Package ‘granulator’

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Type Package

Title Rapid benchmarking of methods for *in silico* deconvolution of bulk RNA-seq data

Version 1.10.0

Description granulator is an R package for the cell type deconvolution of heterogeneous tissues based on bulk RNA-seq data or single cell RNA-seq expression profiles. The package provides a unified testing interface to rapidly run and benchmark multiple state-of-the-art deconvolution methods. Data for the deconvolution of peripheral blood mononuclear cells (PBMCs) into individual immune cell types is provided as well.

URL https://github.com/xanibas/granulator

BugReports https://github.com/xanibas/granulator/issues

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benchmarks

Description

regress computes regression between estimated cell type proportions and the measured cell type proportions (ground truth).

Usage

benchmark(deconvoluted, ground_truth)

Arguments

defconvoluted Output object of the function deconvolute.
ground_truth A matrix containing measured cell type proportions in percentages. Samples names are included in rownames.
**bulkRNAseq_ABIS**

**Value**

Returns a list containing three elements:

- **data**: a list of data frames with celltype matched estimated and predicted proportions
- **stats**: a list of data frames with regression statistics comprising Pearson Correlation Coefficient (‘pcc’), Concordance Correlation Coefficient (‘ccc’), Coefficient of Determination (‘adj.r2’) and Root Mean Square Error (‘rmse’)
- **summary**: a data frame with summary statistics by cell type
- **rank**: ranking of deconvolution algorithms by highest all-to-all correlation of coefficients
- **summay**: summary statistics of regression coefficients by method, signature and cell type
- **rank**: ranking of methods and signatures by highest average regression coefficient
- **combinations**: combination of methods and signatures tested

**Author(s)**

Vincent Kuettel, Sabina Pfister

**Examples**

```r
# load demo PBMCs data
load_ABIS()

# deconvolute
decon <- deconvolute(m = bulkRNAseq_ABIS,
sigMatrix = sigMatrix_ABIS_S0)

# benchmark
bench <- benchmark(deconvoluted = decon,
ground_truth = groundTruth_ABIS)
```

---

**bulkRNAseq_ABIS**

*PBMCs expression profiles (ABIS dataset)*

**Description**

Public dataset (GSE107011) containing the TPM-normalized gene expression values from bulk RNAseq of PBMCs of 12 healthy individuals. We include here only genes selected in the signature matrices.

**Usage**

data(bulkRNAseq_ABIS)

**Format**

A matrix with 1296 rows (genes) and 12 variables (samples)
correlate

Source
GEO

References
Monaco et al. (2019) Cell Reports 26, 1627–1640 (Cell Reports)

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correlate  \hspace{1em} \textit{Pearson correlation of cell type proportions across cell types and methods}

Description
correlate computes Pearson correlations between estimated cell type proportions generated by different methods.

Usage
correlate(deconvoluted, scale = TRUE)

Arguments
deconvoluted \hspace{1em} A list: output object from deconvolute
scale \hspace{1em} Boolean: indicate whether the coefficients should be transformed to standard scores (default: scale = TRUE).

Details
correlation_analysis is particularly useful to assess the performance of the different methods when no ground truth is available. If several methods agree on similar relative abundances of cell types across samples, the results are more likely to reflect true differences in cell type composition.

Value
Returns a list encompassing two data frames:

- the pearson correlation of coefficients with all other coefficients
- summary: summary statistics of all-to-all correlation of coefficients by cell type
- rank: ranking of deconvolution algorithms by highest all-to-all correlation of coefficients
- rank: ranking of deconvolution algorithms by highest average regression all-to-all correlation of coefficients
- combinations: combination of methods and signatures tested

Author(s)
Vincent Kuettel, Sabina Pfister
Examples

```r
# load data
load_ABIS()

# deconvolute
decon <- deconvolute(m = bulkRNAseq_ABIS,
sigMatrix = sigMatrix_ABIS_S0)

# correlate
correl <- correlate(deconvoluted = decon)
```

---

**deconvolute**

*Deconvolution from bulk RNAseq*

**Description**

deconvolute predicts cell type proportions from bulk RNAseq data by applying multiple deconvolution methods.

