# Package 'singleCellTK'

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```
Title Interactive Analysis of Single Cell RNA-Seq Data
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Depends R (>= 3.5), SummarizedExperiment, SingleCellExperiment,
     DelayedArray, Biobase
Description Run common single cell analysis directly through your browser
      including differential expression, downsampling analysis, and clustering.
License MIT + file LICENSE
Encoding UTF-8
biocViews SingleCell, GeneExpression, DifferentialExpression,
      Alignment, Clustering, ImmunoOncology
LazyData TRUE
Imports ape, colourpicker, cluster, ComplexHeatmap, data.table,
     DESeq2, DT, ggplot2, ggtree, gridExtra, GSVA (>= 1.26.0),
     GSVAdata, limma, MAST, matrixStats, methods, multtest, plotly,
     RColorBrewer, Rtsne, S4Vectors, shiny, shinyjs, sva, reshape2,
      AnnotationDbi, shinyalert, circlize, enrichR, shinycssloaders,
      shinythemes, umap
RoxygenNote 6.1.1
Suggests testthat, Rsubread, BiocStyle, knitr, bladderbatch,
      rmarkdown, org.Mm.eg.db, org.Hs.eg.db, scRNAseq, xtable,
      spelling, GSEABase, lintr
VignetteBuilder knitr
URL https://compbiomed.github.io/sctk_docs/
BugReports https://github.com/compbiomed/singleCellTK/issues
Language en-US
git_url https://git.bioconductor.org/packages/singleCellTK
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```

Type Package

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alignSingleCellData Align Single Cell RNA-Seq Data and Create a SCtkExperiment Object

### Description

Align Single Cell RNA-Seq Data and Create a SCtkExperiment Object

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#### **Usage**

```
alignSingleCellData(inputfile1, inputfile2 = NULL, indexPath,
  gtfAnnotation, outputDir = NULL, sampleAnnotations = NULL,
  featureAnnotations = NULL, threads = 1, saveBam = FALSE,
  saveCountFiles = FALSE, isPairedEnd = FALSE)
```

#### **Arguments**

inputfile1 An input file or list of files. Files can be fastq, fastq.gz, or bam, but must all be

of the same type. Sample names will be the full file name, without \_1.fastq.gz,

.fastq.gz, \_1.fastq, .fastq or .bam endings.

inputfile2 If fastq files are provided in input list, a list of corresponding paired fastq files,

if applicable.

indexPath Path to the Rsubread genome index.

gtfAnnotation Path to the GTF gene annotation to use. This must correspond to the genome

specified in indexPath.

outputDir If saveBam or saveCountFiles is TRUE, specify a directory in which to save the

output files.

sampleAnnotations

A data frame of sample annotations, with samples as rows and annotations in columns. The sample names must be identical to and in the same order as the list of files in inputfile1. Alignment statistics will be added to the annotation

data frame.

featureAnnotations

An optional data frame of probe annotations, with probes as rows and probe

annotations in columns.

threads Number of threads to use during alignment. The default is 1.

saveBam If TRUE, bam alignment files will be saved in the outputDir. The default is

FALSE.

saveCountFiles If TRUE, per sample gene count files will be saved in the outputDir. The default

is FALSE.

isPairedEnd If input files are .bam, indicate whether the input bam files are paired end.

#### Value

Object to import into the shiny app.

4 ComBatSCE

calcEffectSizes	Finds the effect sizes for all genes in the original dataset, regardless of significance.
-----------------	---

### Description

Finds the effect sizes for all genes in the original dataset, regardless of significance.

### Usage

```
calcEffectSizes(countMatrix, condition)
```

### **Arguments**

countMatrix Matrix. A simulated counts matrix, sans labels.

condition Factor. The condition labels for the simulated cells. If more than 2 conditions

are given, the first will be compared to all others by default.

#### Value

A vector of cohen's d effect sizes for each gene.

### **Examples**

ComBatSCE

**ComBatSCE** 

### **Description**

Run ComBat on a SCtkExperiment object

### Usage

```
ComBatSCE(inSCE, batch, useAssay = "logcounts",
  par.prior = "Parametric", covariates = NULL, mean.only = FALSE,
  ref.batch = NULL)
```

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### **Arguments**

inSCE	Input SCtkExperiment object. Required
batch	The name of a column in colData to use as the batch variable. Required
useAssay	The assay to use for ComBat. The default is "logcounts"
par.prior	TRUE indicates parametric adjustments will be used, FALSE indicates non-parametric adjustments will be used. Accepted parameters: "Parametric" or "Non-parametric"
covariates	List of other column names in colData to be added to the ComBat model as covariates
mean.only	If TRUE ComBat only corrects the mean of the batch effect
ref.batch	If given, will use the selected batch as a reference for batch adjustment.

#### Value

ComBat matrix based on inputs. You can save this matrix into the SCtkExperiment with assay()

```
if(requireNamespace("bladderbatch", quietly = TRUE)) {
  library(bladderbatch)
  data(bladderdata)
  #subset for testing
  dat <- bladderEset[1:50,]</pre>
  dat <- as(as(dat, "SummarizedExperiment"), "SCtkExperiment")</pre>
  mod <- stats::model.matrix(~as.factor(cancer), data = colData(dat))</pre>
  # parametric adjustment
  combat_edata1 <- ComBatSCE(inSCE = dat, useAssay = "exprs",</pre>
                               batch = "batch", covariates = NULL)
  assay(dat, "parametric_combat") <- combat_edata1</pre>
  # non-parametric adjustment, mean-only version
  combat_edata2 <- ComBatSCE(inSCE = dat, useAssay = "exprs",</pre>
                               batch = "batch", par.prior = "Non-parametric",
                              mean.only = TRUE, covariates = NULL)
  assay(dat, "nonparametric_combat_meanonly") <- combat_edata2</pre>
  # reference-batch version, with covariates
  combat_edata3 <- ComBatSCE(inSCE = dat, useAssay = "exprs",</pre>
                               batch = "batch", covariates = "cancer",
                              ref.batch = 3)
  assay(dat, \ "refbatch\_combat\_wcov") <- \ combat\_edata3
  assays(dat)
```

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convertGeneIDs

Convert Gene IDs

#### **Description**

Convert the gene IDs in a SingleCellExperiment object using Bioconductor org.\*.eg.db data packages. Because annotation databases do not have a 1:1 relationship, this tool removes rows with no corresponding annotation in your desired annotation, and remove any duplicate annotations after conversion.

