Package: SimBu (via r-universe)

November 25, 2026

Title Simulate Bulk RNA-seq Datasets from Single-Cell Datasets

Version 1.16.0

Description SimBu can be used to simulate bulk RNA-seq datasets with known cell type fractions. You can either use your own single-cell study for the simulation or the sfaira database. Different pre-defined simulation scenarios exist, as are options to run custom simulations. Additionally, expression values can be adapted by adding an mRNA bias, which produces more biologically relevant simulations.

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Encoding UTF-8

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BugReports https://github.com/omnideconv/SimBu/issues

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`census` *Applies the Census count transformation on a count matrix*

Description

needs a sparse matrix with cells in columns and genes in rows. You can find the detailed explaination here: http://cole-trapnell-lab.github.io/monocle-release/docs/#census

Usage

```
census(
  matrix,
  exp_capture_rate = 0.25,
  expr_threshold = 0,
  BPPARAM = BiocParallel::bpparam(),
  run_parallel = FALSE
)
```

Arguments

`matrix`	sparse count matrix; cells in columns, genes in rows
`exp_capture_rate`	expected capture rate; default=0.25
`expr_threshold`	expression threshold; default=0
`BPPARAM`	BiocParallel::bpparam() by default; if specific number of threads x want to be used, insert: BiocParallel::MulticoreParam(workers = x)
`run_parallel`	boolean, decide if multi-threaded calculation will be run. FALSE by default

Value

a vector for each cell-type, with a scaling factor which can be used to transform the counts of the matrix

Examples

```
tpm <- Matrix::Matrix(matrix(rpois(3e5, 5), ncol = 300), sparse = TRUE)
tpm <- Matrix::t(1e6 * Matrix::t(tpm) / Matrix::colSums(tpm))
cen <- SimBu::census(tpm)
```

`dataset` *Build SummarizedExperiment using local annotation and count matrix R objects*

Description

Build SummarizedExperiment using local annotation and count matrix R objects

Usage

```
dataset(
  annotation,
  count_matrix = NULL,
  tpm_matrix = NULL,
  name = "SimBu_dataset",
  spike_in_col = NULL,
  additional_cols = NULL,
  filter_genes = TRUE,
  variance_cutoff = 0,
  type_abundance_cutoff = 0,
  scale_tpm = TRUE
)
```

Arguments

`annotation`	(mandatory) dataframe; needs columns 'ID' and 'cell_type'; 'ID' needs to be equal with cell_names in count_matrix
`count_matrix`	(mandatory) sparse count matrix; raw count data is expected with genes in rows, cells in columns
`tpm_matrix`	sparse count matrix; TPM like count data is expected with genes in rows, cells in columns
`name`	name of the dataset; will be used for new unique IDs of cells
`spike_in_col`	which column in annotation contains information on spike_in counts, which can be used to re-scale counts; mandatory for spike_in scaling factor in simulation

additional_cols
: list of column names in annotation, that should be stored as well in dataset object

filter_genes
: boolean, if TRUE, removes all genes with 0 expression over all samples & genes with variance below `variance_cutoff`

variance_cutoff
: numeric, is only applied if `filter_genes` is TRUE: removes all genes with variance below the chosen cutoff (default = 0)

type_abundance_cutoff
: numeric, remove all cells, whose cell-type appears less then the given value. This removes low abundant cell-types

scale_tpm
: boolean, if TRUE (default) the cells in tpm_matrix will be scaled to sum up to 1e6