**Usage**

deconvolute(m, sigMatrix, methods = get_decon_methods(), use_cores = 1)

**Arguments**

`m`  
Bulk RNAseq: a genes (rows) by samples (columns) matrix containing transcript-per-million (TPM)-normalized gene expression values.

`sigMatrix`  
Reference profile: a matrix or a named list of matrices. Each signature matrix should be a genes (rows) by cell types (columns) data frame containing TPM-normalized gene expression values of signature genes.

`methods`  
Deconvolution methods: a character vector containing the names of the deconvolution methods to be applied. By default, all methods are run. Functions are either reimplementations of published methods or wrapper functions for published packages:

- `ols`: ordinary least squares
- `nnls`: non negative least squares regression model. Adapted from Abas et al. (2009)
- `qprog`: quadratic programming without constraints
- `qprogwc`: quadratic programming non-negative and sum-to-one constraints. Adapted from Gong et al. (2015)
- `dtangle`: wrapper for the cell deconvolution function `dtangle` from the package `dtangle`
- `rls`: robust linear regression. Adapted from Monaco et al. (2019)
- `svr`: support vector regression. Adapted from Newman et al. (2015)

`use_cores`  
Number of cores to use for parallel processing
get_decon_methods

Value

Returns a list containing two elements:

• coefficients: estimated cell type coefficients
• proportions: estimated cell type proportions in percentage
• combinations: combination of methods and signatures tested

Author(s)

Vincent Kuettel, Sabina Pfister

Examples

# load demo PBMCs data
load_ABIS()

# generate list of reference profiles to be tested
sigMatrix <- list(
sig1 = sigMatrix_ABIS_S0,
sig2 = sigMatrix_ABIS_S1)

# deconvolute
deon <- deconvolute(m = bulkRNAseq_ABIS,
sigMatrix = sigMatrix)

# get available deconvolution methods
get_decon_methods()

get_decon_methods     Deconvolution methods acronyms

Description

get_decon_methods returns supported deconvolution methods acronyms.

Usage

get_decon_methods()

Value

type containing the acronyms of deconvolution methods.

Author(s)

Vincent Kuettel, Sabina Pfister

Examples

# get available deconvolution methods
get_decon_methods()
**get_TPM**

Convert raw counts to TPM

### Description

get_TPM is used to convert raw counts to TPMs, which is the most suitable normalization for deconvolution.

### Usage

```r
get_TPM(counts, effLen)
```

### Arguments

- **counts**
  
  Bulk RNAseq: a genes (rows) by samples (columns) matrix containing gene raw counts.

- **effLen**
  
  Vector of gene lengths.

### Value

Returns a transcript-per-million (TPM)-normalized matrix.

### Author(s)

Vincent Kuettel, Sabina Pfister

### Examples

```r
# get TPMs from raw counts and gene lengths.
mat <- round(matrix(rexp(200, rate=.01), ncol=20))
len <- round(matrix(rexp(10, rate=.001), ncol=1))+10
tpm <- get_TPM(mat,as.vector(len))
```

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**groundTruth_ABIS**

PBMCS true cell type proportions (ABIS dataset)

### Description

Public dataset (GSE107011) containing the true proportions for all combinations of cell types (PBMCs) for 12 individuals.

### Usage

```r
data(groundTruth_ABIS)
```
### Description

`load_ABIS` is used to load a demo dataset for the deconvolution of PBMCs samples from published data under the accession number GSE107011. The dataset consists of the following datasets:

- `bulkRNAseq_ABIS`: PBMCs expression profiles
- `sigMatrix_ABIS_S0`: Signature matrix for deconvolution of PBMCs in 17 cell types
- `sigMatrix_ABIS_S1`: Signature matrix for deconvolution of PBMCs in 13 cell types
- `sigMatrix_ABIS_S2`: Signature matrix for deconvolution of PBMCs in 11 cell types
- `sigMatrix_ABIS_S3`: Signature matrix for deconvolution of PBMCs in 9 cell types
- `groundTruth_ABIS`: PBMCs true cell type proportions

### Usage

```r
load_ABIS()
```

### Value

Returns string confirming successful loading of the data.