#### Usage

```
convertGeneIDs(inSCE, inSymbol, outSymbol, database = "org.Hs.eg.db")
```

#### **Arguments**

### Value

A SCtkExperiment with converted gene IDs.

### **Examples**

createSCE

Create a SCtkExperiment object

### **Description**

From a file of counts and a file of annotation information, create a SCtkExperiment object.

### Usage

```
createSCE(assayFile = NULL, annotFile = NULL, featureFile = NULL,
  assayName = "counts", inputDataFrames = FALSE,
  createLogCounts = TRUE)
```

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#### **Arguments**

assayFile The path to a text file that contains a header row of sample names, and rows of

raw counts per gene for those samples.

annotFile The path to a text file that contains columns of annotation information for each

sample in the assayFile. This file should have the same number of rows as there

are columns in the assayFile.

featureFile The path to a text file that contains columns of annotation information for each

gene in the count matrix. This file should have the same genes in the same order

as assayFile. This is optional.

assayName The name of the assay that you are uploading. The default is "counts".

inputDataFrames

If TRUE, assayFile and annotFile are read as data frames instead of file paths.

The default is FALSE.

createLogCounts

If TRUE, create a log2(counts+1) normalized assay and include it in the object.

The default is TRUE

#### Value

a SCtkExperiment object

### **Examples**

distinctColors

Generate a distinct palette for coloring different clusters

#### **Description**

Generate a distinct palette for coloring different clusters

#### Usage

```
distinctColors(n, hues = c("red", "cyan", "orange", "blue", "yellow", "purple", "green", "magenta"), saturation.range = <math>c(0.7, 1), value.range = c(0.7, 1))
```

#### **Arguments**

n Integer; Number of colors to generate

hues Character vector of R colors available from the colors() function. These will be used as the base colors for the clustering scheme. Different saturations and

values (i.e. darkness) will be generated for each hue.

8 DownsampleCells

```
saturation.range  Numeric \ vector \ of \ length \ 2 \ with \ values \ between \ 0 \ and \ 1. \ Default: \ c(0.25, \ 1)  value.range  Numeric \ vector \ of \ length \ 2 \ with \ values \ between \ 0 \ and \ 1. \ Default: \ c(0.5, \ 1)
```

#### Value

A vector of distinct colors that have been converted to HEX from HSV.

### **Examples**

```
distinctColors(10)
```

### Description

Estimate numbers of detected genes, significantly differentially expressed genes, and median significant effect size

### Usage

```
DownsampleCells(originalData, useAssay = "counts", minCountDetec = 10,
  minCellsDetec = 3, minCellnum = 10, maxCellnum = 1000, realLabels,
  depthResolution = 10, iterations = 10, totalReads = 1e+06)
```

### **Arguments**

originalData	SCtkExperiment. The SCtkExperiment object storing all assay data from the shiny app.		
useAssay	Character. The name of the assay to be used for subsampling.		
minCountDetec	Numeric. The minimum number of reads found for a gene to be considered detected.		
minCellsDetec	Numeric. The minimum number of cells a gene must have at least 1 read in for it to be considered detected.		
minCellnum	Numeric. The minimum number of virtual cells to include in the smallest simulated dataset.		
maxCellnum	Numeric. The maximum number of virtual cells to include in the largest simulated dataset		
realLabels	Character. The name of the condition of interest. Must match a name from sample data. If only two factors present in the corresponding colData, will default to t-test. If multiple factors, will default to ANOVA.		
depthResolution			
	Numeric. How many different read depth should the script simulate? Will simulate a number of experimental designs ranging from 10 reads to maxReadDepth, with logarithmic spacing.		
iterations	Numeric. How many times should each experimental design be simulated?		
totalReads	Numeric. How many aligned reads to put in each simulated dataset.		

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#### Value

A 3-dimensional array, with dimensions = c(iterations, depthResolution, 3). [,,1] contains the number of detected genes in each simulated dataset, [,,2] contains the number of significantly differentially expressed genes in each simulation, and [,,3] contains the mediansignificant effect size in each simulation. If no genes are significantly differentially expressed, the median effect size defaults to infinity.

### **Examples**

DownsampleDepth

Estimate numbers of detected genes, significantly differentially expressed genes, and median significant effect size

#### **Description**

Estimate numbers of detected genes, significantly differentially expressed genes, and median significant effect size

### Usage

```
DownsampleDepth(originalData, useAssay = "counts", minCount = 10,
  minCells = 3, maxDepth = 1e+07, realLabels, depthResolution = 10,
  iterations = 10)
```

### **Arguments**

iterations

originalData	SCtkExperiment. The SCtkExperiment object storing all assay data from the shiny app.
useAssay	Character. The name of the assay to be used for subsampling.
minCount	Numeric. The minimum number of reads found for a gene to be considered detected.
minCells	Numeric. The minimum number of cells a gene must have at least 1 read in for it to be considered detected.
maxDepth	Numeric. The highest number of total reads to be simulated.
realLabels	Character. The name of the condition of interest. Must match a name from sample data.
depthResolution	
	Numeric. How many different read depth should the script simulate? Will simulate a number of experimental designs ranging from 10 reads to maxReadDepth, with logarithmic spacing.