Value

Return a SummarizedExperiment object

Examples

```
counts <- Matrix::Matrix(matrix(stats::rpois(3e5, 5), ncol = 300), sparse = TRUE)
tpm <- Matrix::Matrix(matrix(stats::rpois(3e5, 5), ncol = 300), sparse = TRUE)
tpm <- Matrix::t(1e6 * Matrix::t(tpm) / Matrix::colSums(tpm))

colnames(counts) <- paste0("cell_", rep(1:300))
colnames(tpm) <- paste0("cell_", rep(1:300))
rownames(counts) <- paste0("gene_", rep(1:1000))
rownames(tpm) <- paste0("gene_", rep(1:1000))

annotation <- data.frame(
  "ID" = paste0("cell_", rep(1:300)),
  "cell_type" = c(rep("T cells CD4", 300))
)

ds <- SimBu::dataset(annotation = annotation, count_matrix = counts, tpm_matrix = tpm, name = "test_dataset")
```

---

dataset_h5ad *Build SummarizedExperiment using a h5ad file for the counts*

Description

Build SummarizedExperiment using a h5ad file for the counts

Usage

```
dataset_h5ad(
  h5ad_file_counts,
  h5ad_file_tpm = NULL,
  cell_id_col = "ID",
  cell_type_col = "cell_type",
  cells_in_obs = TRUE,
  name = "SimBu_dataset",
  spike_in_col = NULL,
  additional_cols = NULL,
  filter_genes = TRUE,
  variance_cutoff = 0,
  type_abundance_cutoff = 0,
  scale_tpm = TRUE
)
```

Arguments

h5ad_file_counts	(mandatory) h5ad file with raw count data
h5ad_file_tpm	h5ad file with TPM count data
cell_id_col	(mandatory) name of column in Seurat meta.data with unique cell ids; 0 for rownames
cell_type_col	(mandatory) name of column in Seurat meta.data with cell type name
cells_in_obs	boolean, if TRUE, cell identifiers are taken from obs layer in anndata object; if FALSE, they are taken from var
name	name of the dataset; will be used for new unique IDs of cells#' @param spike_in_col which column in annotation contains information on spike_in counts, which can be used to re-scale counts; mandatory for spike_in scaling factor in simulation
spike_in_col	which column in annotation contains information on spike_in counts, which can be used to re-scale counts; mandatory for spike_in scaling factor in simulation
additional_cols	list of column names in annotation, that should be stored as well in dataset object
filter_genes	boolean, if TRUE, removes all genes with 0 expression over all samples & genes with variance below variance_cutoff
variance_cutoff	numeric, is only applied if filter_genes is TRUE: removes all genes with variance below the chosen cutoff
type_abundance_cutoff	numeric, remove all cells, whose cell-type appears less then the given value. This removes low abundant cell-types
scale_tpm	boolean, if TRUE (default) the cells in tpm_matrix will be scaled to sum up to 1e6

Value

Return a SummarizedExperiment object

Examples

```
# h5 <- system.file("extdata", "anndata.h5ad", package = "SimBu")
# ds_h5ad <- SimBu::dataset_h5ad(
#  h5ad_file_counts = h5,
#  name = "h5ad_dataset",
#  cell_id_col = "id", # this will use the 'id' column of the metadata as cell identifiers
#  cell_type_col = "group", # this will use the 'group' column of the metadata as cell type info
#  cells_in_obs = TRUE
# ) # in case your cell information is stored in the var layer, switch to FALSE
```

`dataset_merge` *Merge multiple SummarizedExperiment datasets into one*

Description

The objects need to have the same number of assays in order to work.

Usage

```
dataset_merge(
  dataset_list,
  name = "SimBu_dataset",
  spike_in_col = NULL,
  additional_cols = NULL,
  filter_genes = TRUE,
  variance_cutoff = 0,
  type_abundance_cutoff = 0,
  scale_tpm = TRUE
)
```

Arguments

`dataset_list`	(mandatory) list of SummarizedExperiment objects
`name`	name of the new dataset
`spike_in_col`	which column in annotation contains information on spike_in counts, which can be used to re-scale counts; mandatory for spike_in scaling factor in simulation
`additional_cols`	list of column names in annotation, that should be stored as well in dataset object
`filter_genes`	boolean, if TRUE, removes all genes with 0 expression over all samples & genes with variance below `variance_cutoff`
`variance_cutoff`	numeric, is only applied if `filter_genes` is TRUE: removes all genes with variance below the chosen cutoff
`type_abundance_cutoff`	numeric, remove all cells, whose cell-type appears less then the given value. This removes low abundant cell-types
`scale_tpm`	boolean, if TRUE (default) the cells in tpm_matrix will be scaled to sum up to 1e6

