Package `muscat`

March 2, 2024

**Title**  Multi-sample multi-group scRNA-seq data analysis tools

**Description**  `muscat` provides various methods and visualization tools for DS analysis in multi-sample, multi-group, multi-(cell-)subpopulation scRNA-seq data, including cell-level mixed models and methods based on aggregated “pseudobulk” data, as well as a flexible simulation platform that mimics both single and multi-sample scRNA-seq data.

**Type**  Package

**Version**  1.16.0

**Depends**  R (>= 4.3)

**Imports**  BiocParallel, blme, ComplexHeatmap, data.table, DESeq2, dplyr, edgeR, ggplot2, glmmTMB, grDevices, grid, limma, lmerTest, lme4, Matrix, matrixStats, methods, progress, purrr, S4Vectors, scales, scater, scuttle, sctransform, stats, SingleCellExperiment, SummarizedExperiment, variancePartition, viridis

**Suggests**  BiocStyle, countsimQC, cowplot, ExperimentHub, iCOBRA, knitr, phylogram, RColorBrewer, reshape2, rmarkdown, statmod, testthat, UpSetR

**biocViews**  ImmunoOncology, DifferentialExpression, Sequencing, SingleCell, Software, StatisticalMethod, Visualization

**License**  GPL-3

**VignetteBuilder**  knitr

**RoxygenNote**  7.2.3

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**URL**  https://github.com/HelenaLC/muscat

**BugReports**  https://github.com/HelenaLC/muscat/issues

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**git_branch**  RELEASE_3_18

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Description

... 

Usage

aggregateData(
  x,
  assay = NULL,
  by = c("cluster_id", "sample_id"),
  fun = c("sum", "mean", "median", "prop.detected", "num.detected"),
  scale = FALSE,
  verbose = TRUE,
  BPPARAM = SerialParam(progressbar = verbose)
)
Arguments

- `x` a `SingleCellExperiment`.
- `assay` character string specifying the assay slot to use as input data. Defaults to the 1st available (`assayNames(x)[1]`).
- `by` character vector specifying which `colData(x)` columns to summarize by (at most 2!).
- `fun` a character string. Specifies the function to use as summary statistic. Passed to `summarizeAssayByGroup`.
- `scale` logical. Should pseudo-bulks be scaled with the effective library size & multiplied by 1M?
- `verbose` logical. Should information on progress be reported?
- `BPPARAM` a `BiocParallelParam` object specifying how aggregation should be parallelized.

Value

a `SingleCellExperiment`.

- If `length(by) == 2`, each sheet (`assay`) contains pseudobulks for each of `by[1]`, e.g., for each cluster when `by = "cluster_id"`. Rows correspond to genes, columns to `by[2]`, e.g., samples when `by = "sample_id"`.
- If `length(by) == 1`, the returned SCE will contain only a single assay with rows = genes and columns = `by`.

Aggregation parameters (assay, by, fun, scaled) are stored in `metadata()$agg_pars`, and the number of cells that were aggregated are accessible in `int_colData()$n_cells`.

Author(s)

Helena L. Crowell & Mark D Robinson

References


Examples

```r
# pseudobulk counts by cluster-sample
data(example_sce)
pb <- aggregateData(example_sce)
library(SingleCellExperiment)
assayNames(example_sce) # one sheet per cluster
head(assay(example_sce)) # n_genes x n_samples

# scaled CPM
```
calcExprFreqs

Description

Calculates gene expression frequencies

Usage

calcExprFreqs(x, assay = "counts", th = 0)

Arguments

x  a SingleCellExperiment.
assay a character string specifying which assay to use.
th numeric threshold value above which a gene should be considered to be expressed.

Details

calcExprFreq computes, for each sample and group (in each cluster), the fraction of cells that express a given gene. Here, a gene is considered to be expressed when the specified measurement value (assay) lies above the specified threshold value (th).

Value

a SingleCellExperiment containing, for each cluster, an assay of dimensions #genes x #samples giving the fraction of cells that express each gene in each sample. If colData(x) contains a "group_id" column, the fraction of expressing cells in each group will be included as well.