Numeric. How many times should each experimental design be simulated?

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#### Value

A 3-dimensional array, with dimensions = c(iterations, depthResolution, 3). [,,1] contains the number of detected genes in each simulated dataset, [,,2] contains the number of significantly differentially expressed genes in each simulation, and [,,3] contains the mediansignificant effect size in each simulation. If no genes are significantly differentially expressed, the median effect size defaults to infinity.

### **Examples**

enrichRSCE

enrichR Given a list of genes this function runs the enrichR() to perform Gene enrichment

### **Description**

enrichR Given a list of genes this function runs the enrichR() to perform Gene enrichment

### Usage

```
enrichRSCE(inSCE, glist, db = NULL)
```

### **Arguments**

inSCE Input SCtkExperiment object. Required
glist selected genes for enrichment analysis using enrichR(). Required
db selected database name from the enrichR database list. if NULL then enrichR
will be run on all the available databases on the enrichR database.

### Value

enrichRSCE(): returns a data.frame of enrichment terms overlapping in the respective databases along with p-values, z-scores etc.,

```
## Not run:
enrichRSCE(mouseBrainSubsetSCE, "Cmtm5", "GO_Cellular_Component_2017")
## End(Not run)
```

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filterSCData	
--------------	--

Filter Genes and Samples from a Single Cell Object

#### **Description**

Filter Genes and Samples from a Single Cell Object

### Usage

```
filterSCData(inSCE, useAssay = "counts", deletesamples = NULL,
  removeNoExpress = TRUE, removeBottom = 0.5,
  minimumDetectGenes = 1700, filterSpike = TRUE)
```

### **Arguments**

inSCE Input SCtkExperiment object. Required

useAssay Indicate which assay to use for filtering. Default is "counts"

deletesamples List of samples to delete from the object.

removeNoExpress

Remove genes that have no expression across all samples. The default is true

removeBottom Fraction of low expression genes to remove from the single cell object. This

occurs after removeNoExpress. The default is 0.50.

minimumDetectGenes

Minimum number of genes with at least 1 count to include a sample in the single

cell object. The default is 1700.

filterSpike Apply filtering to Spike in controls (indicated by isSpike). The default is TRUE.

### Value

The filtered single cell object.

### **Examples**

generateSimulatedData Generates a single simulated dataset, bootstrapping from the input counts matrix.

### Description

Generates a single simulated dataset, bootstrapping from the input counts matrix.

#### Usage

```
generateSimulatedData(totalReads, cells, originalData, realLabels)
```

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#### **Arguments**

totalReads Numeric. The total number of reads in the simulated dataset, to be split between

all simulated cells.

cells Numeric. The number of virtual cells to simulate.

originalData Matrix. The original raw read count matrix. When used within the Shiny app,

this will be assay(SCEsetObject, "counts").

realLabels Factor. The condition labels for differential expression. If only two factors

present, will default to t-test. If multiple factors, will default to ANOVA.

### Value

A simulated counts matrix, the first row of which contains the 'true' labels for each virtual cell.

### **Examples**

getBiomarker Given a list of genes and a SCtkExperiment object, return the binary

or continuous expression of the genes.

#### **Description**

Given a list of genes and a SCtkExperiment object, return the binary or continuous expression of the genes.

#### Usage

```
getBiomarker(inSCE, gene, binary = "Binary", useAssay = "counts")
```

#### **Arguments**

inSCE Input SCtkExperiment object. Required

gene gene list

binary "Binary" for binary expression or "Continuous" for a gradient. Default: "Bi-

nary"

useAssay Indicate which assay to use. The default is "counts".

### Value

```
getBiomarker(): A data.frame of expression values
```

```
getBiomarker(mouseBrainSubsetSCE, gene="C1qa")
```

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getClusterInputData Get data to use as input clustering algorithms

### Description

Get data to use as input clustering algorithms

### Usage

```
getClusterInputData(inSCE, inputData, useAssay = "logcounts",
   reducedDimName = NULL)
```

#### **Arguments**

inSCE Input SCtkExperiment object. Required

inputData A string ("Raw Data", "PCA Components", "tSNE Components", "UMAP Com-

ponents")

useAssay Indicate which assay to use for PCA. Default is "logcounts"

reducedDimName If clustering on PCA, t-SNE or UMAP data, dimension name. The toolkit will

store data with the pattern <ASSAY>\_<ALGORITHM>.

#### Value

Cluster input data

### **Examples**

getPCA

Get and plot PCA components for a SCtkE object

### **Description**

Selects the 500 most variable genes in the SCE, performs PCA based on them and stores the values in the reducedDims slot of the SCE object.

### Usage

```
getPCA(inSCE, useAssay = "logcounts", reducedDimName = "PCA")
```

#### **Arguments**

inSCE Input SCtkExperiment object. Required

useAssay Indicate which assay to use for PCA. Default is "counts"

reducedDimName Store the PCA data with this name. The default is PCA. The toolkit will store

data with the pattern <ASSAY>\_<ALGORITHM>.

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#### Value

A SCtkE object with the specified reducedDim and pcaVariances updated

#### **Examples**

getTSNE

Run t-SNE dimensionality reduction method on the assay data.

#### **Description**

Run t-SNE dimensionality reduction method on the assay data.