Value

SummarizedExperiment object

Examples

```
counts <- Matrix::Matrix(matrix(stats::rpois(3e5, 5), ncol = 300), sparse = TRUE)
tpm <- Matrix::Matrix(matrix(stats::rpois(3e5, 5), ncol = 300), sparse = TRUE)
tpm <- Matrix::t(1e6 * Matrix::t(tpm) / Matrix::colSums(tpm))

colnames(counts) <- paste0("cell_", rep(1:300))
colnames(tpm) <- paste0("cell_", rep(1:300))
rownames(counts) <- paste0("gene_", rep(1:1000))
rownames(tpm) <- paste0("gene_", rep(1:1000))

annotation <- data.frame(
  "ID" = paste0("cell_", rep(1:300)),
  "cell_type" = c(rep("T cells CD4", 300))
)

ds1 <- SimBu::dataset(annotation = annotation, count_matrix = counts, tpm_matrix = tpm, name = "test_dataset1")
ds2 <- SimBu::dataset(annotation = annotation, count_matrix = counts, tpm_matrix = tpm, name = "test_dataset2")
ds_merged <- SimBu::dataset_merge(list(ds1, ds2))
```

---

dataset_seurat *Build SummarizedExperiment using a Seurat object*

Description

Build SummarizedExperiment using a Seurat object

Usage

```
dataset_seurat(
  seurat_obj,
  counts_layer,
  cell_id_col,
  cell_type_col,
  assay = NULL,
  tpm_layer = NULL,
  name = "SimBu_dataset",
  spike_in_col = NULL,
  additional_cols = NULL,
  filter_genes = TRUE,
  variance_cutoff = 0,
  type_abundance_cutoff = 0,
  scale_tpm = TRUE
)
```

Arguments

seurat_obj	(mandatory) Seurat object with TPM counts
counts_layer	(mandatory) name of assay in Seurat object which contains count data in 'counts' slot
cell_id_col	(mandatory) name of column in Seurat meta.data with unique cell ids
cell_type_col	(mandatory) name of column in Seurat meta.data with cell type name
assay	name of the Seurat objecy assay that should be used. If NULL (default), the currently active assay is used
tpm_layer	name of assay in Seurat object which contains TPM data in 'counts' slot
name	name of the dataset; will be used for new unique IDs of cells
spike_in_col	which column in annotation contains information on spike_in counts, which can be used to re-scale counts; mandatory for spike_in scaling factor in simulation
additional_cols	list of column names in annotation, that should be stored as well in dataset object
filter_genes	boolean, if TRUE, removes all genes with 0 expression over all samples & genes with variance below variance_cutoff
variance_cutoff	numeric, is only applied if filter_genes is TRUE: removes all genes with variance below the chosen cutoff
type_abundance_cutoff	numeric, remove all cells, whose cell-type appears less then the given value. This removes low abundant cell-types
scale_tpm	boolean, if TRUE (default) the cells in tpm_matrix will be scaled to sum up to 1e6