Author(s)

Helena L Crowell & Mark D Robinson
Examples

data(example_sce)
library(SingleCellExperiment)

frq <- calcExprFreqs(example_sce)

# one assay per cluster
assayNames(frq)

# expression frequencies by
# sample & group; 1st cluster:
head(assay(frq))

Description

A SingleCellExperiment containing 10x droplet-based scRNA-seq PBCM data from 8 Lupus patients before and after 6h-treatment with INF-beta (16 samples in total).

The original data has been filtered to

- remove unassigned cells & cell multiplets
- retain only 4 out of 8 samples per experimental group
- retain only 5 out of 8 subpopulations (clusters)
- retain genes with a count $> 1$ in $> 50$ cells
- retain cells with $> 200$ detected genes
- retain at most 100 cells per cluster-sample instance

Assay logcounts corresponds to log-normalized values obtained from logNormCounts with default parameters.

The original measurement data, as well as gene and cell metadata is available through the NCBI GEO accession number GSE96583; code to reproduce this example dataset from the original data is provided in the examples section.

Value

a SingleCellExperiment.

Author(s)

Helena L Crowell

References

Examples

```r
# set random seed for cell sampling
set.seed(2929)

# load data
library(ExperimentHub)
eh <- ExperimentHub()
sce <- eh[["EH2259"]]

# drop unassigned cells & multiplets
sce <- sce[, !is.na(sce$cell)]
sce <- sce[, sce$multiplets == "singlet"]

# keep 4 samples per group
sce$id <- paste0(sce$stim, sce$ind)
inds <- sample(sce$ind, 4)
ids <- paste0(levels(sce$stim), rep(inds, each = 2))
sce <- sce[, sce$id %in% ids]

# keep 5 clusters
kids <- c("B cells", "CD4 T cells", "CD8 T cells",
          "CD14+ Monocytes", "FCGR3A+ Monocytes")
sce <- sce[, sce$cell %in% kids]
sce$cell <- droplevels(sce$cell)

# basic filtering on genes & cells
gs <- rowSums(counts(sce) > 1) > 50
cs <- colSums(counts(sce) > 0) > 200
sce <- sce[gs, cs]

# sample max. 100 cells per cluster-sample
cs_by_ks <- split(colnames(sce), list(sce$cell, sce$id))
cs <- sapply(cs_by_ks, function(u)
             sample(u, min(length(u), 100)))
sce <- sce[, unlist(cs)]

# compute logcounts
library(scater)
sce <- computeLibraryFactors(sce)
sce <- logNormCounts(sce)

# re-format for 'muscat'
sce <- prepSCE(sce,
              kid = "cell",
              sid = "id",
              gid = "stim",
              drop = TRUE)
```
mmDS

DS analysis using mixed-models (MM)

Description

Performs cluster-wise DE analysis by fitting cell-level models.