#### Usage

```
getTSNE(inSCE, useAssay = "logcounts", reducedDimName = "TSNE",
    n_iterations = 1000, perplexity = NULL)
```

#### **Arguments**

inSCE Input SCtkExperiment object. Required
useAssay Indicate which assay to use. The default is "logcounts".
reducedDimName a name to store the results of the dimension reductions
n\_iterations maximum iterations. Default is 1000
perplexity perplexity parameter. Default is 5

#### Value

A SCtkE object with the specified reducedDim and pcaVariances updated

getUMAP 15

getUMAP Uniform Manifold Approximation and Projection(UMAP) for dimension reduction.	algorithm
--	-----------

#### **Description**

Uniform Manifold Approximation and Projection(UMAP) algorithm for dimension reduction.

### Usage

```
getUMAP(inSCE, useAssay = "logcounts", reducedDimName = "UMAP",
    n_neighbors = 5, n_iterations = 200, alpha = 1)
```

#### **Arguments**

inSCE Input SCtkExperiment object. Required
useAssay Indicate which assay to use. The default is "logcounts".

reducedDimName a name to store the results of the dimension reduction coordinates obtained from this method. This is stored in the SingleCellExperiment object in the reduced-Dims slot. Required.

n\_neighbors specify the number of nearest neighbors. Default is 5.

n\_iterations number of iterations performed during layout optimization. Default is 200.

alpha initial value of "learning rate" of layout optimization. Default is 1.

### Value

a SCtkExperiment object with the reduced dimensions updated under reducedDimName specified.

### **Examples**

gsvaSCE

Run GSVA analysis on a SCtkExperiment object.

### Description

Run GSVA analysis on a SCtkExperiment object.

### Usage

```
gsvaSCE(inSCE, useAssay = "logcounts", pathwaySource, pathwayNames, ...)
gsvaPlot(inSCE, gsvaData, plotType, condition = NULL,
    show_column_names = TRUE, show_row_names = TRUE, text_size = 12)
```

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#### **Arguments**

inSCE Input SCtkExperiment object. Required

useAssay Indicate which assay to use. The default is "logcounts"

pathwaySource The pathway source if "Manual Input", the pathwayNames should be rowData

annotations that are (0,1) vectors. If, "MSigDB c2 (Human, Entrez ID only)", the pathwayNames should be pathways from MSigDB c2 or "ALL" to run on

all available pathways.

pathwayNames List of pathway names to run, depending on pathwaySource parameter.

... Parameters to pass to gsva() gsvaData GSVA data to plot. Required.

plotType The type of plot to use, "Violin" or "Heatmap". Required.

condition The condition(s) to use for the Violin plot, or the condition(s) to add as color

bars above the Heatmap. Required for Violin, optional for Heatmap.

show\_column\_names

Display the column labels on the heatmap. The default is TRUE

show\_row\_names Display the row labels on the heatmap. The default is TRUE.

text\_size Text size for plots. The default is 12

#### Value

gsvaSCE(): A data.frame of pathway activity scores from GSVA.

gsvaPlot(): The requested plot of the GSVA results.

#### **Functions**

gsvaPlot: Plot GSVA results.
 Plot GSVA Results

```
utils::data(maits, package = "MAST")
utils::data(c2BroadSets, package = "GSVAdata")
maitslogtpm <- t(maits$expressionmat)</pre>
genesToSubset <- rownames(maitslogtpm)[which(rownames(maitslogtpm) %in%</pre>
                 GSEABase::geneIds(c2BroadSets[["KEGG_PROTEASOME"]]))]
maitslogtpm <- maitslogtpm[rownames(maitslogtpm) %in% genesToSubset, ]</pre>
maitsfeatures <- maits$fdat[rownames(maits$fdat) %in% genesToSubset, ]</pre>
maitsSCE <- createSCE(assayFile = maitslogtpm, annotFile = maits$cdat,</pre>
                       featureFile = maitsfeatures, assayName = "logtpm",
                       inputDataFrames = TRUE, createLogCounts = FALSE)
rowData(maitsSCE)$testbiomarker <- rep(1, nrow(maitsSCE))</pre>
res <- gsvaSCE(inSCE = maitsSCE, useAssay = "logtpm",</pre>
                pathwaySource = "Manual Input", pathwayNames = "testbiomarker",
                parallel.sz = 1)
#Create a small example to run
utils::data(maits, package = "MAST")
utils::data(c2BroadSets, package = "GSVAdata")
maitslogtpm <- t(maits$expressionmat)</pre>
genesToSubset <- rownames(maitslogtpm)[which(rownames(maitslogtpm) %in%</pre>
                  GSEABase::geneIds(c2BroadSets[["KEGG_PROTEASOME"]]))]
maitslogtpm <- maitslogtpm[rownames(maitslogtpm) %in% genesToSubset, ]</pre>
```

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iterateSimulations

Returns significance data from a snapshot.

### **Description**

Returns significance data from a snapshot.

### Usage

```
iterateSimulations(originalData, useAssay = "counts", realLabels,
  totalReads, cells, iterations)
```

### **Arguments**

originalData	SCtkExperiment. The SCtkExperiment object storing all assay data from the shiny app.
useAssay	Character. The name of the assay to be used for subsampling.
realLabels	Character. The name of the condition of interest. Must match a name from sample data.
totalReads	Numeric. The total number of reads in the simulated dataset, to be split between all simulated cells.
cells	Numeric. The number of virtual cells to simulate.
iterations	Numeric. How many times should each experimental design be simulated.

#### Value

A matrix of significance information from a snapshot

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MAST

### Description

Run and visualize MAST analysis on a SCtkExperiment object.