### Author(s)

Vincent Kuettel, Sabina Pfister

### Examples

```r
# load data
load_ABIS()
```
Description

plot_benchmark plots the median correlation scores between estimated and measured cell types across methods and cell types.

Usage

plot_benchmark(benchmarked, metric = "pcc")

Arguments

- `benchmarked` List: output object from function benchmarked.
- `metric` Character: the metric of evaluation. Options include Pearson Correlation Coefficient ('pcc'), Concordance Correlation Coefficient ('ccc'), Coefficient of Determination ('adj.r2') and Root Mean Square Error ('rmse') of the linear regression model.

Value

Plot showing correlations across algorithms and cell types.

Author(s)

Vincent Kuettel, Sabina Pfister

Examples

```r
# load demo PBMCS data
load_ABI()

# deconvolute
decon <- deconvolute(m = bulkRNAseq_ABI,
sigMatrix = sigMatrix_ABI_S0)

# bechmark
bench <- benchmark(deconvoluted = decon,
ground_truth = groundTruth_ABI)

# plot bechmark
plot_benchmark(benchmarked = bench,
metric = 'pcc')
```
**plot_correlate**  

*Plot of correlations between deconvolution methods*

Description

`plot_correlate` is used to visualize the results obtained by `correlation_analysis`.

Usage

```
plot_correlate(correlated, method = "heatmap", legend = TRUE)
```

Arguments

- `correlated`: output object from `correlate`
- `method`: plot type ("heatmap" or "boxplot")
- `legend`: boolean to display color legend

Details

`plot_correlate` plots the correlation of cell type proportions across methods in form of a heatmap or a violin plot. If methods agree, cell type proportions of the same cell type should be strongly correlated. For cell types with weak correlation across methods, corresponding estimated cell type proportions should be interpreted with caution.

Value

Returns a heatmap or violin plot showing the correlation distribution of by different methods/signature matrices for each cell type.

Author(s)

Vincent Kuettel, Sabina Pfister

Examples

```r
# load demo PBMCS data
load_ABIS()

# deconvolute
decon <- deconvolute(m = bulkRNAseq_ABIS,
sigMatrix = sigMatrix_ABIS_S0)

# correlate
correl <- correlate(deconvoluted = decon)

# plot correlate
plot_correlate(correlated = correl,
method="heatmap")
```
Description

plot_deconvolute allows to compare methods across cell types, where the different methods show a high level of agreement or potentially generate diverging proportion estimates.

Usage

plot_deconvolute(
  deconvoluted = deconvoluted,
  scale = TRUE,
  labels = TRUE,
  markers = TRUE
)

Arguments

deconvoluted output object from function deconvolute.
scale Boolean: indicate whether the coefficients should be transformed to standard scores (default: scale = TRUE).
labels Boolean: indicate if x axis labels should be included (default: labels = TRUE).
markers Boolean: indicate if data points markers should be drawn (default: markers = TRUE).

Details

Plots the estimated cell types generated by different deconvolution methods/signature matrices across samples. Scaling is used to directly compare deconvolution outputs across methods.

Value

line plot

Author(s)

Vincent Kuettel, Sabina Pfister

Examples

# load demo PBMCS data
load_ABIS()

# deconvolute
decon <- deconvolute(m = bulkRNAseq_ABIS,
sigMatrix = sigMatrix_ABIS_S0)
Description

`plot_proportions` plots the estimated cell type proportions as computed by a given method and signature matrix.

Usage

```r
plot_proportions(deconvoluted, method = "svr", signature = "sig1")
```

Arguments

- `deconvoluted` Output object from function `deconvolute`.
- `method` Character string with name of method to be regressed.
- `signature` Character string with name of signature to be regressed.

Value

Plot showing regression of estimated versus measured cell type coefficients.

Author(s)

Vincent Kuettel, Sabina Pfister

Examples

```r
# load demo PBMCS data
load_ABIS()

# deconvolute
decon <- deconvolute(m = bulkRNAseq_ABIS,
sigMatrix = sigMatrix_ABIS_S0)

# plot cell type proportions
plot_proportions(deconvoluted = decon,
method = 'svr', signature = 'sig1')
```
plot_regress

**Plot estimated cell type coefficients against the ground truth**

**Description**

plot_regress depicts the measured cell type proportions (x-axis) vs. the estimated proportions (y-axis).