Usage

```r
mmDS(
  x,
  coef = NULL,
  covs = NULL,
  method = c("dream2", "dream", "vst", "poisson", "nbinom", "hybrid"),
  n_cells = 10,
  n_samples = 2,
  min_count = 1,
  min_cells = 20,
  verbose = TRUE,
  BPPARAM = SerialParam(progressbar = verbose),
  vst = c("sctransform", "DESeq2"),
  ddf = c("Satterthwaite", "Kenward-Roger", "lme4"),
  dup_corr = FALSE,
  trended = FALSE,
  bayesian = FALSE,
  blind = TRUE,
  REML = TRUE,
  moderate = FALSE
)
```

```r
.mm_dream(
  x,
  coef = NULL,
  covs = NULL,
  dup_corr = FALSE,
  trended = FALSE,
  ddf = c("Satterthwaite", "Kenward-Roger"),
  verbose = FALSE,
  BPPARAM = SerialParam(progressbar = verbose)
)
```

```r
.mm_dream2(
  x,
  coef = NULL,
  covs = NULL,
  ddf = c("Satterthwaite", "Kenward-Roger"),
  verbose = FALSE,
)```
BPPARAM = SerialParam(progressbar = verbose)
)

.mm_vst(
  x,
  vst = c("sctransform", "DESeq2"),
  coef = NULL,
  covs = NULL,
  bayesian = FALSE,
  blind = TRUE,
  REML = TRUE,
  ddf = c("Satterthwaite", "Kenward-Roger", "lme4"),
  verbose = FALSE,
  BPPARAM = SerialParam(progressbar = verbose)
)

.mm_glmm(
  x,
  coef = NULL,
  covs = NULL,
  family = c("poisson", "nbinom"),
  moderate = FALSE,
  verbose = TRUE,
  BPPARAM = SerialParam(progressbar = verbose)
)

Arguments

x a SingleCellExperiment.
coef character specifying the coefficient to test. If NULL (default), will test the last level of "group_id".
covs character vector of colData(x) column names to use as covariates.
method a character string. Either "dream2" (default, lme4 with voom-weights), "dream" (previous implementation of the dream method), "vst" (variance-stabilizing transformation), "poisson" (poisson GLM-MM), "nbinom" (negative binomial GLM-MM), "hybrid" (combination of pseudobulk and poisson methods) or a function accepting the same arguments.
n_cells number of cells per cluster-sample required to consider a sample for testing.
n_samples number of samples per group required to consider a cluster for testing.
min_count numeric. For a gene to be tested in a given cluster, at least min_cells must have a count >= min_count.
min_cells number (or fraction, if < 1) of cells with a count > min_count required for a gene to be tested in a given cluster.
verbose logical specifying whether messages on progress and a progress bar should be displayed.
BPPARAM a BiocParallelParam object specifying how differential testing should be parallelized.
method to use as variance-stabilizing transformations. "sctransform" for \texttt{vst}; "DESeq2" for \texttt{varianceStabilizingTransformation}.

ddf character string specifying the method for estimating the effective degrees of freedom. For method = "dream", either "Satterthwaite" (faster) or "Kenward-Roger" (more accurate); see \footnotesize{\texttt{?variancePartition::dream}} for details. For method = "vst", method "lme4" is also valid; see \footnotesize{\texttt{contest.lmerModLmerTest}}.

dup_corr logical; whether to use \texttt{duplicateCorrelation}.

trended logical; whether to use expression-dependent variance priors in \footnotesize{\texttt{eBayes}}.

bayesian logical; whether to use bayesian mixed models.

blind logical; whether to ignore experimental design for the vst.

REML logical; whether to maximize REML instead of log-likelihood.

moderate logical; whether to perform empirical Bayes moderation.

family character string specifying which GLMM to fit: "poisson" for \footnotesize{\texttt{bglm}}, "nbinom" for \footnotesize{\texttt{glmmTMB}}.

Details

The \footnotesize{\texttt{.mm_*}} functions (e.g. \footnotesize{\texttt{.mm_dream}}) expect cells from a single cluster, and do not perform filtering or handle incorrect parameters well. Meant to be called by \footnotesize{\texttt{mmDS}} with method = c("dream", "vst") and vst = c("sctransform", "DESeq2") to be applied across all clusters.

method = "dream2" \footnotesize{\texttt{variancePartition}}'s (>=1.14.1) \footnotesize{\texttt{voom-lme4}}-implementation of mixed models for RNA-seq data; function \footnotesize{\texttt{dream}}.

method = "dream" \footnotesize{\texttt{variancePartition}}'s older \footnotesize{\texttt{voom-lme4}}-implementation of mixed models for RNA-seq data; function \footnotesize{\texttt{dream}}.

method = "vst" vst = "sctransform" \footnotesize{\texttt{lmer}} or \footnotesize{\texttt{blmer}} mixed models on vst transformed counts.

Value

a data.frame

Functions

- \footnotesize{\texttt{.mm_dream()}}: see details.
- \footnotesize{\texttt{.mm_dream2()}}: see details.
- \footnotesize{\texttt{.mm_vst()}}: see details.
- \footnotesize{\texttt{.mm_glmm()}}: see details.

Author(s)

Pierre-Luc Germain & Helena L Crowell
References


Examples

