

# Package ‘SPsimSeq’

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**Title** Semi-parametric simulation tool for bulk and single-cell RNA sequencing data

**Version** 1.16.0

**Description** SPsimSeq uses a specially designed exponential family for density estimation to constructs the distribution of gene expression levels from a given real RNA sequencing data (single-cell or bulk), and subsequently simulates a new dataset from the estimated marginal distributions using Gaussian-copulas to retain the dependence between genes. It allows simulation of multiple groups and batches with any required sample size and library size.

**License** GPL-2

**Encoding** UTF-8

**LazyData** true

**URL** <https://github.com/CenterForStatistics-UGent/SPsimSeq>

**Imports** stats, methods, SingleCellExperiment, fitdistrplus, graphics, edgeR, Hmisc, WGCNA, limma, mvtnorm, phyloseq, utils

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SPsimSeq-package	<i>SPsimSeq package</i>
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## Description

SPsimSeq uses a specially designed exponential family for density estimation to constructs the distribution of gene expression levels from a given real RNA sequencing data (single-cell or bulk), and subsequently, simulates a new dataset from the estimated marginal distributions using Gaussian-copulas to retain the dependence between genes. It allows simulation of multiple groups and batches with any required sample size and library size.

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**References**

- Alemu Takele Assefa, Jo Vandesompele, Olivier Thas. (2020). SPsimSeq: semi-parametric simulation of bulk and single cell RNA sequencing data, *Bioinformatics*, , btaa105, <https://doi.org/10.1093/bioinform>

---

buildXmat	<i>An auxiliary function to quickly construct the polyomial matrix, using Horner's rule</i>
-----------	---

---

**Description**

An auxiliary function to quickly construct the polyomial matrix, using Horner's rule

**Usage**

```
buildXmat(x, nc)
```

**Arguments**

x	The base
nc	the number of columns

**Value**

A matrix with increasing powers of x in the columns

---

calculateCPM	<i>Calculates counts per millions of reads, possibly with log-transform</i>
--------------	---

---

**Description**

Calculates counts per millions of reads, possibly with log-transform

**Usage**

```
calculateCPM(X, const.mult, prior.count)
```

**Arguments**

X	raw data matrix
const.mult	a constant to multiply with
prior.count	prior count to be added to the zeroes

**Value**

a normalized data matrix

---

checkInputValidity	<i>Check for data validity</i>
--------------------	--------------------------------

---

**Description**

Check for data validity

**Usage**

```
checkInputValidity(  
  s.data,  
  group,  
  batch,  
  group.config,  
  batch.config,  
  w,  
  log.CPM.transform,  
  prior.count,  
  pDE,  
  lib.size.params,  
  llStat.thrld,  
  result.format  
)
```

**Arguments**

s.data, group, batch, group.config, batch.config, w, log.CPM.transform,  
prior.count, pDE, lib.size.params, llStat.thrld, result.format  
see ?SPsimSeq

**Value**

Throws errors where needed, otherwise returns invisible

---

chooseCandGenes	<i>Select candidate genes</i>
-----------------	-------------------------------

---

**Description**

This function can be used to independently select candidate genes from a given real RNA-srq data (bulk/single) for the SPsimSeq simulation. It chooses genes with various characteristics, such as log-fold-change above a certain threshold.

**Usage**

```

chooseCandGenes(
  cpm.data,
  group,
  lfc.thrld,
  llStat.thrld,
  t.thrld,
  w = w,
  max.frac.zeror.diff = Inf,
  pDE,
  n.genes,
  prior.count
)

```

**Arguments**

cpm.data	logCPM transformed matrix (if log.CPM.transform=FALSE, then it is the source gene expression data)
group	a grouping factor
lfc.thrld	a positive numeric value for the minimum absolute log-fold-change for selecting candidate DE genes in the source data (when group is not NULL and pDE>0)
llStat.thrld	a positive numeric value for the minimum squared test statistics from the log-linear model to select candidate DE genes in the source data (when group is not NULL and pDE>0) containing X as a covariate to select DE genes
t.thrld	a positive numeric value for the minimum absolute t-test statistic for the log-fold-changes of genes for selecting candidate DE genes in the source data (when group is not NULL and pDE>0)
w	a numeric value between 0 and 1. The number of classes to construct the probability distribution will be round(w*n), where n is the total number of samples/cells in a particular batch of the source data
max.frac.zeror.diff	a numeric value >=0 indicating the maximum absolute difference in the fraction of zero counts between the groups for DE genes.
pDE	fraction of DE genes
n.genes	total number of genes
prior.count	a positive constant to be added to the CPM before log transformation, to avoid log(0). The default is 1.

