw	Print the entire contents of the calculation settings slot (calc.params) for CalcVarExpRatio. Default (FALSE) will print a nicely formatted summary.

Value

No return value. Only prints to console.

Examples

```
# Requires CCA to have previously been run
# As CCA requires two datasets, we will split our test object into two just for this example
pbmc1 <- SubsetData(pbmc_small,cells.use = pbmc_small@cell.names[1:40])
pbmc2 <- SubsetData(pbmc_small,cells.use = pbmc_small@cell.names[41:80])
pbmc1@meta.data$group <- "group1"
pbmc2@meta.data$group <- "group2"
pbmc_cca <- RunCCA(pbmc1,pbmc2)
pbmc_cca <- CalcVarExpRatio(pbmc_cca,reduction.type = "pca", grouping.var = "group", dims.use = 1:5)
PrintCalcVarExpRatioParams(object = pbmc_cca)
```

PrintCCAParams *Print CCA Calculation Parameters*

Description

Print the parameters chosen for the latest stored CCA calculation.

Usage

```
PrintCCAParams(object, raw = FALSE)
```

Arguments

object	Seurat object
raw	Print the entire contents of the calculation settings slot (calc.params) for the RunCCA calculation. Default (FALSE) will print a nicely formatted summary.

Value

No return value. Only prints to console.

Examples

```
# As CCA requires two datasets, we will split our test object into two just for this example
pbmc1 <- SubsetData(pbmc_small,cells.use = pbmc_small@cell.names[1:40])
pbmc2 <- SubsetData(pbmc_small,cells.use = pbmc_small@cell.names[41:80])
pbmc1@meta.data$group <- "group1"
pbmc2@meta.data$group <- "group2"
pbmc_cca <- RunCCA(pbmc1,pbmc2)
PrintCCAParams(object = pbmc_cca)
```

PrintDim *Print the results of a dimensional reduction analysis*

Description

Prints a set of genes that most strongly define a set of components

Usage

```
PrintDim(object, reduction.type = "pca", dims.print = 1:5,
  genes.print = 30, use.full = FALSE)
```

Arguments

object	Seurat object
reduction.type	Reduction technique to print results for
dims.print	Number of dimensions to display
genes.print	Number of genes to display
use.full	Use full PCA (i.e. the projected PCA, by default FALSE)

Value

Set of genes defining the components

Examples

```
pbmc_small
PrintDim(object = pbmc_small, reduction.type = "pca")
# Options for how many dimensions and how many genes to print
PrintDim(object = pbmc_small, reduction.type = "pca", dims.print = 1:2, genes.print = 5)
# Can also print for the projected PCA
PrintDim(object = pbmc_small, reduction.type = "pca", use.full = TRUE)
```

PrintDMParams *Print Diffusion Map Calculation Parameters*

Description

Print the parameters chosen for the latest stored diffusion map calculation.

Usage

```
PrintDMParams(object, raw = FALSE)
```

Arguments

object	Seurat object
raw	Print the entire contents of the calculation settings slot (calc.params) for the RunDiffusion calculation. Default (FALSE) will print a nicely formatted summary.

Value

No return value. Only prints to console.

Examples

```
# Run Diffusion on variable genes
pbmc_small <- RunDiffusion(pbmc_small,genes.use = pbmc_small@var.genes)
PrintDMParams(object = pbmc_small)
```

PrintFindClustersParams *Print FindClusters Calculation Parameters*

Description

Print the parameters chosen for the latest FindClusters calculation for each stored resolution.

Usage

```
PrintFindClustersParams(object, resolution, raw = FALSE)
```

Arguments

object	Seurat object
resolution	Optionally specify only a subset of resolutions to print parameters for.
raw	Print the entire contents of the calculation settings slot (calc.params) for the FindClusters calculation. Default (FALSE) will print a nicely formatted summary.

Value

No return value. Only prints to console.

Examples

```
PrintFindClustersParams(object = pbmc_small, raw = TRUE)
```

PrintICA *Print the results of a ICA analysis*

Description

Prints a set of genes that most strongly define a set of independent components

Usage

```
PrintICA(object, ics.print = 1:5, genes.print = 30, use.full = FALSE)
```

Arguments

object	Seurat object
ics.print	Set of ICs to print genes for
genes.print	Number of genes to print for each PC
use.full	Use full PCA (i.e. the projected PCA, by default FALSE)

Value

Only text output

Examples

```
pbmc_small
pbmc_small <- RunICA(object = pbmc_small, ics.compute = 10, ics.print = 0)
pbmc_small <- ProjectDim(object = pbmc_small, reduction.type = "ica", do.print = FALSE)
PrintICA(object = pbmc_small)
# Options for how many dimensions and how many genes to print
PrintICA(object = pbmc_small, ics.print = 1:2, genes.print = 5)
# Can also print for the projected PCA
PrintICA(object = pbmc_small, use.full = TRUE)
```

PrintICAParams *Print ICA Calculation Parameters*

Description

Print the parameters chosen for the latest stored ICA calculation.

Usage

```
PrintICAParams(object, raw = FALSE)
```

Arguments

object	Seurat object
raw	Print the entire contents of the calculation settings slot (calc.params) for the ICA calculation. Default (FALSE) will print a nicely formatted summary.

Value

No return value. Only prints to console.

Examples

```
pbmc_small <- RunICA(object = pbmc_small, ics.compute = 5)
PrintICAParams(object = pbmc_small, raw = TRUE)
```

PrintPCA *Print the results of a PCA analysis*

Description

Prints a set of genes that most strongly define a set of principal components

Usage

```
PrintPCA(object, pcs.print = 1:5, genes.print = 30, use.full = FALSE)
```

Arguments

object	Seurat object
pcs.print	Set of PCs to print genes for
genes.print	Number of genes to print for each PC
use.full	Use full PCA (i.e. the projected PCA, by default FALSE)

Value

Only text output

Examples

```
pbmc_small
PrintPCA(object = pbmc_small)
# Options for how many dimensions and how many genes to print
PrintPCA(object = pbmc_small, pcs.print = 1:2, genes.print = 5)
# Can also print for the projected PCA
PrintPCA(object = pbmc_small, use.full = TRUE)
```

PrintPCAParams *Print PCA Calculation Parameters*

Description

Print the parameters chosen for the latest stored PCA calculation.

Usage

```
PrintPCAParams(object, raw = FALSE)
```

Arguments

object	Seurat object
raw	Print the entire contents of the calculation settings slot (calc.params) for the RunPCA calculation. Default (FALSE) will print a nicely formatted summary.

Value

No return value. Only prints to console.

Examples

```
PrintPCAParams(object = pbmc_small)
```

PrintSNNParams *Print SNN Construction Calculation Parameters*

Description

Print the parameters chosen for the latest stored SNN calculation (via BuildSNN or FindClusters).

Usage

```
PrintSNNParams(object, raw = FALSE)
```

Arguments

object	Seurat object
raw	Print the entire contents of the calculation settings slot (calc.params) for the BuildSNN calculation. Default (FALSE) will print a nicely formatted summary.

Value

No return value. Only prints to console.