### Usage

```
MAST(inSCE, condition = NULL, interest.level = NULL,
  freqExpressed = 0.1, fcThreshold = log2(1.5), p.value = 0.05,
  useThresh = FALSE, useAssay = "logcounts")

thresholdGenes(inSCE, useAssay = "logcounts")

MASTviolin(inSCE, useAssay = "logcounts", fcHurdleSig, samplesize = 49,
  threshP = FALSE, condition)

MASTregression(inSCE, useAssay = "logcounts", fcHurdleSig,
  samplesize = 49, threshP = FALSE, condition)
```

### Arguments

:CCE	Land SCALE and in set about Demand
inSCE	Input SCtkExperiment object. Required
condition	select variable (from the colData) that is used for the model.
interest.level	If the condition of interest has more than two factors, indicate which level should be used to compare to all other samples.
freqExpressed	Filter genes that are expressed in at least this fraction of cells. The default is expression in $0.1$ of samples.
fcThreshold	Minimum fold change for differentially expressed gene.
p.value	p values for selecting the hurdle result, default is 0.05
useThresh	Use adaptive thresholding to filter genes. The default is FALSE.
useAssay	The assay to use for the MAST calculations. The default is "logcounts"
fcHurdleSig	The filtered result from hurdle model
samplesize	The number of most significant genes
threshP	Plot threshold values from adaptive thresholding. Default is FALSE

#### Value

MAST(): A data.frame of differentially expressed genes with p-values.

thresholdGenes(): list of thresholded counts (on natural scale), thresholds, bins, densities estimated on each bin, and the original data from MAST::thresholdSCRNACountMatrix

MASTviolin(): A ggplot object of MAST violin plots.

MASTregression(): A ggplot object of MAST linear regression plots.

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#### **Functions**

- MAST: Run MAST analysis.
- thresholdGenes: Identify adaptive thresholds
- MASTviolin: Visualize MAST results using violin plots
- MASTregression: Visualize MAST results using linear model plots

### **Examples**

```
data("mouseBrainSubsetSCE")
res <- thresholdGenes(mouseBrainSubsetSCE)</pre>
```

 ${\tt mouseBrainSubsetSCE}$ 

Example Single Cell RNA-Seq data in SCtkExperiment Object, GSE60361 subset

### **Description**

A subset of 30 samples from a single cell RNA-Seq experiment from Zeisel, et al. Science 2015. The data was produced from cells from the mouse somatosensory cortex (S1) and hippocampus (CA1). 15 of the cells were identified as oligodendrocytes and 15 of the cell were identified as microglia.

### Usage

mouseBrainSubsetSCE

#### **Format**

**SCtkExperiment** 

### Source

DOI: 10.1126/science.aaa1934

### **Examples**

data("mouseBrainSubsetSCE")

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parseRsubreadLogs

Parse Rsubread Logs for Mapping and Feature Count Statistics

### **Description**

Parse Rsubread Logs for Mapping and Feature Count Statistics

#### Usage

```
parseRsubreadLogs(alignLog = NULL, featurecountLog = NULL,
    sampleName = NULL)
```

### Arguments

alignLog Path to a log file created by the Rsubread align function

featurecountLog

Path to a log file created by the Rsubread feature count function

sampleName Sample name corresponding to the two log files

#### Value

A single line of a data frame with alignment and feature count information

pcaVariances

Get PCA variances

### Description

```
Get PCA variances
Get PCA variances
Set PCA variances
```

### Usage

```
pcaVariances(x, ...)
## S4 method for signature 'SCtkExperiment'
pcaVariances(x)
## S4 replacement method for signature 'SCtkExperiment'
pcaVariances(x) <- value</pre>
```

### **Arguments**

```
x SCtkE object... other parametersvalue The DataFrame of pcaVariances
```

pcaVariances<- 21

#### Value

A data frame of percent variation explained by each PC.

A SCtkExperiment object with the pcaVariances object set.

#### **Examples**

```
data("mouseBrainSubsetSCE")
pcaVariances(mouseBrainSubsetSCE)
```

pcaVariances<-

Set PCA variances

### **Description**

Set PCA variances

### Usage

```
pcaVariances(x, ...) \leftarrow value
```

### **Arguments**

x SCtkE object... other parametersvalue PCA variances DataFrame()

### Value

A SCtkExperiment object with the pcaVariances slot set.

```
data("mouseBrainSubsetSCE")
pcaVariances(mouseBrainSubsetSCE)
#getPCA() sets the pcaVariances
newSCE <- getPCA(mouseBrainSubsetSCE, useAssay = "counts")
#alternatively, set the pcaVariances directly
pca <- prcomp(assay(mouseBrainSubsetSCE, "logcounts"))
percentVar <- pca$sdev ^ 2 / sum(pca$sdev ^ 2)
pcaVariances(mouseBrainSubsetSCE) <- DataFrame(percentVar)</pre>
```

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plotBatchVariance	Plot the percent of the variation that is explained by batch and condi-
	tion in the data

### Description

Visualize the percent variation in the data that is explained by batch and condition if it is given.

#### Usage

```
plotBatchVariance(inSCE, useAssay = "logcounts", batch,
  condition = NULL)
```

### **Arguments**

inSCE Input SCtkExperiment object. Required
useAssay Indicate which assay to use for PCA. Default is "logcounts"
batch The column in the annotation data that corresponds to batch. Required
condition The column in the annotation data that corresponds to condition. Optional

#### Value

A boxplot of variation explained by batch, condition, and batch+condition (if applicable).

### **Examples**

```
if(requireNamespace("bladderbatch", quietly = TRUE)) {
  library(bladderbatch)
  data(bladderdata)
  dat <- as(as(bladderEset, "SummarizedExperiment"), "SCtkExperiment")
  plotBatchVariance(dat, useAssay="exprs", batch="batch", condition = "cancer")
}</pre>
```

plotBiomarker

Given a set of genes, return a ggplot of expression values.