Value

Return a SummarizedExperiment object

Examples

```
counts <- Matrix::Matrix(matrix(stats::rpois(3e5, 5), ncol = 300), sparse = TRUE)
tpm <- Matrix::Matrix(matrix(stats::rpois(3e5, 5), ncol = 300), sparse = TRUE)
tpm <- Matrix::t(1e6 * Matrix::t(tpm) / Matrix::colSums(tpm))

colnames(counts) <- paste0("cell-", rep(1:300))
colnames(tpm) <- paste0("cell-", rep(1:300))
rownames(counts) <- paste0("gene-", rep(1:1000))
rownames(tpm) <- paste0("gene-", rep(1:1000))

annotation <- data.frame(
  "ID" = paste0("cell-", rep(1:300)),
  "cell_type" = c(
    rep("T cells CD4", 50),
    rep("T cells CD8", 50),
    rep("Macrophages", 100),
```

```
    rep("NK cells", 10),
    rep("B cells", 70),
    rep("Monocytes", 20)
  ),
  row.names = paste0("cell-", rep(1:300))
)

seurat_obj <- Seurat::CreateSeuratObject(counts = counts, assay = "gene_expression", meta.data = annotation)
SeuratObject::LayerData(seurat_obj, assay = "gene_expression", layer = "data") <- tpm

ds_seurat <- SimBu::dataset_seurat(
  seurat_obj = seurat_obj,
  counts_layer = "counts",
  cell_id_col = "ID",
  cell_type_col = "cell_type",
  tpm_layer = "data",
  name = "seurat_dataset"
)
```

`dataset_sfaira` *Build SummarizedExperiment using a single sfaira entry ID*

Description

Build SummarizedExperiment using a single sfaira entry ID

Usage

```
dataset_sfaira(
  sfaira_id,
  sfaira_setup,
  name = "SimBu_dataset",
  spike_in_col = NULL,
  additional_cols = NULL,
  force = FALSE,
  filter_genes = TRUE,
  variance_cutoff = 0,
  type_abundance_cutoff = 0,
  scale_tpm = TRUE
)
```

Arguments

`sfaira_id`	(mandatory) ID of a sfaira dataset
`sfaira_setup`	(mandatory) the sfaira setup; given by `setup_sfaira`
`name`	name of the dataset; will be used for new unique IDs of cells
`spike_in_col`	which column in annotation contains information on spike_in counts, which can be used to re-scale counts

additional_cols
: list of column names in annotation, that should be stored as well in dataset object

force
: boolean, if TRUE, datasets without annotation will be downloaded, FALSE otherwise (default)

filter_genes
: boolean, if TRUE, removes all genes with 0 expression over all samples & genes with variance below `variance_cutoff`

variance_cutoff
: numeric, is only applied if `filter_genes` is TRUE: removes all genes with variance below the chosen cutoff

type_abundance_cutoff
: numeric, remove all cells, whose cell-type appears less then the given value. This removes low abundant cell-types

scale_tpm
: boolean, if TRUE (default) the cells in tpm_matrix will be scaled to sum up to 1e6

Value

dataset object

Examples

```
setup_list <- SimBu::setup_sfaira(tempdir())
ds <- SimBu::dataset_sfaira(
 sfaira_id = "homosapiens_lungparenchyma_2019_10x3v2_madissoon_001_10.1186/s13059-019-1906-x",
  sfaira_setup = setup_list,
  name = "test_dataset"
)
```

---

dataset_sfaira_multiple

Build SummarizedExperiment using multiple sfaira entries

Description

You can apply different filters on the whole data-zoo of sfaria; the resulting single-cell datasets will be combined into a single dataset which you can use for simulation Note: only datasets in sfaira with annotation are considered!

Usage

```
dataset_sfaira_multiple(
  organisms = NULL,
  tissues = NULL,
  assays = NULL,
  sfaira_setup,
  name = "SimBu_dataset",
```

```
  spike_in_col = NULL,
  additional_cols = NULL,
  filter_genes = TRUE,
  variance_cutoff = 0,
  type_abundance_cutoff = 0,
  scale_tpm = TRUE
)
```