**Usage**

```r
plot_regress(benchmarked, method = "svr", signature = "sig1")
```

**Arguments**

- `benchmarked`: List: output object from function `benchmarked`.
- `method`: Character string with name of method to be regressed.
- `signature`: Character string with name of signature to be regressed.

**Value**

Plot showing regression of estimated versus measured cell type coefficients.

**Author(s)**

Vincent Kuettel, Sabina Pfister

**Examples**

```r
# load demo PBMCS data
load_ABIS()

# deconvolute
deon <- deconvolute(m = bulkRNAseq_ABIS,
sigMatrix = sigMatrix_ABIS_S0)

# bechmark
t bench <- benchmark(deconvoluted = deon,
ground_truth = groundTruth_ABIS)

# plot regress
plot_regress(benchmarked = bench,
method = "svr", signature = "sig1")
```
**plot_similarity**  

*Plot reference profile similarity matrix*

**Description**

`plot_similarity` plots cell type similarity matrix by computing the Kendall rank correlations between cell type expression profiles. Kendall rank correlation is used to test the similarities in the ordering of data when it is ranked by quantities, and provides a less inflated measure of accuracy than Pearson correlation by accounting for ties in the data.

**Usage**

```r
plot_similarity(sigMatrix)
```

**Arguments**

- `sigMatrix`  
  Signature matrix: a data frame or a named list of data frames. Each signature matrix should be a genes (rows) by cell types (columns) data frame containing TPM-normalized gene expression values of signature genes.

**Value**

Plot showing the Kendall rank correlations similarity matrix.

**Author(s)**

Vincent Kuettel, Sabina Pfister

**Examples**

```r
# load demo PBMCS data
load_ABIS()

# generate list of reference profiles to be tested
sigMatrix <- list(sig1 = sigMatrix_ABIS_S0,
                  sig2 = sigMatrix_ABIS_S2)

# plot similarity
plot_similarity(sigMatrix = sigMatrix)
```
### sigMatrix_ABIS_S0

**Signature matrix for deconvolution of PBMCs in 17 cell types**

**Description**

A dataset containing the TPM-normalized RNA-seq gene expression values for signature genes of 17 PBMCs.

**Usage**

```r
data(sigMatrix_ABIS_S0)
```

**Format**

A matrix with 1296 rows (genes) and 17 variables (cell types)

**Source**

Github

**References**

Monaco et al. (2019) Cell Reports 26, 1627–1640 (Cell Reports)

### sigMatrix_ABIS_S1

**Signature matrix for deconvolution of PBMCs in 13 cell types**

**Description**

A dataset containing the TPM-normalized RNA-seq gene expression values for signature genes of 17 PBMCs.

**Usage**

```r
data(sigMatrix_ABIS_S1)
```

**Format**

A matrix with 1296 rows (genes) and 13 variables (cell types)

**References**

Monaco et al. (2019) Cell Reports 26, 1627–1640 (Cell Reports)
sigMatrix_ABIS_S2  
*Signature matrix for deconvolution of PBMCs in 11 cell types*

**Description**
A dataset containing the TPM-normalized RNA-seq gene expression values for signature genes of 17 PBMCs.

**Usage**
data(sigMatrix_ABIS_S2)

**Format**
A matrix with 1296 rows (genes) and 11 variables (cell types)

**References**
Monaco et al. (2019) Cell Reports 26, 1627–1640 (Cell Reports)

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sigMatrix_ABIS_S3  
*Signature matrix for deconvolution of PBMCs in 9 cell types*

**Description**
A dataset containing the TPM-normalized RNA-seq gene expression values for signature genes of 17 PBMCs.

**Usage**
data(sigMatrix_ABIS_S3)

**Format**
A matrix with 1296 rows (genes) and 9 variables (cell types)

**References**
Monaco et al. (2019) Cell Reports 26, 1627–1640 (Cell Reports)
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