**Value**

a list object containing a set of candidate null and non-null genes and additional results

---

configExperiment	<i>Configure experiment</i>
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---

## Description

Configure experiment

## Usage

```
configExperiment(batch.config, group.config, tot.samples, batch, group)
```

## Arguments

batch.config	a numerical vector for the marginal fraction of samples in each batch. The number of batches to be simulated is equal to the size of the vector. All values must sum to 1.
group.config	a numerical vector for the marginal fraction of samples in each group. The number of groups to be simulated is equal to the size of the vector. All values must sum to 1.
tot.samples	total number of samples to be simulated.
batch, group	batch and grouping vectors

## Value

a list object containing the number of groups and batches to be simulated, and the experiment configuration

## Examples

```
batch = sample(LETTERS[1:3], 20, replace = TRUE)
group = sample(1:3, 20, replace = TRUE)
#---- a design with a total of 10 samples/cells from 1 batch and 1 group
configExperiment(batch.config=1, group.config=1, tot.samples=10,
batch = batch, group = group)

#---- a design with a total of 20 samples/cells from 1 group and 2 batches with
# batch 1 has 15 samples/cells and batch 2 has 5
configExperiment(batch.config = c(15/20, 5/20), group.config = 1,
tot.samples = 20, batch = batch, group = group)

#---- a design with a total of 20 samples/cells from 1 batch and 2 groups with
# group 1 has 10 samples/cells and batch 2 has 10
configExperiment(batch.config=1, group.config=c(0.5, 0.5), tot.samples=20,
batch = batch, group = group)

#---- a design with a total of 30 samples/cells from 2 groups with group 1 has 15 samples
# and group 2 has 15, and three batches with batch 1,2, and 3 have 5, 10, and 15 samples/cells,
# respectively.
configExperiment(batch.config = c(5/30, 10/30, 15/30), group.config = c(0.5, 0.5),
tot.samples = 30, batch = batch, group = group)
```

---

constructDens	<i>Construct the cumulative density</i>
---------------	---

---

**Description**

Construct the cumulative density

**Usage**

```
constructDens(densList.ii, exprmt.design, DE.ind.ii, returnDens = FALSE)
```

**Arguments**

densList.ii	the estimated density parameters
exprmt.design	experiment configuration
DE.ind.ii	a boolean, is the gene to be DE?
returnDens	A boolean, should densities rather than cumulative densities be returned?

**Value**

The cumulative density

---

estLibSizeDistr	<i>Estimate log-normal distribution for the library sizes</i>
-----------------	---

---

**Description**

Estimate log-normal distribution for the library sizes

**Usage**

```
estLibSizeDistr(LS, batch)
```

**Arguments**

LS	observed library sizes
batch	batches

**Value**

Estimated log-normal parameter library sizes

---

evaluateDensities      *Evaluate the densities in the estimated SPsimSeq object*

---

### Description

Evaluate the densities in the estimated SPsimSeq object

### Usage

```
evaluateDensities(SPobj, newData = names(SPobj$detailed.results$densList))
```

### Arguments

SPobj                    The SPsimSeq object, with details retained  
 newData                A character vector of gene names

### Value

a list of estimated densities, breaks and midpoints, one for every gene in newData