Arguments

organisms	(mandatory) list of organisms (only human and mouse available)
tissues	(mandatory) list of tissues
assays	(mandatory) list of assays
sfaira_setup	(mandatory) the sfaira setup; given by setup_sfaira
name	name of the dataset; will be used for new unique IDs of cells
spike_in_col	which column in annotation contains information on spike_in counts, which can be used to re-scale counts
additional_cols	list of column names in annotation, that should be stored as well in dataset object
filter_genes	boolean, if TRUE, removes all genes with 0 expression over all samples & genes with variance below variance_cutoff
variance_cutoff	numeric, is only applied if filter_genes is TRUE: removes all genes with variance below the chosen cutoff
type_abundance_cutoff	numeric, remove all cells, whose cell-type appears less then the given value. This removes low abundant cell-types
scale_tpm	boolean, if TRUE (default) the cells in tpm_matrix will be scaled to sum up to 1e6

Value

dataset object

Examples

```
setup_list <- SimBu::setup_sfaira(tempdir())
ds_human_lung <- SimBu::dataset_sfaira_multiple(
  sfaira_setup = setup_list,
  organisms = "Homo sapiens",
  tissues = "lung parenchyma",
  assay = "10x 3' v2",
  name = "human_lung"
)
```

`merge_simulations` *Combine multiple simulations into one result*

Description

we recommend to only merge simulations from the same dataset object, otherwise the count matrices might not correspond on the gene level

Usage

```
merge_simulations(simulation_list)
```

Arguments

`simulation_list`
: a list of simulations

Value

named list; `bulk` a SummarizedExperiment object, where the assays store the simulated bulk RNAseq datasets. Can hold either one or two assays, depending on how many matrices were present in the dataset `cell-fractions` is a dataframe with the simulated cell-fractions per sample; `scaling_vector` scaling value for each cell in dataset

Examples

```
counts <- Matrix::Matrix(matrix(rpois(3e5, 5), ncol = 300), sparse = TRUE)
tpm <- Matrix::Matrix(matrix(rpois(3e5, 5), ncol = 300), sparse = TRUE)
tpm <- Matrix::t(1e6 * Matrix::t(tpm) / Matrix::colSums(tpm))

colnames(counts) <- paste0("cell_", rep(1:300))
colnames(tpm) <- paste0("cell_", rep(1:300))
rownames(counts) <- paste0("gene_", rep(1:1000))
rownames(tpm) <- paste0("gene_", rep(1:1000))

annotation <- data.frame(
  "ID" = paste0("cell_", rep(1:300)),
  "cell_type" = c(
    rep("T cells CD4", 50),
    rep("T cells CD8", 50),
    rep("Macrophages", 100),
    rep("NK cells", 10),
    rep("B cells", 70),
    rep("Monocytes", 20)
  )
)

dataset <- SimBu::dataset(
  annotation = annotation,
```

```
  count_matrix = counts,
  tpm_matrix = tpm,
  name = "test_dataset"
)

s1 <- SimBu::simulate_bulk(dataset,
  scenario = "even",
  scaling_factor = "NONE",
  nsamples = 10,
  ncells = 100
)

s2 <- SimBu::simulate_bulk(dataset,
  scenario = "even",
  scaling_factor = "NONE",
  nsamples = 10,
  ncells = 100
)

s <- SimBu::merge_simulations(list(s1, s2))
```

`plot_simulation` *Plot the cell-type fractions in your simulated dataset*

Description

Plot the cell-type fractions in your simulated dataset

Usage

```
plot_simulation(simulation)
```

Arguments

`simulation`	a simulation object generated by `simulate_bulk`

Value

a gpplot2 barplot

Examples

```
counts <- Matrix::Matrix(matrix(stats::rpois(3e5, 5), ncol = 300), sparse = TRUE)
tpm <- Matrix::Matrix(matrix(stats::rpois(3e5, 5), ncol = 300), sparse = TRUE)
tpm <- Matrix::t(1e6 * Matrix::t(tpm) / Matrix::colSums(tpm))

colnames(counts) <- paste0("cell_", rep(1:300))
colnames(tpm) <- paste0("cell_", rep(1:300))
rownames(counts) <- paste0("gene_", rep(1:1000))
rownames(tpm) <- paste0("gene_", rep(1:1000))
```

```
annotation <- data.frame(
  "ID" = paste0("cell_", rep(1:300)),
  "cell_type" = c(
    rep("T cells CD4", 50),
    rep("T cells CD8", 50),
    rep("Macrophages", 100),
    rep("NK cells", 10),
    rep("B cells", 70),
    rep("Monocytes", 20)
  )
)

dataset <- SimBu::dataset(
  annotation = annotation,
  count_matrix = counts,
  tpm_matrix = tpm,
  name = "test_dataset"
)

s <- SimBu::simulate_bulk(dataset,
  scenario = "even",
  scaling_factor = "NONE",
  nsamples = 10,
  ncells = 100
)

SimBu::plot_simulation(s)
```