### Description

Given a set of genes, return a ggplot of expression values.

### Usage

```
plotBiomarker(inSCE, gene, binary = "Binary", visual = "PCA",
    shape = "No Shape", x = "PC1", y = "PC2", useAssay = "counts",
    reducedDimName = "PCA")
```

plotDiffEx 23

#### **Arguments**

inSCE Input SCtkExperiment object. Required

gene genelist to run the method on.

binary binary/continuous color for the expression.

visual Type of visualization (PCA, tSNE or UMAP). Default: "PCA"

shape shape parameter for the ggplot.

x x coordinate for PCA y y coordinate for PCA

useAssay Indicate which assay to use. The default is "logcounts".

reducedDimName a name to store the results of the dimension reduction coordinates obtained from

this method. This is stored in the SingleCellExperiment object in the reduced-

Dims slot. Required.

#### Value

A Biomarker plot

### **Examples**

```
data("mouseBrainSubsetSCE")
plotBiomarker(mouseBrainSubsetSCE, gene="C1qa", shape="level1class")
```

plotDiffEx

Plot Differential Expression

### Description

Plot Differential Expression

### Usage

```
plotDiffEx(inSCE, useAssay = "logcounts", condition, geneList,
  clusterRow = TRUE, clusterCol = TRUE, displayRowLabels = TRUE,
  displayColumnLabels = TRUE, displayRowDendrograms = TRUE,
  displayColumnDendrograms = TRUE, annotationColors = NULL,
  scaleExpression = TRUE, columnTitle = "Differential Expression")
```

### Arguments

inSCE	Input data object that contains the data to be plotted. Required
useAssay	Indicate which assay to use. Default is "logcounts"
condition	The condition used for plotting the heatmap. Required
geneList	The list of genes to put in the heatmap. Required
clusterRow	Cluster the rows. The default is TRUE
clusterCol	Cluster the columns. The default is TRUE

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displayRowLabels

Display the row labels on the heatmap. The default is TRUE.

displayColumnLabels

Display the column labels on the heatmap. The default is TRUE

displayRowDendrograms

Display the row dendrograms on the heatmap. The default is TRUE

displayColumnDendrograms

Display the column dendrograms on the heatmap. The default is TRUE.

annotationColors

Set of annotation colors for color bar. If null, no color bar is shown. default is NULL.

scaleExpression

Row scale the heatmap values. The default is TRUE.

columnTitle Title to be displayed at top of heatmap.

#### Value

ComplexHeatmap object for the provided geneList annotated with the condition.

### **Examples**

plotPCA

Plot PCA run data from its components.

### **Description**

Plot PCA run data from its components.

### Usage

```
plotPCA(inSCE, colorBy = "No Color", shape = "No Shape", pcX = "PC1",
    pcY = "PC2", reducedDimName = "PCA", runPCA = FALSE,
    useAssay = "logcounts")
```

### Arguments

inSCE Input SCTKExperiment object. Required. colorBy The variable to color clusters by

shape Shape of the points

pcX User choice for the first principal component
pcY User choice for the second principal component

plotTSNE 25

reducedDimName a name to store the results of the dimension reduction coordinates obtained from

this method. This is stored in the SingleCellExperiment object in the reduced-

Dims slot. Required.

runPCA Run PCA if the reducedDimName does not exist. the Default is FALSE.

useAssay Indicate which assay to use. The default is "logcounts".

#### Value

A PCA plot

#### **Examples**

plotTSNE Plot t-SNE plot on dimensionality reduction data run from t-SNE

method.

#### **Description**

Plot t-SNE plot on dimensionality reduction data run from t-SNE method.

### Usage

```
plotTSNE(inSCE, colorBy = "No Color", shape = "No Shape",
  reducedDimName = "TSNE", runTSNE = FALSE, useAssay = "logcounts")
```

#### **Arguments**

inSCE Input SCtkExperiment object. Required

colorBy color by condition.

shape add shape to each distinct label.

reducedDimName a name to store the results of the dimension reduction coordinates obtained from

this method. This is stored in the SingleCellExperiment object in the reduced-

Dims slot. Required.

runTSNE Run t-SNE if the reducedDimName does not exist. the Default is FALSE.

useAssay Indicate which assay to use. The default is "logcounts".

#### Value

A t-SNE plot

26 saveBiomarkerRes

plotUMAP	Plot UMAP results either on already run results or run first and then plot.
----------	---

#### **Description**

Plot UMAP results either on already run results or run first and then plot.

#### Usage

```
plotUMAP(inSCE, colorBy = "No Color", shape = "No Shape",
  reducedDimName = "UMAP", runUMAP = FALSE, useAssay = "logcounts")
```

### **Arguments**

inSCE Input SCtkExperiment object with saved dimension reduction components or a

variable with saved results. Required

colorBy color by a condition(any column of the annotation data).

shape add shapes to each condition.

reducedDimName saved dimension reduction name in the SCtkExperiment object. Required.

runUMAP If the dimension reduction components are already available set this to FALSE,

otherwise set to TRUE. Default is False.

useAssay Indicate which assay to use. The default is "logcounts"

#### Value

a UMAP plot of the reduced dimensions.

#### **Examples**

saveBiomarkerRes Save biomarker gene information with a custom name when provided with diffex results.

### Description

saveBiomarkerRes Save biomarker gene information with a custom name when provided with diffex results.