### Examples

```
data("zhang.data.sub")
# filter genes with sufficient expression (important step to avoid bugs)
zhang.counts <- zhang.data.sub$counts
MYCN.status <- zhang.data.sub$MYCN.status
# simulate data
sim.data.bulk <- SPsimSeq(n.sim = 1, s.data = zhang.counts,
                          group = MYCN.status, n.genes = 2000, batch.config = 1,
                          group.config = c(0.5, 0.5), tot.samples = 20,
                          pDE = 0.1, lfc.thrld = 0.5, result.format = "list",
                          return.details = TRUE)
outDens = evaluateDensities(sim.data.bulk)
select.genes <- sample(names(outDens), 4)
select.sample = sample(
  seq_along(sim.data.bulk$detailed.results$exprmt.design$sub.groups), 1)
par(mfrow=c(2, 2))
for(i in select.genes){
  plot(outDens[[i]][[select.sample]]$mids, outDens[[i]][[select.sample]]$gy, type = "l",
       xlab = "Outcome", ylab = "Density", main = paste("Gene", i))
}
```

---

expit                    *Evaluate the expit function*

---

### Description

Evaluate the expit function

### Usage

```
expit(x)
```



**Arguments**

x                    the argument

**Value**

the expit of the argument

---

extractMat                    *A function with S4 dispatching to extract the count matrix*

---

**Description**

A function with S4 dispatching to extract the count matrix

**Usage**

```
extractMat(Y, ...)  
  
## S4 method for signature 'SingleCellExperiment'  
extractMat(Y, ...)  
  
## S4 method for signature 'matrix'  
extractMat(Y, ...)  
  
## S4 method for signature 'data.frame'  
extractMat(Y, ...)  
  
## S4 method for signature 'phyloseq'  
extractMat(Y, ...)
```

**Arguments**

Y                    a matrix, data frame, phyloseq object or SingleCellExperiment  
...                    additional arguments, currently ignored

**Value**

A data matrix with samples in the columns and genes in the rows

---

`fitLLmodel`*Fit log linear model for each gene*

---

**Description**

Fit log linear model for each gene

**Usage**

```
fitLLmodel(yy, mu.hat, sig.hat, n)
```

**Arguments**

`yy` a list object containing a result from `obtCount()` function for a single gene  
`mu.hat, sig.hat` Carrier density estimators  
`n` number of observations

**Value**

a list object containing the fitted log linear model and carrier density

---

`fitPoisGlm`*Fast fit Poisson regression*

---

**Description**

Fast fit Poisson regression

**Usage**

```
fitPoisGlm(Ny, x, degree, offset)
```

**Arguments**

`Ny` vector of counts  
`x` regressor  
`degree` degree of the polynomial  
`offset` offset

**Value**

see `glm.fit`

---

fracZeroLogitModel	<i>Extract data and iterate over batches to estimate zero probability models</i>
--------------------	--

---

**Description**

Extract data and iterate over batches to estimate zero probability models

**Usage**

```
fracZeroLogitModel(s.data, batch, cpm.data, n.mean.class, minFracZeroes)
```

**Arguments**

s.data, cpm.data	raw and transformed data
batch	the batch vector
n.mean.class	see zeroProbModel
minFracZeroes	minimum fraction of zeroes before zero-inflation is applied

**Value**

a list of binomial regression parameters

---

genCopula	<i>Generate a copula instance</i>
-----------	-----------------------------------

---

**Description**

Generate a copula instance

**Usage**

```
genCopula(corMats, exprmt.design)
```

**Arguments**

corMats	List of correlation matrices
exprmt.design	Number of batches, and batch vector

**Value**

a list of copula instances

---

geneParmEst                      *Gene level param estimates for density estimation*

---

### Description

Gene level param estimates for density estimation

### Usage

```
geneParmEst(
  cpm.data.i,
  batch,
  group,
  prior.count = prior.count,
  de.ind,
  model.zero.prob,
  w
)
```

### Arguments

cpm.data.i	full vector of genewise observation
batch, group	batch and grouping vectors
prior.count	the prior count for the cpm transform
de.ind	a boolean, is the gene to be DE?
model.zero.prob	a boolean, should zero-density be modelled?
w	weight

### Value

list of density estimates

---

genLibSizes                      *Generate library sizes from log-normal*

---

### Description

Generate library sizes from log-normal

### Usage

```
genLibSizes(fit.ln, exprmt.design)
```

### Arguments

fit.ln	the library size model
exprmt.design	the design

**Value**

The generated library sizes per batch and group

---

matchCopula	<i>Match copulas to estimated SP distribution</i>
-------------	---

---

**Description**

Match copulas to estimated SP distribution

**Usage**

```
matchCopula(cumDens, exprmt.design, copSam, sel.genes.ii)
```

**Arguments**

cumDens	The cumulative densities evaluated
exprmt.design	the design
copSam	the sampled copula
sel.genes.ii	the gene