Examples

```
pbmc_small <- BuildSNN(object = pbmc_small)
PrintSNNParams(object = pbmc_small)
```

PrintTSNEParams *Print TSNE Calculation Parameters*

Description

Print the parameters chosen for the latest stored TSNE calculation.

Usage

```
PrintTSNEParams(object, raw = FALSE)
```

Arguments

object	Seurat object
raw	Print the entire contents of the calculation settings slot (calc.params) for the RunTSNE calculation. Default (FALSE) will print a nicely formatted summary.

Value

No return value. Only prints to console.

Examples

```
PrintTSNEParams(object = pbmc_small)
```

ProjectDim *Project Dimensional reduction onto full dataset*

Description

Takes a pre-computed dimensional reduction (typically calculated on a subset of genes) and projects this onto the entire dataset (all genes). Note that the cell loadings will remain unchanged, but now there are gene loadings for all genes.

Usage

```
ProjectDim(object, reduction.type = "pca", dims.print = 1:5,
  dims.store = 30, genes.print = 30, replace.dim = FALSE,
  do.center = FALSE, do.print = TRUE, assay.type = "RNA")
```

Arguments

object	Seurat object
reduction.type	Reduction to use
dims.print	Number of dims to print genes for
dims.store	Number of dims to store (default is 30)
genes.print	Number of genes with highest/lowest loadings to print for each PC
replace.dim	Replace the existing data (overwrite object@dr$XXX@gene.loadings), not done by default.
do.center	Center the dataset prior to projection (should be set to TRUE)
do.print	Print top genes associated with the projected dimensions
assay.type	Data type, RNA by default. Can be changed for multimodal datasets (i.e. project a PCA done on RNA, onto CITE-seq data)

Value

Returns Seurat object with the projected values in object@dr$XXX@gene.loadings.full

Examples

```
pbmc_small
pbmc_small <- ProjectDim(pbmc_small, reduction.type = "pca")
# Vizualize top projected genes in heatmap
DimHeatmap(pbmc_small,pc.use = 1,use.full = TRUE,do.balanced = TRUE,reduction.type = "pca")
```

ProjectPCA *Project Principal Components Analysis onto full dataset*

Description

Takes a pre-computed PCA (typically calculated on a subset of genes) and projects this onto the entire dataset (all genes). Note that the cell loadings remains unchanged, but now there are gene loading scores for all genes.

Usage

```
ProjectPCA(object, do.print = TRUE, pcs.print = 1:5, pcs.store = 30,
  genes.print = 30, replace.pc = FALSE, do.center = FALSE)
```

Arguments

object	Seurat object
do.print	Print top genes associated with the projected PCs
pcs.print	Number of PCs to print genes for
pcs.store	Number of PCs to store (default is 30)
genes.print	Number of genes with highest/lowest loadings to print for each PC
replace.pc	Replace the existing PCA (overwite object@dr$pca@gene.loadings), not done by default.
do.center	Center the dataset prior to projection (should be set to TRUE)

Value

Returns Seurat object with the projected PCA values in object@dr$pca@gene.loadings.full

Examples

```
pbmc_small
pbmc_small <- ProjectPCA(pbmc_small)
# Vizualize top projected genes in heatmap
PCHeatmap(pbmc_small,pc.use = 1,use.full = TRUE,do.balanced = TRUE)
```

PurpleAndYellow *A purple and yellow color palette*

Description

A purple and yellow color palette

Usage

```
PurpleAndYellow(...)
```

Arguments

...	Extra parameters to CustomPalette

Value

A color palette

See Also

CustomPalette

Examples

```
df <- data.frame(x = rnorm(n = 100, mean = 20, sd = 2), y = rbinom(n = 100, size = 100, prob = 0.2))
plot(df, col = BlackAndWhite())
```

`Read10X` *Load in data from 10X*

Description

Enables easy loading of sparse data matrices provided by 10X genomics.

Usage

```
Read10X(data.dir = NULL)
```

Arguments

`data.dir`	Directory containing the matrix.mtx, genes.tsv, and barcodes.tsv files provided by 10X. A vector or named vector can be given in order to load several data directories. If a named vector is given, the cell barcode names will be prefixed with the name.

Value

Returns a sparse matrix with rows and columns labeled

Examples

```
## Not run:
data_dir <- 'path/to/data/directory'
list.files(data_dir) # Should show barcodes.tsv, genes.tsv, and matrix.mtx
expression_matrix <- Read10X(data.dir = data_dir)
seurat_object = CreateSeuratObject(raw.data = expression_matrix)

## End(Not run)
```

RefinedMapping *Quantitative refinement of spatial inferences*

Description

Refines the initial mapping with more complex models that allow gene expression to vary quantitatively across bins (instead of 'on' or 'off'), and that also considers the covariance structure between genes.

Usage

```
RefinedMapping(object, genes.use)
```

Arguments

object	Seurat object
genes.use	Genes to use to drive the refinement procedure.

Details

Full details given in spatial mapping manuscript.

Value

Seurat object, where mapping probabilities for each bin are stored in object@final.prob

Examples

```
## Not run:
# Note that the PBMC test example object does not contain spatially restricted
# examples below are only demonstrate code
pmbc_small <- RefinedMapping(pbmc_small, genes.use=pbmc_small@var.genes)

## End(Not run)
```

RemoveFromTable *Remove data from a table*

Description

This function will remove any rows from a data frame or matrix that contain certain values

Usage

```
RemoveFromTable(to.remove, data)
```

Arguments

to.remove	A vector of values that indicate removal
data	A data frame or matrix

Value

A data frame or matrix with values removed by row

Examples

```
df <- data.frame(
  x = rnorm(n = 100, mean = 20, sd = 2),
  y = rbinom(n = 100, size = 100, prob = 0.2)
)
nrow(x = df)
nrow (x = RemoveFromTable(to.remove = 20, data = df))
```

RenameIdent *Rename one identity class to another*

Description

Can also be used to join identity classes together (for example, to merge clusters).

Usage

```
RenameIdent(object, old.ident.name = NULL, new.ident.name = NULL)
```

Arguments

object	Seurat object
old.ident.name	The old identity class (to be renamed)
new.ident.name	The new name to apply

Value

A Seurat object where object@ident has been appropriately modified

Examples

```
head(x = pbmc_small@ident)
pbmc_small <- RenameIdent(
  object = pbmc_small,
  old.ident.name = 0,
  new.ident.name = 'cluster_0'
)
head(x = pbmc_small@ident)
```

ReorderIdent *Reorder identity classes*

Description

Re-assigns the identity classes according to the average expression of a particular feature (i.e, gene expression, or PC score) Very useful after clustering, to re-order cells, for example, based on PC scores

Usage

```
ReorderIdent(object, feature = "PC1", rev = FALSE, aggregate.fxn = mean,
  reorder.numeric = FALSE, ...)
```

Arguments

object	Seurat object
feature	Feature to reorder on. Default is PC1
rev	Reverse ordering (default is FALSE)
aggregate.fxn	Function to evaluate each identity class based on (default is mean)
reorder.numeric	Rename all identity classes to be increasing numbers starting from 1 (default is FALSE)
...	additional arguemnts (i.e. use.imputed=TRUE)

Value

A seurat object where the identity have been re-oredered based on the average.