```r
# subset "B cells" cluster
data(example_sce)
b_cells <- example_sce$cluster_id == "B cells"
sub <- example_sce[, b_cells]
sub$cluster_id <- droplevels(sub$cluster_id)

# downsample to 100 genes
gs <- sample(nrow(sub), 100)
sub <- sub[gs, ]

# run DS analysis using cell-level mixed-model
res <- mmDS(sub, method = "dream", verbose = FALSE)
head(res$'B cells')
```

description

pbDS tests for DS after aggregating single-cell measurements to pseudobulk data, by applying bulk RNA-seq DE methods, such as edgeR, DESeq2 and limma.

Usage

```r
pbDS(
  pb,
  method = c("edgeR", "DESeq2", "limma-trend", "limma-voom"),
  design = NULL,
  coef = NULL,
  contrast = NULL,
  min_cells = 10,
  filter = c("both", "genes", "samples", "none"),
  treat = FALSE,
  verbose = TRUE,
  BPPARAM = SerialParam(progressbar = verbose)
)
```
**Arguments**

- **pb**: a `SingleCellExperiment` containing pseudobulks as returned by `aggregateData`.
- **method**: a character string.
- **design**: For methods "edegR" and "limma", a design matrix with row & column names(!) created with `model.matrix`; For "DESeq2", a formula with variables in `colData(pb)`. Defaults to ~ group_id or the corresponding `model.matrix`.
- **coef**: passed to `glmQLFTest, contrasts.fit, results` for method = "edgeR", "limma-x", "DESeq2", respectively. Can be a list for multiple, independent comparisons.
- **contrast**: a matrix of contrasts to test for created with `makeContrasts`.
- **min_cells**: a numeric. Specifies the minimum number of cells in a given cluster-sample required to consider the sample for differential testing.
- **filter**: character string specifying whether to filter on genes, samples, both or neither.
- **treat**: logical specifying whether empirical Bayes moderated-t p-values should be computed relative to a minimum fold-change threshold. Only applicable for methods "limma-x" (treat) and "edgeR" (glmTreat), and ignored otherwise.
- **verbose**: logical. Should information on progress be reported?
- **BPPARAM**: a `BiocParallelParam` object specifying how differential testing should be parallelized.

**Value**

A list containing

- a data.frame with differential testing results,
- a `DGEList` object of length nb.-clusters, and
- the design matrix, and contrast or coef used.

**Author(s)**

Helena L Crowell & Mark D Robinson

**References**


**Examples**

```r
# simulate 5 clusters, 20% of DE genes
data(example_sce)

# compute pseudobulk sum-counts & run DS analysis
pb <- aggregateData(example_sce)
res <- pbDS(pb, method = "limma-trend")
```
names(res)
names(res$table)
head(res$table$stim$`B cells``)

# count nb. of DE genes by cluster
vapply(res$table$stim, function(u)
  sum(u$p_adj.loc < 0.05), numeric(1))

# get top 5 hits for ea. cluster w/ abs(logFC) > 1
library(dplyr)
lapply(res$table$stim, function(u)
  filter(u, abs(logFC) > 1) %>%
    arrange(p_adj.loc) %>%
    slice(seq_len(5)))

---

**pbFlatten**

**pbFlatten** Flatten pseudobulk SCE

**Description**

Flattens a pseudobulk SingleCellExperiment as returned by aggregateData such that all cell subpopulations are represented as a single assay.

**Usage**

```
pbFlatten(pb, normalize = TRUE)
```

**Arguments**

- **pb**
  a pseudobulk SingleCellExperiment as returned by aggregateData, with different subpopulations as assays.
- **normalize**
  logical specifying whether to compute a logcpm assay.

**Value**

a SingleCellExperiment.

**Examples**