`save_simulation` *Save the expression matrix of a simulated pseudo-bulk dataset to a file*

Description

Save the expression matrix of a simulated pseudo-bulk dataset to a file

Usage

```
save_simulation(simulation, filename, assay = "bulk_counts")
```

Arguments

`simulation`	the result of simulate_bulk()
`filename`	the filename where to save the expression matrix to
`assay`	name of the assay in simulation to save, default to bulk_counts

Value

write a file

Examples

```
counts <- Matrix::Matrix(matrix(stats::rpois(3e5, 5), ncol = 300), sparse = TRUE)
tpm <- Matrix::Matrix(matrix(stats::rpois(3e5, 5), ncol = 300), sparse = TRUE)
tpm <- Matrix::t(1e6 * Matrix::t(tpm) / Matrix::colSums(tpm))

colnames(counts) <- paste0("cell_", rep(1:300))
colnames(tpm) <- paste0("cell_", rep(1:300))
rownames(counts) <- paste0("gene_", rep(1:1000))
rownames(tpm) <- paste0("gene_", rep(1:1000))

annotation <- data.frame(
  "ID" = paste0("cell_", rep(1:300)),
  "cell_type" = c(
    rep("T cells CD4", 50),
    rep("T cells CD8", 50),
    rep("Macrophages", 100),
    rep("NK cells", 10),
    rep("B cells", 70),
    rep("Monocytes", 20)
  )
)

dataset <- SimBu::dataset(
  annotation = annotation,
  count_matrix = counts,
  tpm_matrix = tpm,
  name = "test_dataset"
)

s <- SimBu::simulate_bulk(dataset,
  scenario = "even",
  scaling_factor = "NONE",
  nsamples = 10,
  ncells = 100
)

save_simulation(s, tempfile())
```

---

setup_sfaira *setup the sfaira package*

Description

If you want to download datasets from Sfaira, you need to specify a directory where the datasets are saved into. Additionally, when this function is called for the first time, a conda environment will be established and sfaira along all of its dependencies are installed. This can take some time but will be only performed one single time, as the environment can be re-used.

Usage

```
setup_sfaira(basedir)
```

Arguments

basedir	name of the directory, where the raw files will be downloaded into

Value

list with sfaira file directories; must be used as input for other sfaira based functions

Examples

```
setup_list <- setup_sfaira(basedir = tempdir())
```

---

sfaira_overview *Gives an overview of the possible datasets you can use from the sfaira database*

Description

Gives an overview of the possible datasets you can use from the sfaira database

Usage

```
sfaira_overview(setup_list)
```

Arguments

setup_list	the sfaira setup; given by setup_sfaira

Value

a dataframe with information on each dataset

Examples

```
setup_list <- setup_sfaira(basedir = tempdir())
# all_datasets <- sfaira_overview(setup_list)
```

`SimBu` *SimBu: Bias-aware simulation of bulk RNA-seq data with variable cell type composition*

Description

As complex tissues are typically composed of various cell types, deconvolution tools have been developed to computationally infer their cellular composition from bulk RNA sequencing (RNA-seq) data. To comprehensively assess deconvolution performance, gold-standard datasets are indispensable. The simulation of 'pseudo-bulk' data, generated by aggregating single-cell RNA-seq (scRNA-seq) expression profiles in pre-defined proportions, offers a scalable and cost-effective way of generating these gold-standard datasets. SimBu was developed to simulate pseudo-bulk samples based on various simulation scenarios, designed to test specific features of deconvolution methods. A unique feature of SimBu is the modelling of cell-type-specific mRNA bias using experimentally-derived or data-driven scaling factors.