Examples

```
head(x = pbmc_small@ident)
pbmc_small <- ReorderIdent(object = pbmc_small)
head(x = pbmc_small@ident)
```

RunCCA *Perform Canonical Correlation Analysis*

Description

Runs a canonical correlation analysis using a diagonal implementation of CCA. For details about stored CCA calculation parameters, see PrintCCAParams.

Usage

```
RunCCA(object, object2, group1, group2, group.by, num.cc = 20, genes.use,
  scale.data = TRUE, rescale.groups = FALSE, ...)
```

Arguments

object	Seurat object
object2	Optional second object. If object2 is passed, object1 will be considered as group1 and object2 as group2.
group1	First set of cells (or IDs) for CCA
group2	Second set of cells (or IDs) for CCA
group.by	Factor to group by (column vector stored in object@meta.data)
num.cc	Number of canonical vectors to calculate
genes.use	Set of genes to use in CCA. Default is object@var.genes. If two objects are given, the default is the union of both variable gene sets that are also present in both objects.
scale.data	Use the scaled data from the object
rescale.groups	Rescale each set of cells independently
...	Extra parameters (passed onto MergeSeurat in case with two objects passed, passed onto ScaleData in case with single object and rescale.groups set to TRUE)

Value

Returns Seurat object with the CCA stored in the @dr$cca slot. If one object is passed, the same object is returned. If two are passed, a combined object is returned.

See Also

MergeSeurat

Examples

```
pbmc_small
# As CCA requires two datasets, we will split our test object into two just for this example
pbmc1 <- SubsetData(pbmc_small,cells.use = pbmc_small@cell.names[1:40])
pbmc2 <- SubsetData(pbmc_small,cells.use = pbmc_small@cell.names[41:80])
pbmc1@meta.data$group <- "group1"
pbmc2@meta.data$group <- "group2"
pbmc_cca <- RunCCA(pbmc1,pbmc2)
# Print results
PrintDim(pbmc_cca,reduction.type = 'cca')
```

RunDiffusion *Run diffusion map*

Description

Run diffusion map

Usage

```
RunDiffusion(object, cells.use = NULL, dims.use = 1:5, genes.use = NULL,
  reduction.use = "pca", q.use = 0.01, max.dim = 2, scale.clip = 10,
  reduction.name = "dm", reduction.key = "DM", ...)
```

Arguments

object	Seurat object
cells.use	Which cells to analyze (default, all cells)
dims.use	Which dimensions to use as input features
genes.use	If set, run the diffusion map procedure on this subset of genes (instead of running on a set of reduced dimensions). Not set (NULL) by default
reduction.use	Which dimensional reduction (PCA or ICA) to use for the diffusion map input. Default is PCA
q.use	Quantile to clip diffusion map components at. This addresses an issue where 1-2 cells will have extreme values that obscure all other points. 0.01 by default
max.dim	Max dimension to keep from diffusion calculation
scale.clip	Max/min value for scaled data. Default is 3
reduction.name	dimensional reduction name, specifies the position in the object$dr list. dm by default
reduction.key	dimensional reduction key, specifies the string before the number for the dimension names. DM by default
...	Additional arguments to the diffuse call

Value

Returns a Seurat object with a diffusion map

Examples

```
pbmc_small
# Run Diffusion on variable genes
pbmc_small <- RunDiffusion(pbmc_small,genes.use = pbmc_small@var.genes)
# Run Diffusion map on first 10 PCs
pbmc_small <- RunDiffusion(pbmc_small,genes.use = pbmc_small@var.genes)
# Plot results
DMPlot(pbmc_small)
```

RunICA *Run Independent Component Analysis on gene expression*

Description

Run fastica algorithm from the ica package for ICA dimensionality reduction. For details about stored ICA calculation parameters, see PrintICAParams.

Usage

```
RunICA(object, ic.genes = NULL, ics.compute = 50, use.imputed = FALSE,
  rev.ica = FALSE, print.results = TRUE, ics.print = 1:5,
  genes.print = 50, ica.function = "icafast", seed.use = 1,
  reduction.name = "ica", reduction.key = "IC", ...)
```

Arguments

object	Seurat object
ic.genes	Genes to use as input for ICA. Default is object@var.genes
ics.compute	Number of ICs to compute
use.imputed	Run ICA on imputed values (FALSE by default)
rev.ica	By default, computes the dimensional reduction on the cell x gene matrix. Setting to true will compute it on the transpose (gene x cell matrix).
print.results	Print the top genes associated with each dimension
ics.print	ICs to print genes for
genes.print	Number of genes to print for each IC
ica.function	ICA function from ica package to run (options: icafast, icaimax, icajade)
seed.use	Random seed to use for fastica
reduction.name	dimensional reduction name, specifies the position in the object$dr list. ica by default
reduction.key	dimensional reduction key, specifies the string before the number for the dimension names. IC by default
...	Additional arguments to be passed to fastica

Value

Returns Seurat object with an ICA calculation stored in object@dr$ica

Examples

```
pbmc_small
# Run ICA on variable genes (default)
pbmc_small <- RunICA(pbmc_small, ics.compute=5)
# Run ICA on different gene set (in this case all genes)
pbmc_small <- RunICA(pbmc_small, ic.genes = rownames(pbmc_small@data))
# Plot results
ICAPlot(pbmc_small)
```

RunPCA *Run Principal Component Analysis on gene expression using IRLBA*

Description

Run a PCA dimensionality reduction. For details about stored PCA calculation parameters, see `PrintPCAParams`.

Usage

```
RunPCA(object, pc.genes = NULL, pcs.compute = 20, use.imputed = FALSE,
  rev.pca = FALSE, weight.by.var = TRUE, do.print = TRUE,
  pcs.print = 1:5, genes.print = 30, reduction.name = "pca",
  reduction.key = "PC", assay.type = "RNA", ...)
```

Arguments

`object`	Seurat object
`pc.genes`	Genes to use as input for PCA. Default is object@var.genes
`pcs.compute`	Total Number of PCs to compute and store (20 by default)
`use.imputed`	Run PCA on imputed values (FALSE by default)
`rev.pca`	By default computes the PCA on the cell x gene matrix. Setting to true will compute it on gene x cell matrix.
`weight.by.var`	Weight the cell embeddings by the variance of each PC (weights the gene loadings if rev.pca is TRUE)
`do.print`	Print the top genes associated with high/low loadings for the PCs
`pcs.print`	PCs to print genes for
`genes.print`	Number of genes to print for each PC
`reduction.name`	dimensional reduction name, specifies the position in the object$dr list. pca by default

reduction.key	dimensional reduction key, specifies the string before the number for the dimension names. PC by default
assay.type	Data type, RNA by default. Can be changed for multimodal
...	Additional arguments to be passed to IRLBA

Value

Returns Seurat object with the PCA calculation stored in object@dr$pca.

