Package ‘SimBu’

May 4, 2024

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

Version  1.6.0

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.

License  GPL-3 + file LICENSE

Encoding  UTF-8

Roxygen  list(markdown = TRUE)

RoxygenNote  7.2.3

Imports  basilisk, BiocParallel, data.table, dplyr, ggplot2, tools, Matrix (>= 1.3.3), methods, phyloseq, proxyC, RColorBrewer, RCurl, reticulate, sparseMatrixStats, SummarizedExperiment, tidyr

Suggests  curl, knitr, matrixStats, rmarkdown, Seurat (>= 5.0.0), SeuratObject (>= 5.0.0), testthat (>= 3.0.0)

URL  https://github.com/omnideconv/SimBu

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

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StagedInstall  no

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calc_scaling_vector Calculate scaling factor for a dataset

Description

Each scaling factor has a default matrix it will try to use (counts or TPM). If the required matrix is not available, the other one is used and a warning is given.

Usage

calc_scaling_vector(
    data,
    scaling_factor,
    custom_scaling_vector,
    scaling_factor_single_cell,
    BPPARAM,
census

run_parallel
)

Arguments

data dataset object
scaling_factor name of scaling factor; possible are: census, spike_in, read_number, custom or NONE for no scaling factor
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
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
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 named vector with a scaling value for each cell in the dataset

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 explanation 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**

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

---

census_monocle  

=Census calculation as implemented in monocle=

**Description**


**Usage**

census_monocle(expr_matrix, exp_capture_rate, expr_threshold)

**Arguments**

expr_matrix  

TPM matrix

exp_capture_rate

expected capture rate; default=0.25

expr_threshold

expression threshold; default=0

**Value**

vector with estimated mRNA values per cell in expr_matrix
check_annotation

check for correct column names in annotation file and replace them if necessary

Description

check for correct column names in annotation file and replace them if necessary

Usage

check_annotation(annotatioan, cell_column = "cell_type", id_column = 1)

Arguments

annotation dataframe; annotation dataframe

cell_column name of cell-type column; default is "cell_type"

id_column name of cell ID column; default is 1, which uses the rownames

Value

annotation dataframe with correct column names

check_if_tpm

Checks, if a matrix is TPM-like (columns sum up to 1e6)

Description

Checks, if a matrix is TPM-like (columns sum up to 1e6)

Usage

check_if_tpm(tpm_matrix, lower_limit = 7e05)

Arguments

tpm_matrix matrix to check

lower_limit the lowest sum value, a cell may have

Value

boolean
compare_matrix_with_annotation

*Check if annotation and matrix have same cells*

Description

Otherwise intersection of both is used

Usage

```r
compare_matrix_with_annotation(m, annotation)
```

Arguments

- `m` matrix, column names are cells
- `annotation` data.frame, rownames are genes, cell names are in ID column

Value

intersected matrix

dataset

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

Description

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

Usage

```r
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

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>annotation</td>
<td>(mandatory) dataframe; needs columns 'ID' and 'cell_type'; 'ID' needs to be equal with cell_names in count_matrix</td>
</tr>
<tr>
<td>count_matrix</td>
<td>(mandatory) sparse count matrix; raw count data is expected with genes in rows, cells in columns</td>
</tr>
<tr>
<td>tpm_matrix</td>
<td>sparse count matrix; TPM like count data is expected with genes in rows, cells in columns</td>
</tr>
<tr>
<td>name</td>
<td>name of the dataset; will be used for new unique IDs of cells</td>
</tr>
<tr>
<td>spike_in_col</td>
<td>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</td>
</tr>
<tr>
<td>additional_cols</td>
<td>list of column names in annotation, that should be stored as well in dataset object</td>
</tr>
<tr>
<td>filter_genes</td>
<td>boolean, if TRUE, removes all genes with 0 expression over all samples &amp; genes with variance below variance_cutoff</td>
</tr>
<tr>
<td>variance_cutoff</td>
<td>numeric, is only applied if filter_genes is TRUE: removes all genes with variance below the chosen cutoff (default = 0)</td>
</tr>
<tr>
<td>type_abundance_cutoff</td>
<td>numeric, remove all cells, whose cell-type appears less then the given value. This removes low abundant cell-types</td>
</tr>
<tr>
<td>scale_tpm</td>
<td>boolean, if TRUE (default) the cells in tpm_matrix will be scaled to sum up to 1e6</td>
</tr>
</tbody>
</table>

Value

Return a `SummarizedExperiment` object

Examples

```r
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")
```
**Description**

Build `SummarizedExperiment` using a h5ad file for the counts

**Usage**

```r
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
- `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`
dataset_merge

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 than 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
)
dataset_merge

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))

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))
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 object 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
dataset_sfaira

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
dataset_sfaira

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
dataset_sfaira_multiple

Examples

```r
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

```r
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**
umeric, is only applied if **filter_genes** is TRUE: removes all genes with
variance below the chosen cutoff

