Package ‘granulator’

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Type Package

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

Version 1.12.0

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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benchmark

Regress estimated cell type proportions against the ground truth

Description

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

Usage

benchmark(deconvoluted, ground_truth)

Arguments

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

Returns a list containing three elements:

- data: a list of data frames with cell type 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
- summary: 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)

**correlate**

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**

decovluted: A list: output object from deconvolute

scale: 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
decn <- 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` form 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
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
decon <- deconvolute(m = bulkRNAseq_ABIS,
sigMatrix = sigMatrix)

generate_reconcile_output methods

Description

generate_reconcile_output methods returns supported deconvolution methods acronyms.

Usage

generate_reconcile_output methods()

Value

vector containing the acronyms of deconvolution methods.

Author(s)

Vincent Kuettel, Sabina Pfister

Examples

# get available deconvolution methods
generate_reconcile_output 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 de-convolution.

**Usage**

`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))
```

---

**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**

`data(groundTruth_ABIS)`
**load_ABIS**

**Format**

A matrix with 12 rows (samples) and 24 variables (cell types)

**Source**

Github

**References**

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

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**load_ABIS**

*Load demo PBMCs deconvolution data*

**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**

`load_ABIS()`

**Value**

Returns string confirming successful loading of the data.

**Author(s)**

Vincent Kuettel, Sabina Pfister

**Examples**

```r
# load data
load_ABIS()
```
**plot_benchmark**

*Plot benchmarking analysis scores*

**Description**

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

**Usage**

```r
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_ABIS()

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

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

# 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

# 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

```r
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

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

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

# plot deconvolute
plot_deconvolute(deconvoluted = decon)

plot_proportions  
Plot estimated cell type proportions

Description

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

Usage

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

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

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

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

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

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

# 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
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
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)
Index

* datasets
  bulkRNAseq_ABIS, 3
  groundTruth_ABIS, 7
  sigMatrix_ABIS_S0, 15
  sigMatrix_ABIS_S1, 15
  sigMatrix_ABIS_S2, 16
  sigMatrix_ABIS_S3, 16

benchmark, 2
bulkRNAseq_ABIS, 3

correlate, 4
deconvolute, 5
dtangle, 5

gt_decon_methods, 6
gt_TPM, 7
groundTruth_ABIS, 7

load_ABIS, 8

plot_benchmark, 9
plot_correlate, 10
plot_deconvolute, 11
plot_proportions, 12
plot_regress, 13
plot_similarity, 14

sigMatrix_ABIS_S0, 15
sigMatrix_ABIS_S1, 15
sigMatrix_ABIS_S2, 16
sigMatrix_ABIS_S3, 16