```
data(example_sce)
library(SingleCellExperiment)
pb_stack <- aggregateData(example_sce)
(pb_flat <- pbFlatten(pb_stack))
ncol(pb_flat) == ncol(pb_stack)*length(assays(pb_stack))
```
pbHeatmap

Heatmap of cluster-sample pseudobulks

Description
...

Usage

pbHeatmap(
  x,
  y,
  k = NULL,
  g = NULL,
  c = NULL,
  top_n = 20,
  fdr = 0.05,
  lfc = 1,
  sort_by = "p_adj.loc",
  decreasing = FALSE,
  assay = "logcounts",
  fun = mean,
  normalize = TRUE,
  row_anno = TRUE,
  col_anno = TRUE
)

Arguments

x          a SingleCellExperiment.
y          a list of DS analysis results as returned by pbDS or mmDS.
k          character vector; specifies which cluster ID(s) to retain. Defaults to levels(x$cluster_id).
g          character vector; specifies which genes to retain. Defaults to considering all genes.
c          character string; specifies which contrast/coefficient to retain. Defaults to names(y$table)[1].
top_n      single numeric; number of genes to retain per cluster.
fdr, lfc   single numeric; FDR and logFC cutoffs to filter results by. The specified FDR threshold is applied to p_adj.loc values.
sort_by    character string specifying a numeric results table column to sort by; "none" to retain original ordering.
decreasing logical; whether to sort in decreasing order of sort_by.
assay      character string; specifies which assay to use; should be one of assayNames(x).
fun

function to use as summary statistic, e.g., mean, median, sum (depending on the input assay).

normalize

logical; whether to apply a z-normalization to each row (gene) of the cluster-sample pseudobulk data.

col

character vector of colors or color mapping function generated with `colorRamp2`. Passed to argument `col` in `Heatmap` (see `?ComplexHeatmap::Heatmap` for details).

row_anno, col_anno

logical; whether to render annotations of cluster and group IDs, respectively.

Value

a `HeatmapList-class` object.

Author(s)

Helena L. Crowell

Examples

```r
# compute pseudobulks & run DS analysis
data(example_sce)
pb <- aggregateData(example_sce)
res <- pbDS(pb)

# cluster-sample expression means
pbHeatmap(example_sce, res)

# include only a single cluster
pbHeatmap(example_sce, res, k = "B cells")

# plot specific gene across all clusters
pbHeatmap(example_sce, res, g = "ISG20")
```

---

**pbMDS**

Pseudobulk-level MDS plot

**Description**

Renders a multidimensional scaling (MDS) where each point represents a cluster-sample instance; with points colored by cluster ID and shaped by group ID.

**Usage**

`pbMDS(x)`
Arguments

\( x \)  
\(\text{a SingleCellExperiment}\) containing cluster-sample pseudobulks as returned by \texttt{aggregateData} with argument \( \texttt{by} = \text{c("cluster_id", "sample_id")} \).

Value

\(\text{a ggplot object.}\)

Author(s)

Helena L. Crowell & Mark D Robinson

Examples

\begin{verbatim}
data(example_sce)
pb <- aggregateData(example_sce)
pbMDS(pb)
\end{verbatim}

Description

...

Usage

\begin{verbatim}
prepSCE(
  x,
  kid = "cluster_id",
  sid = "sample_id",
  gid = "group_id",
  drop = FALSE
)
\end{verbatim}

Arguments

\( x \)  
\(\text{a SingleCellExperiment.}\)

\(\text{kid, sid, gid}\)  
character strings specifying the \texttt{colData(x)} columns containing cluster assignments, unique sample identifiers, and group IDs (e.g., treatment).

\(\text{drop}\)  
logical. Specifies whether \texttt{colData(x)} columns besides those specified as \texttt{cluster_id, sample_id, group_id} should be retained (default \(\text{drop} = \text{FALSE}\)) or removed (\(\text{drop} = \text{TRUE}\)).

Value

\(\text{a SingleCellExperiment.}\)
Author(s)
Helena L. Crowell

Examples

# generate random counts
ng <- 50
nc <- 200

generate some cell metadata
gids <- sample(c("groupA", "groupB"), nc, TRUE)
sids <- sample(paste0("sample", seq_len(3)), nc, TRUE)
kids <- sample(paste0("cluster", seq_len(5)), nc, TRUE)
batch <- sample(seq_len(3), nc, TRUE)
cd <- data.frame(group = gids, id = sids, cluster = kids, batch)

# construct SCE
library(scuttle)
sce <- mockSCE(ncells = nc, ngenes = ng)
colData(sce) <- cbind(colData(sce), cd)

# prep. for workflow
sce <- prepSCE(sce, kid = "cluster", sid = "id", gid = "group")
head(colData(sce))
metadata(sce)$experiment_info
sce

prepSim
SCE preparation for simData

Description

prepSim prepares an input SCE for simulation with muscat’s simData function by

1. basic filtering of genes and cells
2. (optional) filtering of subpopulation-sample instances
3. estimation of cell (library sizes) and gene parameters (dispersions and sample-specific means), respectively.

Usage

prepSim(
x, 
  min_count = 1, 
  min_cells = 10, 
  min_genes = 100, 
  min_size = 100,
prepSim