Dataset generation

You will need an annotated scRNA-seq dataset (as matrix file, h5ad file, Seurat object), which is the baseline for the simulations. Use the dataset_* functions to generate a SummarizedExperiment, that holds all important information. It is also possible to access scRNA-seq datasets through the public database Sfaira, by using the functions dataset_sfaira() and dataset_sfaira_multiple().

Simulation

Use the simulate_bulk() function to generate multiple pseudo-bulk samples, which will be returned as a SummarizedExperiment. You can adapt the cell type fractions in each sample by changing the scenario parameter.

Visulaization

Inspect the cell type composition of your simulations with the plot_simulation() function.

`simulate_bulk` *Simulate whole pseudo-bulk RNAseq dataset*

Description

This function allows you to create a full pseudo-bulk RNAseq dataset. You need to provide a SummarizedExperiment from which the cells will be sampled for the simulation. Also a `scenario` has to be selected, where you can choose how the cells will be sampled and a `scaling_factor` on how the read counts will be transformed proir to the simulation.

Usage

```
simulate_bulk(
  data,
  scenario = c("even", "random", "mirror_db", "weighted", "pure", "custom"),
  scaling_factor = c("NONE", "census", "spike_in", "custom", "read_number",
    "expressed_genes", "annotation_column", "epic", "abis", "quantiseq"),
  scaling_factor_single_cell = TRUE,
  weighted_cell_type = NULL,
  weighted_amount = NULL,
  pure_cell_type = NULL,
  custom_scenario_data = NULL,
  custom_scaling_vector = NULL,
  balance_even_mirror_scenario = 0.01,
  remove_bias_in_counts = FALSE,
  remove_bias_in_counts_method = "read-number",
  norm_counts = FALSE,
  nsamples = 100,
  ncells = 1000,
  total_read_counts = NULL,
  whitelist = NULL,
  blacklist = NULL,
  seed = NA,
  BPPARAM = BiocParallel::bpparam(),
  run_parallel = FALSE
)
```

Arguments

`data`	(mandatory) SummarizedExperiment object
`scenario`	(mandatory) select on of the pre-defined cell-type fraction scenarios; possible are: `even`,`random`,`mirror_db`,`pure`,`weighted`; you can also use the `custom` scenario, where you need to set the `custom_scenario_data` parameter.
`scaling_factor`	(mandatory) name of scaling factor; possible are: `census`, `spike_in`, `read_number`, `expressed_genes`, `custom`, `epic`, `abis`, `quantiseq` or `NONE` for no scaling factor
`scaling_factor_single_cell`	boolean: decide if a scaling value for each single cell is calculated (default) or the median of all scaling values for each cell type is calculated
`weighted_cell_type`	name of cell-type used for `weighted` scenario
`weighted_amount`	fraction of cell-type used for `weighted` scenario; must be between `0` and `0.99`
`pure_cell_type`	name of cell-type for `pure` scenario
`custom_scenario_data`	dataframe; needs to be of size `nsamples` x number_of_cell_types, where each sample is a row and each entry is the cell-type fraction. Rows need to sum up to 1.

custom_scaling_vector
: named vector with custom scaling values for cell-types. Cell-types that do not occur in this vector but are present in the dataset will be set to 1; mandatory for custom scaling factor

balance_even_mirror_scenario
: balancing value for the uniform and mirror_db scenarios: increasing it will result in more diverse simulated fractions. To get the same fractions in each sample, set to 0. Default is 0.01.