**Value**

the outcome values as a vector

---

obtCorMatsBatch	<i>A function to obtain copulas or uniform random variables</i>
-----------------	---

---

**Description**

A function to obtain copulas or uniform random variables

**Usage**

```
obtCorMatsBatch(cpm.data, batch)
```

**Arguments**

cpm.data	the transformed data matrix
batch	the batch indicators

**Value**

The estimated correlation matrices per batch

---

obtCount	<i>Calculates height and mid points of a distribution</i>
----------	---

---

**Description**

Calculates height and mid points of a distribution

**Usage**

```
obtCount(Y, w)
```

**Arguments**

Y	a vector of gene expression data for a particular gene (in log CPM)
w	a numeric value between 0 and 1 or NULL referring the number of classes to be created

**Value**

a list object containing class breaks, mid points and counts

---

parmEstDensVec	<i>Density estimation on a single vector</i>
----------------	--

---

**Description**

Density estimation on a single vector

**Usage**

```
parmEstDensVec(
  Y0,
  model.zero.prob,
  min.val,
  w,
  prev.min.val = 0.25,
  min.count.nonnull = 3
)
```

**Arguments**

Y0	the vector of observations
model.zero.prob, min.val, w	see geneParmEst()
prev.min.val	minimum prevalence of minimum values
min.count.nonnull	minimum count for estimation

**Value**

density estimates

---

prepareSPsimOutputs    *A function to prepare outputs*

---

**Description**

A function to prepare outputs

**Usage**

```
prepareSPsimOutputs(sim.dat, exprmt.design, DE.ind, result.format, LL)
```

**Arguments**

sim.dat	The simulated data
exprmt.design	the design
DE.ind	the differential abundance indicator
result.format	the desired output format
LL	simulated library sizes

**Value**

the data in the desired format

---

samZeroID                    *Return ID for observations to be set to zero*

---

**Description**

Return ID for observations to be set to zero

**Usage**

```
samZeroID(fracZero.logit.list, logLL, gene)
```

**Arguments**

fracZero.logit.list	The estimated zero model
logLL	the logged library sizes
gene	the gene name

**Value**

A boolean, should a zero be introduced or not?





**Value**

a vector of selected genes

---

SPsimPerGene

*A function that generates the simulated data for a single gene*

---

**Description**

A function that generates the simulated data for a single gene

**Usage**

```
SPsimPerGene(
  cumDens,
  exprmt.design,
  sel.genes.ii,
  log.CPM.transform,
  prior.count,
  LL,
  copSam,
  model.zero.prob,
  fracZero.logit.list,
  const.mult
)
```

**Arguments**

cumDens	cumulative density
exprmt.design	the experiment design
sel.genes.ii	selected gene
log.CPM.transform	a boolean, is log-CPM transform required?
prior.count	the prior count
LL	the library sizes
copSam	the generated copula
model.zero.prob	a boolean, should the zeroes be modelled separately
fracZero.logit.list	The zero model
const.mult	a large constant for the CPM transform, normally 1e6

**Value**

Simulated cpm values

---

 SPsimSeq

*A function to simulate bulk or single cell RNA sequencing data*


---

### Description

This function simulates (bulk/single cell) RNA-seq dataset from semi-parametrically estimated distributions of gene expression levels in a given real source RNA-seq dataset

### Usage

```
SPsimSeq(
  n.sim = 1,
  s.data,
  batch = rep(1, ncol(s.data)),
  group = rep(1, ncol(s.data)),
  n.genes = 1000,
  batch.config = 1,
  group.config = 1,
  pDE = 0.1,
  cand.DE.genes = NULL,
  lfc.thrld = 0.5,
  t.thrld = 2.5,
  llStat.thrld = 5,
  tot.samples = ncol(s.data),
  model.zero.prob = FALSE,
  genewiseCor = TRUE,
  log.CPM.transform = TRUE,
  lib.size.params = NULL,
  variable.lib.size = FALSE,
  w = NULL,
  result.format = "SCE",
  return.details = FALSE,
  verbose = TRUE,
  prior.count = 1,
  const.mult = 1e+06,
  n.mean.class = 0.2,
  minFracZeroes = 0.25
)
```