```r
  group_keep = NULL,
  verbose = TRUE
)
```

**Arguments**

- `x` a `SingleCellExperiment`.
- `min_count`, `min_cells` used for filtering of genes; only genes with a count $>\text{min\_count}$ in $\geq \text{min\_cells}$ will be retained.
- `min_genes` used for filtering cells; only cells with a count $>0$ in $\geq \text{min\_genes}$ will be retained.
- `min_size` used for filtering subpopulation-sample combinations; only instances with $\geq \text{min\_size}$ cells will be retained. Specifying `min\_size = NULL` skips this step.
- `group_keep` character string; if `nlevels(x$\text{group\_id}) > 1`, specifies which group of samples to keep (see details). The default `NULL` retains samples from `levels(x$\text{group\_id})[1]`; otherwise, if `colData(x)$\text{group\_id}` is not specified, all samples will be kept.
- `verbose` logical; should information on progress be reported?

**Details**

For each gene $g$, `prepSim` fits a model to estimate sample-specific means $\beta_g^s$, for each sample $s$, and dispersion parameters $\phi_g$ using edgeR’s `estimateDisp` function with default parameters. Thus, the reference count data is modeled as NB distributed:

$$Y_{gc} \sim \text{NB}(\mu_{gc}, \phi_g)$$

for gene $g$ and cell $c$, where the mean $\mu_{gc} = \exp(\sum \beta_g^s(c) \cdot \lambda_c)$. Here, $\beta_g^s(c)$ is the relative abundance of gene $g$ in sample $s(c)$, $\lambda_c$ is the library size (total number of counts), and $\phi_g$ is the dispersion.

**Value**

a `SingleCellExperiment` containing, for each cell, library size (`colData(x)$offset`) and, for each gene, dispersion and sample-specific mean estimates (`rowData(x)$\text{dispersion}` and `$\text{beta\_sample\_id}$`, respectively).

**Author(s)**

Helena L. Crowell

**References**

Examples

# estimate simulation parameters
data(example_sce)
ref <- prepSim(example_sce)

# tabulate number of genes/cells before vs. after
ns <- cbind(
  before = dim(example_sce),
  after = dim(ref))
rownames(ns) <- c("#genes", "#cells")
ns

library(SingleCellExperiment)
head(rowData(ref)) # gene parameters
head(colData(ref)) # cell parameters

resDS provides a simple wrapper to format cluster-level differential testing results into an easily filterable table, and to optionally append gene expression frequencies by cluster-sample & -group, as well as cluster-sample-wise CPM.

Usage

resDS(
  x,
  y,
  bind = c("row", "col"),
  frq = FALSE,
  cpm = FALSE,
  digits = 3,
  sep = "__",
  ...
)

Arguments

x a SingleCellExperiment.
y a list of DS testing results as returned by pbDS or mmDS.
bind character string specifying the output format (see details).
frq logical or a pre-computed list of expression frequencies as returned by calcExprFreqs.
cpm logical specifying whether CPM by cluster-sample should be appended to the output result table(s).
digits  integer value specifying the number of significant digits to maintain.
sep    character string to use as separator when constructing new column names.
...    optional arguments passed to calcExprFreqs if frq = TRUE.

Details

When bind = "col", the list of DS testing results at y$table will be merge vertically (by column) into a single table in tidy format with column contrast/coef specifying the comparison.

Otherwise, when bind = "row", an identifier of the respective contrast or coefficient will be appended to the column names, and all tables will be merge horizontally (by row).

Expression frequencies pre-computed with calcExprFreqs may be provided with frq. Alternatively, when frq = TRUE, expression frequencies can be computed directly, and additional arguments may be passed to calcExprFreqs (see examples below).

Value

returns a 'data.frame'.

Author(s)

Helena L Crowell & Mark D Robinson

Examples

# compute pseudobulks (sum of counts)
data(example_sce)
pb <- aggregateData(example_sce, 
  assay = "counts", fun = "sum")

# run DS analysis (edgeR on pseudobulks)
res <- pbDS(pb, method = "edgeR")

head(resDS(example_sce, res, bind = "row")) # tidy format
head(resDS(example_sce, res, bind = "col", digits = Inf))

# append CPMs & expression frequencies
head(resDS(example_sce, res, cpm = TRUE))
head(resDS(example_sce, res, frq = TRUE))

# pre-computed expression frequencies & append
frq <- calcExprFreqs(example_sce, assay = "counts", th = 0)
head(resDS(example_sce, res, frq = frq))
Description

Simulation of complex scRNA-seq data

Usage