remove_bias_in_counts
: boolean; if TRUE the internal mRNA bias that is present in count data will be *removed* using the number of reads mapped to each cell. Default to FALSE

remove_bias_in_counts_method
: 'read-number' (default) or 'gene-number'; method with which the mRNA bias in counts will be removed

norm_counts
: boolean; if TRUE the samples simulated with counts will be normalized to CPMs, default is FALSE

nsamples
: numeric; number of samples in pseudo-bulk RNAseq dataset (default = 100)

ncells
: numeric; number of cells in each dataset (default = 1000)

total_read_counts
: numeric; sets the total read count value for each sample

whitelist
: list; give a list of cell-types you want to keep for the simulation; if NULL, all are used

blacklist
: list; give a list of cell-types you want to remove for the simulation; if NULL, all are used; is applied after whitelist

seed
: numeric; specifiy a seed for RNG. This effects cell sampling; with a fixed seed you will always sample the same cells for each sample (seed value is incrased by 1 for each sample). Default = NA (two simulation runs will sample different cells).

BPPARAM
: BiocParallel::bpparam() by default; if specific number of threads x want to be used, insert: BiocParallel::MulticoreParam(workers = x)

run_parallel
: boolean, decide if multi-threaded calculation will be run. FALSE by default

Value

named list; bulk a SummarizedExperiment object, where the assays store the simulated bulk RNAseq datasets. Can hold either one or two assays, depending on how many matrices were present in the dataset cell-fractions is a dataframe with the simulated cell-fractions per sample; scaling_vector scaling value for each cell in dataset

Examples

```
# generate sample single-cell data to work with:

counts <- Matrix::Matrix(matrix(stats::rpois(3e5, 5), ncol = 300), sparse = TRUE)
tpm <- Matrix::Matrix(matrix(stats::rpois(3e5, 5), ncol = 300), sparse = TRUE)
tpm <- Matrix::t(1e6 * Matrix::t(tpm) / Matrix::colSums(tpm))
```

```
colnames(counts) <- paste0("cell_", rep(1:300))
colnames(tpm) <- paste0("cell_", rep(1:300))
rownames(counts) <- paste0("gene_", rep(1:1000))
rownames(tpm) <- paste0("gene_", rep(1:1000))

annotation <- data.frame(
  "ID" = paste0("cell_", rep(1:300)),
  "cell_type" = c(
    rep("T cells CD4", 50),
    rep("T cells CD8", 50),
    rep("Macrophages", 100),
    rep("NK cells", 10),
    rep("B cells", 70),
    rep("Monocytes", 20)
  )
)

dataset <- SimBu::dataset(
  annotation = annotation,
  count_matrix = counts,
  tpm_matrix = tpm,
  name = "test_dataset"
)

# this creates a basic pseudo-bulk dataset with uniform cell-type distribution
# and no additional transformation of the data with 10 samples and 2000 cells each

s <- SimBu::simulate_bulk(dataset,
  scenario = "even",
  scaling_factor = "NONE",
  nsamples = 10,
  ncells = 100
)

# use a blacklist to exclude certain cell-types for the simulation
s <- SimBu::simulate_bulk(dataset,
  scenario = "even",
  scaling_factor = "NONE",
  nsamples = 10,
  ncells = 2000,
  blacklist = c("Monocytes", "Macrophages")
)


# use the pure scenario to only have B cells
s <- SimBu::simulate_bulk(dataset,
  scenario = "pure",
  scaling_factor = "NONE",
  nsamples = 10,
  ncells = 100,
  pure_cell_type = "B cells"
)
```

```
# simulate a dataset with custom cell-type fraction for each of the 3 samples
fractions <- data.frame(
  "B cells" = c(0.2, 0.4, 0.2),
  "T cells CD4" = c(0.4, 0.2, 0.1),
  "Macrophages" = c(0.4, 0.4, 0.7), check.names = FALSE
)
s <- SimBu::simulate_bulk(dataset,
  scenario = "custom",
  scaling_factor = "NONE",
  nsamples = 3,
  ncells = 2000,
  custom_scenario_data = fractions
)
```

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