### Arguments

<code>n.sim</code>	an integer for the number of simulations to be generated
<code>s.data</code>	a real source dataset (a <code>SingleCellExperiment</code> class or a matrix/data.frame of counts with genes in rows and samples in columns)
<code>batch</code>	NULL or a vector containing batch indicators for each sample/cell in the source data
<code>group</code>	NULL or a vector containing group indicators for each sample/cell in the source data
<code>n.genes</code>	a numeric value for the total number of genes to be simulated

<code>batch.config</code>	a numerical vector containing fractions for the composition of samples/cells per batch. The fractions must sum to 1. The number of batches to be simulated is equal to the size of the vector. (Example, <code>batch.config=c(0.6, 0.4)</code> means simulate 2 batches with 60% of the simulated samples/cells in batch 1 and the rest 40% in the second batch. Another example, <code>batch.config=c(0.3, 0.35, 0.25)</code> means simulate 3 batches with the first, second and third batches contain 30%, 35% and 25% of the samples/cells, respectively).
<code>group.config</code>	a numerical vector containing fractions for the composition of samples/cells per group. Similar definition to 'batch.config'. The number of groups to be simulated is equal to the size of the vector. The fractions must sum to 1.
<code>pDE</code>	a numeric value between 0 and 1 indicating the desired fraction of DE genes in the simulated data
<code>cand.DE.genes</code>	a list object containing candidate null and non-null (DE/predictor) genes. If NULL (the default), an internal function determines candidate genes based on log-fold-change and other statistics. The user can also pass a list of candidate null and non-null genes (they must be disjoint). In particular, the list should contain two character vectors (for the name of the features/genes in the source data) with names 'null.genes' and 'nonnull.genes'. For example, <code>cand.DE.genes=list(null.genes=c('A', 'B'), nonnull.genes=c('C', 'D'))</code> .
<code>lfc.thrld</code>	a positive numeric value for the minimum absolute log-fold-change for selecting candidate DE genes in the source data (when group is not NULL, <code>pDE&gt;0</code> and <code>cand.DE.genes</code> is NULL)
<code>t.thrld</code>	a positive numeric value for the minimum absolute t-test statistic for the log-fold-changes of genes for selecting candidate DE genes in the source data (when group is not NULL, <code>pDE&gt;0</code> and <code>cand.DE.genes</code> is NULL)
<code>llStat.thrld</code>	a positive numeric value for the minimum squared test statistics from the log-linear model to select candidate DE genes in the source data (when group is not NULL, <code>pDE&gt;0</code> and <code>cand.DE.genes</code> is NULL)
<code>tot.samples</code>	a numerical value for the total number of samples/cells to be simulated.
<code>model.zero.prob</code>	a logical value whether to model the zero expression probability separately (suitable for simulating of single-cell RNA-seq data or zero-inflated data)
<code>genewiseCor</code>	a logical value, if TRUE (default) the simulation will retain the gene-to-gene correlation structure of the source data using Gaussian-copulas. Note that if it is TRUE, the program will be slow or it may fail for a limited memory size.
<code>log.CPM.transform</code>	a logical value. If TRUE, the source data will be transformed into $\log\text{-}(\text{CPM}+\text{const})$ before estimating the probability distributions
<code>lib.size.params</code>	NULL or a named numerical vector containing parameters for simulating library sizes from log-normal distribution. If <code>lib.size.params=NULL</code> (default), then the package will fit a log-normal distribution for the library sizes in the source data to simulate new library sizes. If the user would like to specify the parameters of the log-normal distribution for the desired library sizes, then the log-mean and log-SD params of <code>rlnorm()</code> functions can be passed using this argument. Example, <code>lib.size.params=c(meanlog=10, sdlog=0.2)</code> . See also <code>?rlnorm</code> .
<code>variable.lib.size</code>	a logical value. If FALSE (default), then the expected library sizes are simulated once and remains the same for every replication (if <code>n.sim&gt;1</code> ).