```r
simData(
    x,
    ng = nrow(x),
    nc = ncol(x),
    ns = NULL,
    nk = NULL,
    probs = NULL,
    dd = TRUE,
    p_dd = diag(6)[1,],
    paired = FALSE,
    p_ep = 0.5,
    p_dp = 0.3,
    p_dm = 0.5,
    p_type = 0,
    lfc = 2,
    rel_lfc = NULL,
    phylo_tree = NULL,
    phylo_pars = c(ifelse(is.null(phylo_tree), 0, 0.1), 3),
    force = FALSE
)
```

Arguments

- `x`: a `SingleCellExperiment`.
  - number of genes to simulate. Importantly, for the library sizes computed by `prepSim (= exp(x$offset))` to make sense, the number of simulated genes should match with the number of genes in the reference. To simulate a reduced number of genes, e.g. for testing and development purposes, please set `force = TRUE`.

- `ng`: number of cells to simulate.

- `nc`: number of samples to simulate; defaults to as many as available in the reference to avoid duplicated reference samples. Specifically, the number of samples will be set to `n = nlevels(x$sample_id)` when `dd = FALSE`, `n` per group when `dd`, `paired = TRUE`, and `floor(n/2)` per group when `dd = TRUE`, `paired = FALSE`. When a larger number samples should be simulated, set `force = TRUE`.

- `nk`: number of clusters to simulate; defaults to the number of available reference clusters (`nlevels(x$cluster_id)`).
**probs**  
a list of length 3 containing probabilities of a cell belonging to each cluster, sample, and group, respectively. List elements must be NULL (equal probabilities) or numeric values in [0, 1] that sum to 1.

**dd**  
whether or not to simulate differential distributions; if TRUE, two groups are simulated and ns corresponds to the number of samples per group, else one group with ns samples is simulated.

**p_dd**  
numeric vector of length 6. Specifies the probability of a gene being EE, EP, DE, DP, DM, or DB, respectively.

**paired**  
logical specifying whether a paired design should be simulated (both groups use the same set of reference samples) or not (reference samples are drawn at random).

**p_ep, p_dp, p_dm**  
numeric specifying the proportion of cells to be shifted to a different expression state in one group (see details).

**p_type**  
numeric. Probability of EE/EP gene being a type-gene. If a gene is of class "type" in a given cluster, a unique mean will be used for that gene in the respective cluster.

**lfc**  
numeric value to use as mean logFC (logarithm base 2) for DE, DP, DM, and DB type of genes.

**rel_lfc**  
numeric vector of relative logFCs for each cluster. Should be of length nlevels(x$cluster_id) with levels(x$cluster_id) as names. Defaults to factor of 1 for all clusters.

**phylo_tree**  
newick tree text representing cluster relations and their relative distance. An explanation of the syntax can be found here. The distance between the nodes, except for the original branch, will be translated in the number of shared genes between the clusters belonging to these nodes (this relation is controlled with phylo_pars). The distance between two clusters is defined as the sum of the branches lengths separating them.

**phylo_pars**  
vector of length 2 providing the parameters that control the number of type genes. Passed to an exponential PDF (see details).

**force**  
logical specifying whether to force simulation when ng and/or ns don’t match the number of available reference genes and samples, respectively.

---

**Details**

`simData` simulates multiple clusters and samples across 2 experimental conditions from a real scRNA-seq data set.