### Usage

```
saveBiomarkerRes(inSCE, diffex, biomarkerName, method, ntop = 25,
   logFC = NULL, pVal = NULL)
```

saveDiffExResults 27

#### Arguments

inSCE Input SCtkExperiment object. Required

diffex results table saved from the differential expression analysis. Required. biomarkerName name of the biomarker result to be saved under in rowData(). Required.

method name of the diffex method used to generate the results. Options are DESeq2,

Limma and ANOVA. Required

ntop number of top N genes. Default is 25. Required

logFC logfold-change cutoff applied to save biomarker results. Optional

pVal adjusted p-value cutoff. Optional

#### Value

a new SCE object with the diffex result saved in the rowData using the "biomarkerName"

### **Examples**

saveDiffExResults Save Differential Expression Results with a custom name.

### Description

saveDiffExResults Save Differential Expression Results with a custom name.

#### **Usage**

```
saveDiffExResults(inSCE, diffex, name, method)
```

#### **Arguments**

inSCE Input SCtkExperiment object. Required

diffex results table saved from the differential expression analysis. Required

name of the result to be saved under in rowData(). Required

method name of the diffex method used to generate the results. Options are DESeq2,

limma and ANOVA. Required

#### Value

a new SCE object with the diffex result saved in the rowData using the "name"

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#### **Examples**

scDiffEx

Perform differential expression analysis on a SCtkExperiment object

#### **Description**

Perform differential expression analysis on a SCtkExperiment object

### Usage

```
scDiffEx(inSCE, useAssay = "logcounts", condition, covariates = NULL,
    significance = 0.05, ntop = 500, usesig = TRUE, diffexmethod,
    levelofinterest = NULL, analysisType = NULL, controlLevel = NULL,
    adjust = "fdr")

scDiffExDESeq2(inSCE, useAssay = "counts", condition,
    analysisType = "biomarker", levelofinterest = NULL,
    controlLevel = NULL, covariates = NULL, adjust = "fdr")

scDiffExlimma(inSCE, useAssay = "logcounts", condition,
    analysisType = "biomarker", levelofinterest = NULL,
    covariates = NULL, adjust = "fdr")

scDiffExANOVA(inSCE, useAssay = "logcounts", condition,
    covariates = NULL, adjust = "fdr")
```

#### **Arguments**

inSCE	Input SCtkExperiment object. Required
useAssay	Indicate which assay to use. Default is "logcounts" for limma and ANOVA, and "counts" for DESeq2.
condition	The name of the condition to use for differential expression. Must be a name of a column from colData that contains at least two labels. Required
covariates	Additional covariates to add to the model. Default is NULL
significance	FDR corrected significance cutoff for differentially expressed genes. Required
ntop	Number of top differentially expressed genes to display in the heatmap. Required
usesig	If TRUE, only display genes that meet the significance cutoff, up to ntop genes. Required

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diffexmethod The method for performing differential expression analysis. Available options are DESeq2, limma, and ANOVA. Required

levelofinterest

If the condition has more than two labels, levelofinterest should contain one factor for condition. The differential expression results will use levelofinterest depending on the analysisType parameter.

analysisType For conditions with more than two levels, limma and DESeq2 can be run using

multiple methods. For DESeq2, choose "biomarker" to compare the levelofinterest to all other samples. Choose "contrast" to compare the levelofinterest to a controlLevel (see below). Choose "fullreduced" to perform DESeq2 in LRT mode. For limma, Choose "biomarker" to compare the levelofinterest to all other samples. Choose "coef" to select a coefficient of interest with levelofinterest (see

below). Choose "allcoef" to test if any coefficient is different from zero.

controlLevel If the condition has more than two labels, controlLevel should contain one factor

from condition to use as the control.

adjust Method for p-value correction. See options in p.adjust(). The default is fdr.

#### Value

A data frame of gene names and adjusted p-values

#### **Functions**

- scDiffExDESeq2: Perform differential expression analysis with DESeq2
- scDiffExlimma: Perform differential expression analysis with limma
- scDiffExANOVA: Perform differential expression analysis with ANOVA

```
data("mouseBrainSubsetSCE")
res <- scDiffEx(mouseBrainSubsetSCE,</pre>
                 useAssay = "logcounts",
                 "level1class",
                 diffexmethod = "limma")
data("mouseBrainSubsetSCE")
#sort first 100 expressed genes
ord <- rownames(mouseBrainSubsetSCE)[</pre>
  order(rowSums(assay(mouseBrainSubsetSCE, "counts")),
        decreasing = TRUE)][1:100]
#subset to those first 100 genes
subset <- mouseBrainSubsetSCE[ord, ]</pre>
res <- scDiffExDESeq2(subset, condition = "level1class")</pre>
data("mouseBrainSubsetSCE")
res <- scDiffExlimma(mouseBrainSubsetSCE, condition = "level1class")</pre>
data("mouseBrainSubsetSCE")
res <- scDiffExANOVA(mouseBrainSubsetSCE, condition = "level1class")</pre>
```

30 SCtkExperiment-class

SCtkExperiment

Create a SCtkExperiment

#### **Description**

Create a SCtkExperiment

#### Usage

```
SCtkExperiment(..., pcaVariances = S4Vectors::DataFrame())
```

#### **Arguments**

... SingleCellExperiment and SummarizedExperiment components pcaVariances The percent variation contained in each PCA dimension

#### Value

A SingleCellExperiment like object with an addition pcaVariances slot.

### **Examples**

SCtkExperiment-class A lightweight

A lightweight S4 extension to the SingleCellExperiment class to store additional information.

### Description

A lightweight S4 extension to the SingleCellExperiment class to store additional information.

### Arguments

value The DataFrame of pcaVariances

### Value

A SingleCellExperiment like object with an addition pcaVariances slot.

singleCellTK 31

#### **Slots**

pcaVariances The percent variation contained in each PCA dimension

#### **Examples**

 $\verb|singleCellTK|$ 

Run the single cell analysis app

### **Description**

Use this function to run the single cell analysis app.