Examples

```
pbmc_small
# Run PCA on variable genes (default)
pbmc_small <- RunPCA(pbmc_small)
# Run PCA on different gene set (in this case all genes)
pbmc_small=RunPCA(pbmc_small,pc.genes = rownames(pbmc_small@data))
# Run PCA but compute more than 20 dimensions
pbmc_small=RunPCA(pbmc_small,pcs.compute=30)
# Plot results
PCAPlot(pbmc_small)
```

RunTSNE *Run t-distributed Stochastic Neighbor Embedding*

Description

Run t-SNE dimensionality reduction on selected features. Has the option of running in a reduced dimensional space (i.e. spectral tSNE, recommended), or running based on a set of genes. For details about stored TSNE calculation parameters, see PrintTSNEParams.

Usage

```
RunTSNE(object, reduction.use = "pca", cells.use = NULL, dims.use = 1:5,
  genes.use = NULL, seed.use = 1, do.fast = TRUE, add.iter = 0,
  dim.embed = 2, distance.matrix = NULL, reduction.name = "tsne",
  reduction.key = "tSNE_", ...)
```

Arguments

object	Seurat object
reduction.use	Which dimensional reduction (e.g. PCA, ICA) to use for the tSNE. Default is PCA
cells.use	Which cells to analyze (default, all cells)
dims.use	Which dimensions to use as input features
genes.use	If set, run the tSNE on this subset of genes (instead of running on a set of reduced dimensions). Not set (NULL) by default

seed.use	Random seed for the t-SNE
do.fast	If TRUE, uses the Barnes-hut implementation, which runs faster, but is less flexible. TRUE by default.
add.iter	If an existing tSNE has already been computed, uses the current tSNE to seed the algorithm and then adds additional iterations on top of this
dim.embed	The dimensional space of the resulting tSNE embedding (default is 2). For example, set to 3 for a 3d tSNE
distance.matrix	If set, runs tSNE on the given distance matrix instead of data matrix (experimental)
reduction.name	dimensional reduction name, specifies the position in the object$dr list. tsne by default
reduction.key	dimensional reduction key, specifies the string before the number for the dimension names. tSNE_ by default
...	Additional arguments to the tSNE call. Most commonly used is perplexity (expected number of neighbors default is 30)

Value

Returns a Seurat object with a tSNE embedding in object@dr$tsne@cell.embeddings

Examples

```
pbmc_small
# Run tSNE on first five PCs, note that for test dataset (only 80 cells)
# we can't use default perplexity of 30
pbmc_small <- RunTSNE(pbmc_small, reduction.use = "pca", dims.use = 1:5, perplexity=10)
# Run tSNE on first five independent components from ICA
pbmc_small <- RunICA(pbmc_small,ics.compute=5)
pbmc_small <- RunTSNE(pbmc_small, reduction.use = "ica", dims.use = 1:5, perplexity=10)
# Plot results
TSNEPlot(pbmc_small)
```

SampleUMI *Sample UMI*

Description

Downsample each cell to a specified number of UMIs. Includes an option to upsample cells below specified UMI as well.

Usage

```
SampleUMI(data, max.umi = 1000, upsample = FALSE, progress.bar = TRUE)
```

Arguments

data	Matrix with the raw count data
max.umi	Number of UMIs to sample to
upsample	Upsamples all cells with fewer than max.umi
progress.bar	Display the progress bar

Value

Matrix with downsampled data

Examples

```
raw_data = as.matrix(x = pbmc_small@raw.data)
downsampled = SampleUMI(data = raw_data)
head(x = downsampled)
```

SaveClusters *Save cluster assignments to a TSV file*

Description

Save cluster assignments to a TSV file

Usage

```
SaveClusters(object, file)
```

Arguments

object	Seurat object with cluster assignments
file	Path to file to write cluster assignments to

Value

No return value. Writes clusters assignments to specified file.

Examples

```
## Not run:
pbmc_small
file.loc <- "~/Desktop/cluster_assignments.tsv"
SaveClusters(object = pbmc_small, file = file.loc)

## End(Not run)
```

`ScaleData` *Scale and center the data.*

Description

Scales and centers genes in the dataset. If variables are provided in vars.to.regress, they are individually regressed against each gene, and the resulting residuals are then scaled and centered.

Usage

```
ScaleData(object, genes.use = NULL, data.use = NULL, vars.to.regress,
  model.use = "linear", use.umi = FALSE, do.scale = TRUE,
  do.center = TRUE, scale.max = 10, block.size = 1000,
  min.cells.to.block = 3000, display.progress = TRUE, assay.type = "RNA",
  do.cpp = TRUE, check.for.norm = TRUE)
```

Arguments

`object`	Seurat object
`genes.use`	Vector of gene names to scale/center. Default is all genes in object@data.
`data.use`	Can optionally pass a matrix of data to scale, default is object@data[genes.use,]
`vars.to.regress`	Variables to regress out (previously latent.vars in RegressOut). For example, nUMI, or percent.mito.
`model.use`	Use a linear model or generalized linear model (poisson, negative binomial) for the regression. Options are 'linear' (default), 'poisson', and 'negbinom'
`use.umi`	Regress on UMI count data. Default is FALSE for linear modeling, but automatically set to TRUE if model.use is 'negbinom' or 'poisson'
`do.scale`	Whether to scale the data.
`do.center`	Whether to center the data.
`scale.max`	Max value to return for scaled data. The default is 10. Setting this can help reduce the effects of genes that are only expressed in a very small number of cells. If regressing out latent variables and using a non-linear model, the default is 50.
`block.size`	Default size for number of genes to scale at in a single computation. Increasing block.size may speed up calculations but at an additional memory cost.
`min.cells.to.block`	If object contains fewer than this number of cells, don't block for scaling calculations.
`display.progress`	Displays a progress bar for scaling procedure
`assay.type`	Assay to scale data for. Default is RNA. Can be changed for multimodal analyses.

do.cpp	By default (TRUE), most of the heavy lifting is done in c++. We've maintained support for our previous implementation in R for reproducibility (set this to FALSE) as results can change slightly due to differences in numerical precision which could affect downstream calculations.
check.for.norm	Check to see if data has been normalized, if not, output a warning (TRUE by default)

Details

ScaleData now incorporates the functionality of the function formerly known as RegressOut (which regressed out given the effects of provided variables and then scaled the residuals). To make use of the regression functionality, simply pass the variables you want to remove to the vars.to.regress parameter.

Setting center to TRUE will center the expression for each gene by subtracting the average expression for that gene. Setting scale to TRUE will scale the expression level for each gene by dividing the centered gene expression levels by their standard deviations if center is TRUE and by their root mean square otherwise.

Value

Returns a seurat object with object@scale.data updated with scaled and/or centered data.

Examples

```
pbmc_small <- ScaleData(object = pbmc_small)
## Not run:
# To regress out certain effects
pbmc_small = ScaleData(object = pbmc_small, vars.to.regress = effects_list)

## End(Not run)
```

ScaleDataR *Old R based implementation of ScaleData. Scales and centers the data*

Description

Old R based implementation of ScaleData. Scales and centers the data

Usage

```
ScaleDataR(object, genes.use = NULL, data.use = NULL, do.scale = TRUE,
  do.center = TRUE, scale.max = 10)
```

Arguments

object	Seurat object
genes.use	Vector of gene names to scale/center. Default is all genes in object@data.
data.use	Can optionally pass a matrix of data to scale, default is object@data[genes.use,]
do.scale	Whether to scale the data.
do.center	Whether to center the data.
scale.max	Max value to accept for scaled data. The default is 10. Setting this can help reduce the effects of genes that are only expressed in a very small number of cells.