w	see ?hist
result.format	a character value for the type of format for the output. Choice can be 'SCE' for SingleCellExperiment class or "list" for a list object that contains the simulated count, column information and row information.
return.details	a logical value. If TRUE, detailed results including estimated parameters and densities will be returned
verbose	a logical value, if TRUE a message about the status of the simulation will be printed on the console
prior.count	a positive constant to be added to the CPM before log transformation, to avoid log(0). The default is 1.
const.mult	A constant by which the count are scaled. Usually 1e6 to get CPM
n.mean.class	a fraction of the number of genes for the number of groups to be created for the mean log CPM of genes
minFracZeroes	minimum fraction of zeroes before a zero inflation model is fitted

## Details

This function uses a specially designed exponential family for density estimation to constructs the distribution of gene expression levels from a given real gene expression data (e.g. single-cell or bulk sequencing data), and subsequently, simulates a new from the estimated distributions.#' For simulation of single-cell RNA-seq data (or any zero inflated gene expression data), the program involves an additional step to explicitly account for the high abundance of zero counts (if required). This step models the probability of zero counts as a function the mean expression of the gene and the library size of the cell (both in log scale) to add excess zeros. This can be done by using *model.zero.prob=TRUE*. Note that, for extremely large size data, it is recommended to use a random sample of cells to reduce computation time. To enable this, add the argument *subset.data=TRUE* and you can specify the number of cells to be used using *n.samples* argument. For example *n.samples=400*. Given known groups of samples/cells in the source data, DGE is simulated by independently sampling data from distributions constructed for each group separately. In particular, this procedure is applied on a set of genes with absolute log-fold-change in the source data more than a given threshold (*lfc.thrld*). Moreover, when the source dataset involves samples/cells processed in different batches, our simulation procedure incorporates this batch effect in the simulated data, if required. Different experimental designs can be simulated using the group and batch configuration arguments to simulate biological/experimental conditions and batches, respectively. Also, it is important to filter the source data so that genes with sufficient expression will be used to estimate the probability distributions.

## Value

a list of SingleCellExperiment/list objects each containing simulated counts (not normalized), sample/cell level information in colData, and gene/feature level information in rowData.

## References

- Assefa, A. T., Vandesompele, J., & Thas, O. (2020). SPsimSeq: semi-parametric simulation of bulk and single cell RNA sequencing data. *Bioinformatics*, doi: <https://doi.org/10.1093/bioinformatics/btaa105>.
- Efron, B., & Tibshirani, R. (1996). Using specially designed exponential families for density estimation. *The Annals of Statistics*, 24(6), 2431-2461.

**Examples**

```

#-----
# Example 1: simulating bulk RNA-seq

# load the Zhang bulk RNA-seq data (available with the package)
data("zhang.data.sub")

zhang.counts <- zhang.data.sub$counts
MYCN.status <- zhang.data.sub$MYCN.status

# We simulate only a single data (n.sim = 1) with the following property
# - 1000 genes ( n.genes = 1000)
# - 40 samples (tot.samples = 40)
# - the samples are equally divided into 2 groups each with 20 samples
#   (group.config = c(0.5, 0.5))
# - all samples are from a single batch (batch = NULL, batch.config = 1)
# - we add 10% DE genes (pDE = 0.1)
# - the DE genes have a log-fold-change of at least 0.5 in
#   the source data (lfc.thrld = 0.5)
# - we do not model the zeroes separately, they are the part of density
#   estimation (model.zero.prob = FALSE)

# simulate data
set.seed(6452)
sim.data.bulk <- SPsimSeq(n.sim = 1, s.data = zhang.counts,
                        group = MYCN.status, n.genes = 1000, batch.config = 1,
                        group.config = c(0.5, 0.5), tot.samples = 40,
                        pDE = 0.1, lfc.thrld = 0.5, result.format = "list")

head(sim.data.bulk$counts[[1]][, seq_len(5)]) # count data
head(sim.data.bulk$colData) # sample info
head(sim.data.bulk$rowData) # gene info

#-----
# Example 2: simulating single cell RNA-seq from a single batch (read-counts)
# we simulate only a single scRNA-seq data (n.sim = 1) with the following property
# - 2000 genes (n.genes = 2000)
# - 100 cells (tot.samples = 100)
# - the cells are equally divided into 2 groups each with 50 cells
#   (group.config = c(0.5, 0.5))
# - all cells are from a single batch (batch = NULL, batch.config = 1)
# - we add 10% DE genes (pDE = 0.1)
# - the DE genes have a log-fold-change of at least 0.5
# - we model the zeroes separately (model.zero.prob = TRUE)
# - the output will be in SingleCellExperiment class object (result.format = "SCE")

library(SingleCellExperiment)

# load the NGP nutlin data (available with the package, processed with
# SMARTer/C1 protocol, and contains read-counts)
data("scNGP.data")

# filter genes with sufficient expression (important step to avoid bugs)
treatment <- ifelse(scNGP.data$characteristics..treatment=="nutlin",2,1)

set.seed(654321)