### Usage

```
singleCellTK(inSCE = NULL, includeVersion = TRUE, theme = "yeti")
```

#### **Arguments**

inSCE The input SCtkExperiment class object

includeVersion Include the version number in the SCTK header. The default is TRUE.

theme The bootswatch theme to use for the singleCellTK UI. The default is 'flatly'.

### Value

The shiny app will open

```
#Upload data through the app
if(interactive()){
    singleCellTK()
}

#Load the app with a SCtkExperiment object
if(interactive()){
    data("mouseBrainSubsetSCE")
    singleCellTK(mouseBrainSubsetSCE)
}
```

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subDiffEx	Passes the output of generateSimulatedData() to differential expression tests, picking either t-tests or ANOVA for data with only two con-
	ditions or multiple conditions, respectively.

### **Description**

Passes the output of generateSimulatedData() to differential expression tests, picking either t-tests or ANOVA for data with only two conditions or multiple conditions, respectively.

### Usage

```
subDiffEx(tempData)
subDiffExttest(countMatrix, class.labels, test.type = "t.equalvar")
subDiffExANOVA(countMatrix, condition)
```

#### **Arguments**

tempData	Matrix. The output of generateSimulatedData(), where the first row contains condition labels.	
countMatrix	Matrix. A simulated counts matrix, sans labels.	
class.labels	Factor. The condition labels for the simulated cells. Will be coerced into 1's and 0's.	
test.type	Type of test to perform. The default is t.equalvar.	
condition	Factor. The condition labels for the simulated cells.	

#### Value

subDiffEx(): A vector of fdr-adjusted p-values for all genes. Nonviable results (such as for genes with 0 counts in a simulated dataset) are coerced to 1.

subDiffExttest(): A vector of fdr-adjusted p-values for all genes. Nonviable results (such as for genes with 0 counts in a simulated dataset) are coerced to 1.

subDiffExANOVA(): A vector of fdr-adjusted p-values for all genes. Nonviable results (such as for genes with 0 counts in a simulated dataset) are coerced to 1.

#### **Functions**

- subDiffEx: Get PCA components for a SCtkE object
- subDiffExttest: Runs t-tests on all genes in a simulated dataset with 2 conditions, and adjusts for FDR.
- subDiffExANOVA: Runs ANOVA on all genes in a simulated dataset with more than 2 conditions, and adjusts for FDR.

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#### **Examples**

```
data("mouseBrainSubsetSCE")
res <- generateSimulatedData(</pre>
         totalReads = 1000, cells=10,
         originalData = assay(mouseBrainSubsetSCE, "counts"),
          realLabels = colData(mouseBrainSubsetSCE)[, "level1class"])
tempSigDiff <- subDiffEx(res)</pre>
data("mouseBrainSubsetSCE")
#sort first 100 expressed genes
ord <- rownames(mouseBrainSubsetSCE)[</pre>
  order(rowSums(assay(mouseBrainSubsetSCE, "counts")),
        decreasing = TRUE)][1:100]
#subset to those first 100 genes
subset <- mouseBrainSubsetSCE[ord, ]</pre>
res <- generateSimulatedData(totalReads = 1000, cells=10,</pre>
                               originalData = assay(subset, "counts"),
                               realLabels = colData(subset)[, "level1class"])
realLabels <- res[1, ]</pre>
output <- res[-1, ]</pre>
fdr <- subDiffExttest(output, realLabels)</pre>
data("mouseBrainSubsetSCE")
#sort first 100 expressed genes
ord <- rownames(mouseBrainSubsetSCE)[</pre>
  order(rowSums(assay(mouseBrainSubsetSCE, "counts")),
        decreasing = TRUE)][1:100]
# subset to those first 100 genes
subset <- mouseBrainSubsetSCE[ord, ]</pre>
res <- generateSimulatedData(totalReads = 1000, cells=10,</pre>
                               originalData = assay(subset, "counts"),
                               realLabels = colData(subset)[, "level2class"])
realLabels <- res[1, ]</pre>
output <- res[-1, ]</pre>
fdr <- subDiffExANOVA(output, realLabels)</pre>
```

summarizeTable

Summarize SCtkExperiment

### Description

Creates a table of summary metrics from an input SCtkExperiment.

#### Usage

```
summarizeTable(inSCE, useAssay = "counts", expressionCutoff = 1700)
```

### Arguments

inSCE Input SCtkExperiment object. Required useAssay Indicate which assay to summarize. Default is "counts" expressionCutoff

Count number of samples with fewer than expressionCutoff genes. The default is 1700.

visPlot

#### Value

A data frame object of summary metrics.

### **Examples**

```
data("mouseBrainSubsetSCE")
summarizeTable(mouseBrainSubsetSCE)
```

visPlot visPlot

### Description

Given a plotting method with condition and gene list, return the respective visualization plot(s).

### Usage

```
visPlot(inSCE, useAssay, method, condition, glist, facetWrap = TRUE,
    scaleHMap = TRUE, convertFactor = FALSE)
```

### **Arguments**

inSCE	Input SCtkExperiment object. Required
useAssay	The assay to use in the visualization plot. Required
method	Visualization method. Available options are boxplot, scatterplot, or heatmap. Required
condition	colData annotation of the experiment. Required
glist	selected genes for visualization. Maximum 25 genes. Required
facetWrap	facet wrap according to genes for boxplot, scatterplot and barplot. Default is FALSE. Optional
scaleHMap	scale heatmap expression values. Default is TRUE. Optional
convertFactor	If the condition is not a factor, convert it to a factor before plotting. The default is FALSE

#### Value

A visualization plot

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