Value

Returns a seurat object with object@scale.data updated with scaled and/or centered data.

Examples

```
## Not run:
pbmc_small <- ScaleDataR(object = pbmc_small)

## End(Not run)
```

SetAllIdent *Switch identity class definition to another variable*

Description

Switch identity class definition to another variable

Usage

```
SetAllIdent(object, id = NULL)
```

Arguments

object	Seurat object
id	Variable to switch identity class to (for example, 'DBclust.ident', the output of density clustering) Default is orig.ident - the original annotation pulled from the cell name.

Value

A Seurat object where object@ident has been appropriately modified

Examples

```
head(x = pbmc_small@ident)
pbmc_small <- SetAllIdent(object = pbmc_small, id = 'orig.ident')
head(x = pbmc_small@ident)
```

SetAssayData *Assay Data Mutator Function*

Description

Store information for specified assay, for multimodal analysis. new.data needs to have cells as the columns and measurement features (e.g. genes, proteins, etc ...) as rows. Additionally, all the cell names in the new.data must match the cell names in the object (object@cell.names).

Usage

```
SetAssayData(object, assay.type, slot, new.data)
```

Arguments

object	Seurat object
assay.type	Type of assay to fetch data for (default is RNA)
slot	Specific information to pull (i.e. raw.data, data, scale.data,...). Default is data
new.data	New data to insert

Value

Seurat object with updated slot

Examples

```
# Simulate CITE-Seq results
df <- t(x = data.frame(
  x = round(x = rnorm(n = 80, mean = 20, sd = 2)),
  y = round(x = rbinom(n = 80, size = 100, prob = 0.2))
))
pbmc_small = SetAssayData(
  object = pbmc_small,
  assay.type = 'CITE',
  new.data = df,
  slot = 'raw.data'
)
```

SetClusters *Set Cluster Assignments*

Description

Easily set the cluster assignments using the output of GetClusters() — a dataframe with cell names as the first column and cluster assignments as the second.

Usage

```
SetClusters(object, clusters = NULL)
```

Arguments

object	Seurat object
clusters	A dataframe containing the cell names and cluster assignments to set for the object.

Value

Returns a Seurat object with the identities set to the cluster assignments that were passed.

Examples

```
pbmc_small
# Get clusters as a dataframe with GetClusters.
clusters <- GetClusters(object = pbmc_small)
# Use SetClusters to set cluster IDs
pbmc_small <- SetClusters(object = pbmc_small, clusters = clusters)
```

SetDimReduction *Dimensional Reduction Mutator Function*

Description

Set information for specified stored dimensional reduction analysis

Usage

```
SetDimReduction(object, reduction.type, slot, new.data)
```

Arguments

object	Seurat object
reduction.type	Type of dimensional reduction to set
slot	Specific information to set (must be one of the following: "cell.embeddings", "gene.loadings", "gene.loadings.full", "sdev", "key", "misc")
new.data	New data to set

Value

Seurat object with updated slot

Examples

```
pbmc_small
# Simulate adding a new dimensional reduction
new.cell.embeddings <- GetCellEmbeddings(object = pbmc_small, reduction.type = "pca")
new.gene.loadings <- GetGeneLoadings(object = pbmc_small, reduction.type = "pca")
SetDimReduction(
  object = pbmc_small,
  reduction.type = "new.pca",
  slot = "cell.embeddings",
  new.data = new.cell.embeddings
)
SetDimReduction(
  object = pbmc_small,
  reduction.type = "new.pca",
  slot = "gene.loadings",
  new.data = new.gene.loadings
)
```

SetIdent *Set identity class information*

Description

Sets the identity class value for a subset (or all) cells

Usage

```
SetIdent(object, cells.use = NULL, ident.use = NULL)
```

Arguments

object	Seurat object
cells.use	Vector of cells to set identity class info for (default is all cells)
ident.use	Vector of identity class values to assign (character vector)

Value

A Seurat object where object@ident has been appropriately modified

Examples

```
cluster2 <- WhichCells(object = pbmc_small, ident = 2)
pbmc_small@ident[cluster2]
pbmc_small <- SetIdent(
  object = pbmc_small,
  cells.use = cluster2,
  ident.use = 'cluster_2'
)
pbmc_small@ident[cluster2]
```

---

seurat *The Seurat Class*

Description

The Seurat object is the center of each single cell analysis. It stores all information associated with the dataset, including data, annotations, analyes, etc. All that is needed to construct a Seurat object is an expression matrix (rows are genes, columns are cells), which should be log-scale

Details

Each Seurat object has a number of slots which store information. Key slots to access are listed below.

Slots

`raw.data` The raw project data

`data` The normalized expression matrix (log-scale)

`scale.data` scaled (default is z-scoring each gene) expression matrix; used for dimmensional reduction and heatmap visualization

`var.genes` Vector of genes exhibiting high variance across single cells

`is.expr` Expression threshold to determine if a gene is expressed (0 by default)

`ident` THe 'identity class' for each cell

`meta.data` Contains meta-information about each cell, starting with number of genes detected (nGene) and the original identity class (orig.ident); more information is added using `AddMetaData`

`project.name` Name of hte project (for record keeping)

`dr` List of stored dimmensional reductions; named by technique

`assay` List of additional assays for multimodal analysis; named by technique

`hvg.info` The output of the mean/variability analysis for all genes

`imputed` Matrix of imputed gene scores

`cell.names` Names of all single cells (column names of the expression matrix)

`cluster.tree` List where the first element is a phylo object containing the phylogenetic tree relating different identity classes

`snn` Spare matrix object representation of the SNN graph

`calc.params` Named list to store all calculation-related parameter choices

`kmeans` Stores output of gene-based clustering from `DoKMeans`

`spatial` Stores internal data and calculations for spatial mapping of single cells

`misc` Miscellaneous spot to store any data alongisde the object (for example, gene lists)

`version` Version of package used in object creation

`Seurat-deprecated` *Deprecated function(s) in the Seurat package*

Description

These functions are provided for compatibility with older version of the Seurat package. They may eventually be completely removed.