```

```
# simulate data (we simulate here only a single data, n.sim = 1)
sim.data.sc <- SPsimSeq(n.sim = 1, s.data = scNGP.data, group = treatment,
  n.genes = 2000, batch.config = 1, group.config = c(0.5, 0.5),
  tot.samples = 100, pDE = 0.1, lfc.thrld = 0.5, model.zero.prob = TRUE,
  result.format = "SCE")

sim.data.sc1 <- sim.data.sc[[1]]
class(sim.data.sc1)
head(counts(sim.data.sc1)[, seq_len(5)])
colData(sim.data.sc1)
rowData(sim.data.sc1)
```

---

zeroProbModel	<i>Predict zero probability using logistic rgression</i>
---------------	--

---

### Description

Predict zero probability using logistic rgression

### Usage

```
zeroProbModel(cpm.data, logL, zeroMat, n.mean.class)
```

### Arguments

cpm.data	log CPM matrix
logL	log library size of the source data
zeroMat	the matrix of zero indicators
n.mean.class	a fraction of the number of genes for the number of groups to be created for the mean log CPM of genes

### Value

The coefficients of the estimated logistic regression

---

zhang.data.sub	<i>Neuroblastoma bulk RNA-seq data retrieved from Zhang et (2015).</i>
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### Description

The data contains 498 neuroblastoma tumors. In short, unstranded poly(A)+ RNA sequencing was performed on the HiSeq 2000 instrument (Illumina). Paired-end reads with a length of 100 nucleotides were obtained. To quantify the full transcriptome, raw fastq files were processed with Kallisto v0.42.4 (index build with GRCh38-Ensembl v85). The pseudo-alignment tool Kallisto was chosen above other quantification methods as it is performing equally good but faster. For this study, a subset of 172 tumors (samples) with high-risk disease were selected, forming two groups: the MYCN amplified ( $n_1 = 91$ ) and MYCN non-amplified ( $n_2 = 81$ ) tumours. Sometimes we refer this dataset to us the Zhang data or the Zhang neuroblastoma data. In this package, a subset of 5000 genes (randomly selected) are made available for illustration purpose only.

### Usage

```
data(zhang.data.sub)
```

### Format

A list object

### Source

[GEOaccessionGSE49711](#)

### References

1. Zhang W, Yu Y, Hertwig F, Thierry-Mieg J, Zhang W, Thierry-Mieg D, Wang J, Furlanello C, Devanarayan V, Cheng J, et al. Comparison of RNA-seq and microarray-based models for clinical endpoint prediction. *Genome Biol.* 2015;16(133) <https://doi.org/10.1186/s13059-015-0694-1>
2. Assefa, A. T., De Paepe, K., Everaert, C., Mestdagh, P., Thas, O., & Vandesompele, J. (2018). Differential gene expression analysis tools exhibit substandard performance for long non-coding RNA-sequencing data. *GENOME BIOLOGY*, 19.

**counts** gene counts

**group** MYCN (0 for MYCN non-amplified and 1 for MYCN amplified)

### Examples

```
data("zhang.data.sub")  
str(zhang.data.sub)
```

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