The simulation of type genes can be performed in 2 ways; (1) via `p_type` to simulate independent clusters, OR (2) via `phylo_tree` to simulate a hierarchical cluster structure.

For (1), a subset of `p_type` % of genes are selected per cluster to use a different references genes than the remainder of clusters, giving rise to cluster-specific NB means for count sampling.

For (2), the number of shared/type genes at each node are given by \(a \times G \times e^{(-b \times d)}\), where

- `a` – controls the percentage of shared genes between nodes. By default, at most 10% of the genes are reserved as type genes (when \(b = 0\)). However, it is advised to tune this parameter depending on the input `prep_sce`.

- `b` – determines how the number of shared genes decreases with increasing distance \(d\) between clusters (defined through `phylo_tree`).
Value

a SingleCellExperiment containing multiple clusters & samples across 2 groups as well as the following metadata:

cell metadata (colData(.)) a DataFrame containing, containing, for each cell, it's cluster, sample, and group ID.

gene metadata (rowData(.)) a DataFrame containing, for each gene, it's class (one of "state", "type", "none") and specificity (specs: NA for genes of type "state", otherwise a character vector of clusters that share the given gene).

experiment metadata (metadata(.)) experiment_info a data.frame summarizing the experimental design.

n_cells the number of cells for each sample.

gene_info a data.frame containing, for each gene in each cluster, it's differential distribution category, mean logFC (NA for genes for categories "ee" and "ep"), gene used as reference (sim_gene), dispersion sim_disp, and simulation means for each group sim_mean.A/B.

ref_sids/kids kids the sample/cluster IDs used as reference.

args a list of the function call’s input arguments.

Author(s)

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References


Examples

data(example_sce)
library(SingleCellExperiment)

# prep. SCE for simulation
ref <- prepSim(example_sce)

# simulate data
(sim <- simData(ref, nc = 200,
  p_dd = c(0.9, 0, 0.1, 0, 0, 0),
  ng = 100, force = TRUE,
  probs = list(NULL, NULL, c(1, 0))))

# simulation metadata
head(gi <- metadata(sim)$gene_info)

# should be ~10% DE
table(gi$category)
# unbalanced sample sizes
sim <- simData(ref, nc = 100, ns = 2,
               probs = list(NULL, c(0.25, 0.75), NULL),
               ng = 10, force = TRUE)
table(sim$sample_id)

# one group only
sim <- simData(ref, nc = 100,
               probs = list(NULL, NULL, c(1, 0)),
               ng = 10, force = TRUE)
levels(sim$group_id)

# HIERARCHICAL CLUSTER STRUCTURE
# define phylogram specifying cluster relations
phylo_tree <- "(("cluster1":0.1,"cluster2":0.1):0.4,"cluster3":0.5);"
# verify syntax & visualize relations
library(phylogram)
plot(read.dendrogram(text = phylo_tree))

# let's use a more complex phylogeny
phylo_tree <- "(("cluster1":0.4,"cluster2":0.4):0.4,"cluster3":
               0.5,("cluster4":0.2,"cluster5":0.2,"cluster6":0.2):0.4):0.4);"
plot(read.dendrogram(text = phylo_tree))

# simulate clusters accordingly
sim <- simData(ref,
                phylo_tree = phylo_tree,
                phylo_pars = c(0.1, 3),
                ng = 500, force = TRUE)
# view information about shared 'type' genes
table(rowData(sim)$class)
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