Usage

```
vlnPlot(...)
```

Arguments

`...`	Parameters to be passed to the modern version of the function

Details

`vlnPlot`	now a synonym for `VlnPlot`
`subsetData`	now a synonym for `SubsetData`
`pca`	now a synonym for `RunPCA`
`PCA`	now a synonym for `PCA`
`project.pca`	now a synonym for `ProjectPCA`
`viz.pca`	now a synonym for `VizPCA`
`set.ident`	now a synonym for `SetIdent`
`pca.plot`	now a synonym for `PCAPlot`
`pcHeatmap`	now a synonym for `PCHeatmap`
`jackStraw`	now a synonym for `JackStraw`
`jackStrawPlot`	now a synonym for `JackStrawPlot`
`run_tsne`	now a synonym for `RunTSNE`
`tsne.plot`	now a synonym for `TSNEPlot`
`find.markers`	now a synonym for `FindMarkers`
`find_all_markers`	now a synonym for `FindAllMarkers`
`genePlot`	now a synonym for `GenePlot`

feature.plot	now a synonym for FeaturePlot
buildClusterTree	now a synonym for BuildClusterTree
plotClusterTree	now a synonym for PlotClusterTree
plotNoiseModel	has been removed and may be replaced at a later date
add_samples	now a synonym for AddSamples
subsetCells	now deleted
project.samples	has been removed and may be replaced at a later date
run_diffusion	now a synonym for RunDiffusion
ica	now a synonym for RunICA
ICA	now a synonym for RunICA
cluster.alpha	now a synonym for AverageDetectionRate
average.pca	now a synonym for AveragePCA
average.expression	now a synonym for AverageExpression
icTopGenes	now a synonym for ICTopGenes
pcTopGenes	now a synonym for PCTopGenes
pcTopCells	now a synonym for PCTopCells
fetch.data	now a synonym for FetchData
viz.ica	now a synonym for VizIca
regulatorScore	now deleted
find.markers.node	now a synonym for FindMarkersNode
diffExp.test	now a synonym for DiffExpTest
tobit.test	now a synonym for TobitTest
batch.gene	has been removed and may be restored at a later date
marker.test	now a synonym for MarkerTest
which.cells	now a synonym for WhichCells
set.all.ident	now a synonym for SetAllIdent
rename.ident	now a synonym for RenameIdent
posterior.plot	now a synonym for PosteriorPlot
map.cell	has been deprecated
get.centroids	now a synonym for GetCentroids
refined.mapping	now a synonym for RefinedMapping
initial.mapping	now a synonym for InitialMapping
calc.insitu	now a synonym for CalcInsitu
fit.gene.k	now a synonym for FitGeneK
fit.gene.mix	now a synonym for FitGeneMix
addSmoothedScore	now a synonym for AddSmoothedScore
addImputedScore	now a synonym for AddImputedScore
getNewScore	has been removed without replacement
calcNoiseModels	has been removed and may be replaced at a later date
feature.plot.keynote	has been removed without replacement
feature.heatmap	now a synonym for FeatureHeatmap
ica.plot	now a synonym for ICAPlot
spatial.de	has been removed and may be replaced at a later date
DBclust_dimension	now a synonym for DBClustDimension
Kclust_dimension	now a synonym for KClustDimension
pca.sig.genes	now a synonym for PCASigGenes
doHeatMap	now a synonym for DoHeatMap
icHeatmap	now a synonym for ICHeatmap

doKMeans	now a synonym for DoKMeans
genes.in.cluster	now a synonym for GenesInCluster
kMeansHeatmap	now a synonym for KMeansHeatmap
cell.cor.matrix	has been removed and may be replaced at a later date
gene.cor.matrix	has been removed and may be replaced at a later date
calinskiPlot	has been removed and may be replaced at a later date
dot.plot	now a synonym for DotPlot
addMetaData	now a synonym for AddMetaData
removePC	has been removed and may be replaced at a later date
geneScorePlot	now deleted
cellPlot	now a synonym for CellPlot
jackStraw.permutation.test	has been deleted
jackStrawMC	has been deleted
jackStrawFull	has been deleted
PCAFast	now a synonym for PCA
writ.table	has been removed without replacement
jackRandom	has been removed without replacement
MeanVarPlot	now a synonym for FindVariableGenes
myPalette	now a synonym for CustomPalette
minusr	now a synonym for SubsetRow
minusc	now a synonym for SubsetColumn
RegressOut	now part of ScaleData
VizClassification	has been removed without replacement

---

show *show method for seurat*

Description

show method for seurat

Usage

```
## S4 method for signature 'seurat'
show(object)
```

Arguments

object	A Seurat object

Shuffle *Shuffle a vector*

Description

Shuffle a vector

Usage

```
Shuffle(x)
```

Arguments

x	A vector

Value

A vector with the same values of x, just in random order

Examples

```
v <- seq(10)
v2 <- Shuffle(x = v)
v2
```

SplitDotPlotGG *Split Dot plot visualization*

Description

Intuitive way of visualizing how gene expression changes across different identity classes (clusters). The size of the dot encodes the percentage of cells within a class, while the color encodes the AverageExpression level of 'expressing' cells (green is high). Splits the cells into two groups based on a grouping variable. Still in BETA

Usage

```
SplitDotPlotGG(object, grouping.var, genes.plot, gene.groups,
  cols.use = c("green", "red"), col.min = -2.5, col.max = 2.5,
  dot.min = 0, dot.scale = 6, group.by, plot.legend = FALSE,
  do.return = FALSE, x.lab.rot = FALSE)
```

Arguments

object	Seurat object
grouping.var	Grouping variable for splitting the dataset
genes.plot	Input vector of genes
gene.groups	Add labeling bars to the top of the plot
cols.use	colors to plot
col.min	Minimum scaled average expression threshold (everything smaller will be set to this)
col.max	Maximum scaled average expression threshold (everything larger will be set to this)
dot.min	The fraction of cells at which to draw the smallest dot (default is 0.05).
dot.scale	Scale the size of the points, similar to cex
group.by	Factor to group the cells by
plot.legend	plots the legends
do.return	Return ggplot2 object
x.lab.rot	Rotate x-axis labels

Value

default, no return, only graphical output. If do.return=TRUE, returns a ggplot2 object

Examples

```
# Create a simulated grouping variable
pbmc_small@meta.data$groups <- sample(
  x = c("g1", "g2"),
  size = length(x = pbmc_small@cell.names),
  replace = TRUE
)
SplitDotPlotGG(pbmc_small, grouping.var = "groups", genes.plot = pbmc_small@var.genes[1:5])
```

StashIdent *Set identity class information*

Description

Stashes the identity in data.info to be retrieved later. Useful if, for example, testing multiple clustering parameters

Usage

```
StashIdent(object, save.name = "oldIdent")
```

Arguments

object	Seurat object
save.name	Store current object@ident under this column name in object@meta.data. Can be easily retrived with SetAllIdent

Value

A Seurat object where object@ident has been appropriately modified

Examples

```
head(x = pbmc_small@meta.data)
pbmc_small <- StashIdent(object = pbmc_small, save.name = 'cluster.ident')
head(x = pbmc_small@meta.data)
```

SubsetColumn *Return a subset of columns for a matrix or data frame*

Description

Return a subset of columns for a matrix or data frame

Usage

```
SubsetColumn(data, code, invert = FALSE)
```

Arguments

data	Matrix or data frame with column names
code	Pattern for matching within column names
invert	Invert the search?

Value

Returns a subset of data. If invert = TRUE, returns data where colnames do not contain code, otherwise returns data where colnames contain code

Examples

```
head(as.matrix(SubsetColumn(data = pbmc_small@raw.data, code = 'ATGC'))[, 1:4])
```

SubsetData *Return a subset of the Seurat object*

Description

Creates a Seurat object containing only a subset of the cells in the original object. Takes either a list of cells to use as a subset, or a parameter (for example, a gene), to subset on.

