# Package 'singleCellTK'

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Type Package

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.

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**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, shinyBS, sva, reshape2, AnnotationDbi, shinyalert, circlize, enrichR, celda, shinycssloaders, shinythemes, umap

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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

#### alignSingleCellData

### 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.	
sampleAnnotatio		
	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.

### Examples

calcEffectSizes

### 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)
```

#### **ComBatSCE**

#### 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()

#### Examples

```
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</pre>
  assays(dat)
}
```

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

inSCE	Input SCtkExperiment object. Required
inSymbol	The input symbol type
outSymbol	The output symbol type
database	The org.*.eg.db database to use. The default is org.Hs.eg.db

### 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)
```

#### distinctColors

#### 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 = c(0.7, 1),
  value.range = c(0.7, 1))
```

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.

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)

DownsampleCells	Estimate numbers of detected genes, significantly differentially ex-
	pressed genes, and median significant effect size

### 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)
```

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.	

#### DownsampleDepth

### 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 ex-
	pressed 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)
```

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

### 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 per-
	form 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.,

### Examples

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

## End(Not run)

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filterSCData

#### 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)
```

### 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

```
data("mouseBrainSubsetSCE")
res <- generateSimulatedData(
    totalReads = 1000, cells=10,
    originalData = assay(mouseBrainSubsetSCE, "counts"),
    realLabels = colData(mouseBrainSubsetSCE)[, "level1class"])</pre>
```

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

### Value

getBiomarker(): A data.frame of expression values

### Examples

getBiomarker(mouseBrainSubsetSCE, gene="C1qa")

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 Components")
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>.</algorithm></assay>

### 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")

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>.</algorithm></assay>

### 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

#### Examples

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getUMAP

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

### Description

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

### Usage

### 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)
```

#### 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_nam	nes
	Display the column labels on the heatmap. The default is TRUE
<pre>show_row_names</pre>	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

### Examples

```
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>
```

#### iterateSimulations

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

### Examples

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

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.

#### 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>
```

mouseBrainSubsetSCE	Example	Single	Cell	RNA-Seq	data	in	SCtkExperiment	Object,
	GSE6036	1 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")

parseRsubreadLogs Parse Rsubread L

#### 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	6
	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>

х	SCtkE object
	other parameters
value	The DataFrame of pcaVariances

#### pcaVariances<-

#### 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, ...) <- value</pre>

#### Arguments

х	SCtkE object
	other parameters
value	PCA variances DataFrame()

### Value

A SCtkExperiment object with the pcaVariances slot set.

### Examples

```
data("mouseBrainSubsetSCE")
pcaVariances(mouseBrainSubsetSCE)
#getPCA() sets the pcaVariances
newSCE <- getPCA(mouseBrainSubsetSCE, useAssay = "counts")</pre>
```

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

plotBatchVariance

### 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", shape = "No Shape",
  useAssay = "counts", reducedDimName = "PCA", x = NULL, y = NULL,
  comp1 = NULL, comp2 = NULL)
```

#### plotDiffEx

### Arguments

inSCE	Input SCtkExperiment object. Required
gene	genelist to run the method on.
binary	binary/continuous color for the expression.
shape	shape parameter for the ggplot.
useAssay	Indicate which assay to use. The default is "logcounts".
reducedDimName	results name of dimension reduction coordinates obtained from this method. This is stored in the SingleCellExperiment object in the reducedDims slot. Re- quired.
x	PCA component to be used for plotting(if applicable). Default is first PCA component for PCA data and NULL otherwise.
У	PCA component to be used for plotting(if applicable). Default is second PCA component for PCA data and NULL otherwise.
comp1	label for x-axis
comp2	label for y-axis

### Value

A Biomarker plot

### Examples

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

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")
```

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			
displayRowLabel	S			
	Display the row labels on the heatmap. The default is TRUE.			
displayColumnLa	bels			
	Display the column labels on the heatmap. The default is TRUE			
displayRowDendr	ograms			
	Display the row dendrograms on the heatmap. The default is TRUE			
displayColumnDe	ndrograms			
	Display the column dendrograms on the heatmap. The default is TRUE.			
annotationColor	S			
	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

plotDimRed

Plot results either on already run results of reduced dimensions data.

### Description

Plot results either on already run results of reduced dimensions data.

### Usage

```
plotDimRed(inSCE, colorBy = "No Color", shape = "No Shape",
  reducedDimName = NULL, useAssay = "logcounts", comp1 = NULL,
  comp2 = NULL, pcX = NULL, pcY = NULL)
```

#### plotPCA

### 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.
useAssay	Indicate which assay to use. The default is "logcounts"
comp1	label for x-axis
comp2	label for y-axis
рсХ	PCA component to be used for plotting(if applicable). Default is first PCA component for PCA data and NULL otherwise.
рсҮ	PCA component to be used for plotting(if applicable). Default is second PCA component for PCA data and NULL otherwise.

### Value

a ggplot of the reduced dimensions.

### Examples

```
plotDimRed(inSCE = mouseBrainSubsetSCE, colorBy = "No Color", shape = "No Shape",
    reducedDimName = "TSNE_counts", useAssay = "counts",
    comp1 = "tSNE1", comp2 = "tSNE2")
```

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")
```

inSCE	Input SCTKExperiment object. Required.
colorBy	The variable to color clusters by
shape	Shape of the points
рсХ	User choice for the first principal component
рсҮ	User choice for the second principal component
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 of	n 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

### Examples

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plotUMAP

### 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	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)
```

### 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	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	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"

#### scDiffEx

### 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")
```

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

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 levelofin- terest 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

### Examples

```
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>
```

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 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.

### Slots

pcaVariances The percent variation contained in each PCA dimension

#### Examples

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

### Examples

```
#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 conditions 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.

#### 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"
expressionCutof	f
	Count number of samples with fewer than expressionCutoff genes. The default
	is 1700.

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#### 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 = NULL, 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

### Examples

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