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

**scale_tpm**oolean, if TRUE (default) the cells in tpm_matrix will be scaled to sum up to
1e6

**Value**
dataset object

**Examples**

```r
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"
)
```

---

**dmode**

*use gaussian kernel to calculate the mode of transcript counts*

**Description**

use gaussian kernel to calculate the mode of transcript counts

**Usage**

`dmode(x)`

**Arguments**

- `x` vector of numeric values

**Value**

most commonly occurring (log-transformed) TPM value
**download_sfaira**

*download a specific dataset from sfaira by an ID*

---

**Description**

download a specific dataset from sfaira by an ID

**Usage**

```r
download_sfaira(
  setup_list,
  ids,
  force = FALSE,
  synapse_user = NULL,
  synapse_pw = NULL
)
```

**Arguments**

- `setup_list` the sfaira setup; given by `setup_sfaira`
- `ids` the IDs of the datasets
- `force` logical; TRUE if you want to force the download, even though no cell-type annotation exists for this dataset. Default if FALSE
- `synapse_user` character; username for synapse portal (https://www.synapse.org)
- `synapse_pw` character; password for synapse portal (https://www.synapse.org)

**Value**

matrix, gene names and cell IDs

---

**download_sfaira_multiple**

*download multiple datasets from sfaira using filters for organism, tissue and/or assay*

---

**Description**

similar to the filters on the sfaira website (https://theislab.github.io/sfaira-portal/Datasets)
Usage

download_sfaira_multiple(
    setup_list,
    organisms = NULL,
    tissues = NULL,
    assays = NULL,
    force = FALSE
)

Arguments

setup_list the sfaira setup; given by setup_sfaira
organisms list of organisms (only human and mouse available)
tissues list of tissues
assays list of assays
force logical; TRUE if you want to force to download all datasets, otherwise only the
ones with cell-type annotation will be returned. Default if FALSE

Value

annotated data object, contains count matrix and annotation

filter_matrix

filter one (or two) expression matrix by genes

Description

filter one (or two) expression matrix by genes

Usage

filter_matrix(m1, m2 = NULL, filter_genes = TRUE, variance_cutoff = 0)

Arguments

m1 Matrix 1
m2 Matrix 2 (optional)
filter_genes boolean
variance_cutoff numeric, genes below this variance value are removed

Value

filtered matrix
**generate_summarized_experiment**

*Generate SummarizedExperiment using multiple parameters*

**Description**

Generate SummarizedExperiment using multiple parameters

**Usage**

```r
generate_summarized_experiment(
  annotation,
  count_matrix,
  tpm_matrix,
  name,
  spike_in_col,
  additional_cols,
  filter_genes,
  variance_cutoff,
  type_abundance_cutoff,
  scale_tpm
)
```

**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
- `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
**h5ad_to_adata**

**Value**

Return a `SummarizedExperiment` object

---

**Description**

Use basilisk environment to read h5ad file and access anndata object

**Usage**

```r
h5ad_to_adata(h5ad_path, cells_in_obs)
```

**Arguments**

- `h5ad_path`:
  - path to h5ad file
- `cells_in_obs`:
  - boolean, if TRUE, cell identifiers are taken from obs layer in anndata object; if FALSE, they are taken from var

**Value**

matrix contained on h5ad file as dgCMatrix

---

**merge_scaling_factor**

**Create scaling vector from custom or pre-defined scaling factor**

---

**Description**

Create scaling vector from custom or pre-defined scaling factor

**Usage**

```r
merge_scaling_factor(data, scaling_factor_values, scaling_factor_name)
```

**Arguments**

- `data`:
  - dataset
- `scaling_factor_values`:
  - named list of scaling values
- `scaling_factor_name`:
  - name of scaling factor method

**Value**

scaling vector
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,
```r
plot_simulation

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 ggplot2 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", 70),
    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**

```r
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())
```

**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**

```r
setup_sfaira(basedir)
```
sfaira_overview

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

```r
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

```r
sfaira_overview(setup_list)
```

Arguments

setup_list  
the sfaira setup; given by `setup_sfaira`

Value

a dataframe with information on each dataset

Examples

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

Visualization

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

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 prior 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 sce-
nario, 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 fac-
tor

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.
**simulate_bulk**

- **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; specify 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 increased 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**

```r
# 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
)

### simulate_sample

**simulate single pseudo-bulk sample**

**Description**

function to sample cells according to given cell-type fractions. This creates a single pseudo-bulk sample by calculating the mean expression value per gene over all sampled cells. Note: if total_read_counts is used, the cell-fractions are applied to the number of counts, not the number of cells!

**Usage**

```r
simulate_sample(
data,    # SummarizedExperiment object
scaling_vector,    # vector with scaling values for each cell; calculated by the calc_scaling_vector function
simulation_vector, # named vector with wanted cell-types and their fractions
total_cells,       # numeric; number of total cells for this simulation
total_read_counts, # numeric; total number of counts for all cells
remove_bias_in_counts, # logical; remove bias due to sequencing depth
remove_bias_in_counts_method, # character; method used to remove bias
norm_counts, # logical; normalize to equal expression for each cell
seed
)
```

**Arguments**

- `data`: SummarizedExperiment object
- `scaling_vector`: vector with scaling values for each cell; calculated by the calc_scaling_vector function
- `simulation_vector`: named vector with wanted cell-types and their fractions
- `total_cells`: numeric; number of total cells for this simulation
- `total_read_counts`: numeric; total number of counts for all cells
- `remove_bias_in_counts`: logical; remove bias due to sequencing depth
- `remove_bias_in_counts_method`: character; method used to remove bias
- `norm_counts`: logical; normalize to equal expression for each cell
- `seed`:
simulate_sample

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

**remove_bias_in_counts**
boolean; if TRUE (default) the internal mRNA bias that is present in count data will be removed using the number of reads mapped to each cell

**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

**seed**
umeric; fix this value if you want the same cells to be sampled

**Value**
returns two vectors (one based on counts, one based on tpm; depends on which matrices are present in data) with expression values for all genes in the provided dataset
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