Usage

```
SubsetData(object, cells.use = NULL, subset.name = NULL, ident.use = NULL,
  ident.remove = NULL, accept.low = -Inf, accept.high = Inf,
  do.center = FALSE, do.scale = FALSE, max.cells.per.ident = Inf,
  random.seed = 1, ...)
```

Arguments

object	Seurat object
cells.use	A vector of cell names to use as a subset. If NULL (default), then this list will be computed based on the next three arguments. Otherwise, will return an object consisssting only of these cells
subset.name	Parameter to subset on. Eg, the name of a gene, PC1, a column name in object@meta.data, etc. Any argument that can be retreived using FetchData
ident.use	Create a cell subset based on the provided identity classes
ident.remove	Subtract out cells from these identity classes (used for filtration)
accept.low	Low cutoff for the parameter (default is -Inf)
accept.high	High cutoff for the parameter (default is Inf)
do.center	Recenter the new object@scale.data
do.scale	Rescale the new object@scale.data. FALSE by default
max.cells.per.ident	Can be used to downsample the data to a certain max per cell ident. Default is inf.
random.seed	Random seed for downsampling
...	Additional arguments to be passed to FetchData (for example, use.imputed=TRUE)

Value

Returns a Seurat object containing only the relevant subset of cells

Examples

```
pbmc1 <- SubsetData(object = pbmc_small, cells.use = pbmc_small@cell.names[1:40])
pbmc1
```

SubsetRow *Return a subset of rows for a matrix or data frame*

Description

Return a subset of rows for a matrix or data frame

Usage

```
SubsetRow(data, code, invert = FALSE)
```

Arguments

data	Matrix or data frame with row names
code	Pattern for matching within row names
invert	Invert the search?

Value

Returns a subset of data. If invert = TRUE, returns data where rownames do not contain code, otherwise returns data where rownames contain code

Examples

```
cd_genes <- SubsetRow(data = pbmc_small@raw.data, code = 'CD')
head(as.matrix(cd_genes)[, 1:4])
```

TobitTest *Differential expression testing using Tobit models*

Description

Identifies differentially expressed genes between two groups of cells using Tobit models, as proposed in Trapnell et al., Nature Biotechnology, 2014

Usage

```
TobitTest(object, cells.1, cells.2, genes.use = NULL, print.bar = TRUE,
  assay.type = "RNA")
```

Arguments

object	Seurat object
cells.1	Group 1 cells
cells.2	Group 2 cells
genes.use	Genes to test. Default is to use all genes
print.bar	Print a progress bar once expression testing begins (uses pbapply to do this)
assay.type	Type of assay to fetch data for (default is RNA)

Value

Returns a p-value ranked matrix of putative differentially expressed genes.

Examples

```
pbmc_small
## Not run:
TobitTest(pbmc_small, cells.1 = WhichCells(object = pbmc_small, ident = 1),
            cells.2 = WhichCells(object = pbmc_small, ident = 2))

## End(Not run)
```

TSNEPlot *Plot tSNE map*

Description

Graphs the output of a tSNE analysis Cells are colored by their identity class.

Usage

```
TSNEPlot(object, do.label = FALSE, pt.size = 1, label.size = 4,
  cells.use = NULL, colors.use = NULL, ...)
```

Arguments

object	Seurat object
do.label	FALSE by default. If TRUE, plots an alternate view where the center of each cluster is labeled
pt.size	Set the point size
label.size	Set the size of the text labels
cells.use	Vector of cell names to use in the plot.
colors.use	Manually set the color palette to use for the points
...	Additional parameters to DimPlot, for example, which dimensions to plot.

Details

This function is a wrapper for DimPlot. See ?DimPlot for a full list of possible arguments which can be passed in here.

See Also

DimPlot

Examples

```
TSNEPlot(object = pbmc_small)
```

`UpdateSeuratObject` *Update old Seurat object to accomodate new features*

Description

Updates Seurat objects to new structure for storing data/calculations.

Usage

```
UpdateSeuratObject(object)
```

Arguments

`object`	Seurat object

Value

Returns a Seurat object compatible with latest changes

Examples

```
## Not run:
updated_seurat_object = UpdateSeuratObject(object = old_seurat_object)

## End(Not run)
```

`ValidateClusters` *Cluster Validation*

Description

Methods for validating the legitimacy of clusters using classification. SVMs are used as the basis for the classification. Merging is done based on the connectivity from an SNN graph.

Usage

```
ValidateClusters(object, pc.use = NULL, top.genes = 30,
  min.connectivity = 0.01, acc.cutoff = 0.9, verbose = TRUE)
```

Arguments

`object`	Seurat object
`pc.use`	Which PCs to use to define genes in model construction
`top.genes`	Use the top X genes for each PC in model construction
`min.connectivity`	Threshold of connectedness for comparison of two clusters
`acc.cutoff`	Accuracy cutoff for classifier
`verbose`	Controls whether to display progress and merging results

Value

Returns a Seurat object, object@ident has been updated with new cluster info

Examples

```
pbmc_small
# May throw warnings when cluster sizes are particularly small
## Not run:
pbmc_small <- FindClusters(object = pbmc_small, reduction.type = "pca",
                           dims.use = 1:10, resolution = 1.1, save.SNN = TRUE)
pbmc_small <- ValidateClusters(pbmc_small, pc.use = 1:10)

## End(Not run)
```

ValidateSpecificClusters *Specific Cluster Validation*

Description

Methods for validating the legitimacy of two specific clusters using classification. SVMs are used as the basis for the classification. Merging is done based on the connectivity from an SNN graph.

Usage

```
ValidateSpecificClusters(object, cluster1 = NULL, cluster2 = 1,
  pc.use = 2, top.genes = 30, acc.cutoff = 0.9)
```

Arguments

object	Seurat object
cluster1	First cluster to check classification
cluster2	Second cluster to check with classification
pc.use	Which PCs to use for model construction
top.genes	Use the top X genes for model construction
acc.cutoff	Accuracy cutoff for classifier

Value

Returns a Seurat object, object@ident has been updated with new cluster info

Examples

```
## Not run:
pbmc_small
pbmc_small <- FindClusters(object = pbmc_small, reduction.type = "pca",
                           dims.use = 1:10, resolution = 1.1, save.SNN = TRUE)
pbmc_small <- ValidateSpecificClusters(pbmc_small, cluster1 = 1,
                                       cluster2 = 2,  pc.use = 1:10)

## End(Not run)
```

`VariableGenePlot` *View variable genes*

Description

View variable genes

Usage

```
VariableGenePlot(object, do.text = TRUE, cex.use = 0.5,
  cex.text.use = 0.5, do.spike = FALSE, pch.use = 16, col.use = "black",
  spike.col.use = "red", plot.both = FALSE, do.contour = TRUE,
  contour.lwd = 3, contour.col = "white", contour.lty = 2,
  x.low.cutoff = 0.1, x.high.cutoff = 8, y.cutoff = 1,
  y.high.cutoff = Inf)
```

Arguments

`object`	Seurat object
`do.text`	Add text names of variable genes to plot (default is TRUE)
`cex.use`	Point size
`cex.text.use`	Text size
`do.spike`	FALSE by default. If TRUE, color all genes starting with ^ERCC a different color
`pch.use`	Pch value for points
`col.use`	Color to use
`spike.col.use`	if do.spike, color for spike-in genes
`plot.both`	Plot both the scaled and non-scaled graphs.
`do.contour`	Draw contour lines calculated based on all genes
`contour.lwd`	Contour line width
`contour.col`	Contour line color
`contour.lty`	Contour line type
`x.low.cutoff`	Bottom cutoff on x-axis for identifying variable genes
`x.high.cutoff`	Top cutoff on x-axis for identifying variable genes
`y.cutoff`	Bottom cutoff on y-axis for identifying variable genes
`y.high.cutoff`	Top cutoff on y-axis for identifying variable genes

Examples

```
VariableGenePlot(object = pbmc_small)
```

VizDimReduction *Visualize Dimensional Reduction genes*

Description

Visualize top genes associated with reduction components

Usage

```
VizDimReduction(object, reduction.type = "pca", dims.use = 1:5,
  num.genes = 30, use.full = FALSE, font.size = 0.5, nCol = NULL,
  do.balanced = FALSE)
```

Arguments

object	Seurat object
reduction.type	Reduction technique to visualize results for
dims.use	Number of dimensions to display
num.genes	Number of genes to display
use.full	Use reduction values for full dataset (i.e. projected dimensional reduction values)
font.size	Font size
nCol	Number of columns to display
do.balanced	Return an equal number of genes with + and - scores. If FALSE (default), returns the top genes ranked by the scores absolute values

Value

Graphical, no return value

Examples

```
VizDimReduction(object = pbmc_small)
```

VizICA *Visualize ICA genes*

Description

Visualize top genes associated with principal components

Usage

```
VizICA(object, ics.use = 1:5, num.genes = 30, use.full = FALSE,
  font.size = 0.5, nCol = NULL, do.balanced = FALSE)
```

Arguments

object	Seurat object
ics.use	Number of ICs to display
num.genes	Number of genes to display
use.full	Use full ICA (i.e. the projected ICA, by default FALSE)
font.size	Font size
nCol	Number of columns to display
do.balanced	Return an equal number of genes with both + and - IC scores. If FALSE (by default), returns the top genes ranked by the score's absolute values

Value

Graphical, no return value

Examples

```
pbmc_small <- RunICA(object = pbmc_small, ics.compute = 25, print.results = FALSE)
VizICA(object = pbmc_small)
```

VizPCA *Visualize PCA genes*

Description

Visualize top genes associated with principal components

Usage

```
VizPCA(object, pcs.use = 1:5, num.genes = 30, use.full = FALSE,
  font.size = 0.5, nCol = NULL, do.balanced = FALSE)
```

Arguments

object	Seurat object
pcs.use	Number of PCs to display
num.genes	Number of genes to display
use.full	Use full PCA (i.e. the projected PCA, by default FALSE)
font.size	Font size
nCol	Number of columns to display
do.balanced	Return an equal number of genes with both + and - PC scores. If FALSE (by default), returns the top genes ranked by the score's absolute values

Value

Graphical, no return value

Examples

```
VizPCA(object = pbmc_small)
```

VlnPlot *Single cell violin plot*

Description

Draws a violin plot of single cell data (gene expression, metrics, PC scores, etc.)

Usage

```
VlnPlot(object, features.plot, ident.include = NULL, nCol = NULL,
  do.sort = FALSE, y.max = NULL, same.y.lims = FALSE, size.x.use = 16,
  size.y.use = 16, size.title.use = 20, adjust.use = 1,
  point.size.use = 1, cols.use = NULL, group.by = NULL, y.log = FALSE,
  x.lab.rot = FALSE, y.lab.rot = FALSE, legend.position = "right",
  single.legend = TRUE, remove.legend = FALSE, do.return = FALSE,
  return.plotlist = FALSE, ...)
```

Arguments

object	Seurat object
features.plot	Features to plot (gene expression, metrics, PC scores, anything that can be retreived by FetchData)
ident.include	Which classes to include in the plot (default is all)
nCol	Number of columns if multiple plots are displayed

do.sort	Sort identity classes (on the x-axis) by the average expression of the attribute being potted
y.max	Maximum y axis value
same.y.lims	Set all the y-axis limits to the same values
size.x.use	X axis title font size
size.y.use	Y axis title font size
size.title.use	Main title font size
adjust.use	Adjust parameter for geom_violin
point.size.use	Point size for geom_violin
cols.use	Colors to use for plotting
group.by	Group (color) cells in different ways (for example, orig.ident)
y.log	plot Y axis on log scale
x.lab.rot	Rotate x-axis labels
y.lab.rot	Rotate y-axis labels
legend.position	Position the legend for the plot
single.legend	Consolidate legend the legend for all plots
remove.legend	Remove the legend from the plot
do.return	Return a ggplot2 object (default : FALSE)
return.plotlist	Return the list of individual plots instead of compiled plot.
...	additional parameters to pass to FetchData (for example, use.imputed, use.scaled, use.raw)

Value

By default, no return, only graphical output. If do.return=TRUE, returns a list of ggplot objects.

Examples

```
VlnPlot(object = pbmc_small, features.plot = 'PC1')
```

WhichCells *Identify cells matching certain criteria*

Description

Returns a list of cells that match a particular set of criteria such as identity class, high/low values for particular PCs, ect..

Usage

```
WhichCells(object, ident = NULL, ident.remove = NULL, cells.use = NULL,
  subset.name = NULL, accept.low = -Inf, accept.high = Inf,
  accept.value = NULL, max.cells.per.ident = Inf, random.seed = 1)
```

Arguments

object	Seurat object
ident	Identity classes to subset. Default is all identities.
ident.remove	Indentity classes to remove. Default is NULL.
cells.use	Subset of cell names
subset.name	Parameter to subset on. Eg, the name of a gene, PC1, a column name in object@meta.data, etc. Any argument that can be retreived using FetchData
accept.low	Low cutoff for the parameter (default is -Inf)
accept.high	High cutoff for the parameter (default is Inf)
accept.value	Returns all cells with the subset name equal to this value
max.cells.per.ident	Can be used to downsample the data to a certain max per cell ident. Default is inf.
random.seed	Random seed for downsampling

Value

A vector of cell names

Examples

```
WhichCells(object = pbmc_small, ident = 2)
```

WilcoxDETest *Differential expression using Wilcoxon Rank Sum*

Description

Identifies differentially expressed genes between two groups of cells using a Wilcoxon Rank Sum test

Usage

```
WilcoxDETest(object, cells.1, cells.2, min.cells = 3, genes.use = NULL,
  print.bar = TRUE, assay.type = "RNA", ...)
```

Arguments

object	Seurat object
cells.1	Group 1 cells
cells.2	Group 2 cells
min.cells	Minimum number of cells expressing the gene in at least one of the two groups
genes.use	Genes to use for test
print.bar	Print a progress bar
assay.type	Type of assay to perform DE for (default is RNA)
...	Extra parameters passed to wilcox.test

Value

Returns a p-value ranked matrix of putative differentially expressed genes.

Examples

```
pbmc_small
WilcoxDETest(pbmc_small, cells.1 = WhichCells(object = pbmc_small, ident = 1),
            cells.2 = WhichCells(object = pbmc_small, ident = 2))
```

Index