Package: DifferentialRegulation (via r-universe)

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Type Package

Title Differentially regulated genes from scRNA-seq data

Version 2.12.0

Description DifferentialRegulation is a method for detecting differentially regulated genes between two groups of samples (e.g., healthy vs. disease, or treated vs. untreated samples), by targeting differences in the balance of spliced and unspliced mRNA abundances, obtained from single-cell RNA-sequencing (scRNA-seq) data. From a mathematical point of view, DifferentialRegulation accounts for the sample-to-sample variability, and embeds multiple samples in a Bayesian hierarchical model. Furthermore, our method also deals with two major sources of mapping uncertainty: i) 'ambiguous' reads, compatible with both spliced and unspliced versions of a gene, and ii) reads mapping to multiple genes. In particular, ambiguous reads are treated separately from spliced and unsplced reads, while reads that are compatible with multiple genes are allocated to the gene of origin. Parameters are inferred via Markov chain Monte Carlo (MCMC) techniques (Metropolis-within-Gibbs).

biocViews DifferentialSplicing, Bayesian, Genetics, RNASeq, Sequencing, DifferentialExpression, GeneExpression, MultipleComparison, Software, Transcription, StatisticalMethod, Visualization, SingleCell, GeneTarget

License GPL-3

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Contents

DifferentialRegulation-package . . . 2
compute_PB_counts . . . 3
DifferentialRegulation . . . 5
DifferentialRegulation_bulk . . . 9
load_bulk_EC . . . 12
load_bulk_US . . . 13
load_EC . . . 14
load_USA . . . 16
plot_bulk_pi . . . 17
plot_bulk_traceplot . . . 18
plot_pi . . . 19
plot_traceplot . . . 20

Index **21**

`DifferentialRegulation-package`

Differentially regulated genes from scRNA-seq data

Description

DifferentialRegulation is a method for detecting differentially regulated genes between two groups of samples (e.g., healthy vs. disease, or treated vs. untreated samples), by targeting differences in the balance of spliced and unspliced mRNA abundances, obtained from single-cell RNA-sequencing (scRNA-seq) data. From a mathematical point of view, DifferentialRegulation accounts for the sample-to-sample variability, and embeds multiple samples in a Bayesian hierarchical model. Furthermore, our method also deals with two major sources of mapping uncertainty: i) 'ambiguous'

reads, compatible with both spliced and unspliced versions of a gene, and ii) reads mapping to multiple genes. In particular, ambiguous reads are treated separately from spliced and unsplced reads, while reads that are compatible with multiple genes are allocated to the gene of origin. Parameters are inferred via Markov chain Monte Carlo (MCMC) techniques (Metropolis-within-Gibbs).

Details

The DESCRIPTION file: This package was not yet installed at build time.
Questions relative to DifferentialRegulation should be reported as a new issue at https://github.com/SimoneTiberi/Differential

To access the vignettes, type: `browseVignettes("DifferentialRegulation")`.

Index: This package was not yet installed at build time.

Author(s)

Simone Tiberi <simone.tiberi@uzh.ch>

`compute_PB_counts` *Discover differentially regulated genes*

Description

`compute_PB_counts` computese the pseudo-bulk (PB) counts, needed to perform differential testing by `DifferentialRegulation`.

Usage

```
compute_PB_counts(
  sce,
  EC_list,
  design,
  sample_col_name = "sample",
  group_col_name = "group",
  sce_cluster_name = "cell_type",
  min_cells_per_cluster = 100,
  min_counts_per_gene_per_group = 20,
  min_counts_ECs = 0
)
```

Arguments

`sce`	a `SingleCellExperiment` object, computed via `load_USA`.
`EC_list`	a `list`, computed via `load_EC`.
`design`	a `data.frame` indicating the design of the experiment with one row for each sample; 'design' must contain a column with the sample id and one with the group id.

`sample_col_name`
: a character ("sample" by default), indicating the column name of the 'design' element which stores the sample id.

`group_col_name`
: a character ("group" by default), indicating the column name of the 'design' element which stores the group id.

`sce_cluster_name`
: a character ("cell_type" by default), indicating the name of the 'colData(sce)' element, which stores the cluster id of each cell (i.e., colData(sce)$name_cluster).

`min_cells_per_cluster`
: cell cluster (e.g., cell-type) filter. 'min_cells_per_cluster' is the minimum number of cells, across all samples and groups, for a cell cluster to be considered. Cell clusters with less than 'min_cells_per_cluster' cells will not be analyzed.

`min_counts_per_gene_per_group`
: minimum number of counts per gene, in each cell, across all samples of every group. In each cell cluster, only genes with at least 'min_counts_per_gene_per_group' counts in both groups of samples will be analyzed.

`min_counts_ECs`
: equivalence classes (ECs) filter. 'min_counts_ECs' indicates the minimum number of counts (across all cells in a cell cluster) for each equivalence class; by default all ECs are considered (min_counts_ECs = 0). ECs with less or equal than 'min_counts_ECs' will be discarded. Increasing 'min_counts_ECs' will marginally decrease computational cost computational at the cost of a marginal loss in performance.

Value

A `list` of objects required perform differential testing by `DifferentialRegulation`.

Author(s)

Simone Tiberi <simone.tiberi@unibo.it>

See Also

`load_EC`, `load_USA`, `DifferentialRegulation`, `plot_pi`

Examples

```
# load internal data to the package:
data_dir = system.file("extdata", package = "DifferentialRegulation")

# specify samples ids:
sample_ids = paste0("organoid", c(1:3, 16:18))
# set directories of each sample input data (obtained via alevin-fry):
base_dir = file.path(data_dir, "alevin-fry", sample_ids)
file.exists(base_dir)

# set paths to USA counts, cell id and gene id:
# Note that alevin-fry needs to be run with '--use-mtx' option
# to store counts in a 'quants_mat.mtx' file.
```

```
path_to_counts = file.path(base_dir,"/alevin/quants_mat.mtx")
path_to_cell_id = file.path(base_dir,"/alevin/quants_mat_rows.txt")
path_to_gene_id = file.path(base_dir,"/alevin/quants_mat_cols.txt")

# load USA counts:
sce = load_USA(path_to_counts,
               path_to_cell_id,
               path_to_gene_id,
               sample_ids)

# define the design of the study:
design = data.frame(sample = sample_ids,
                    group = c( rep("3 mon", 3), rep("6 mon", 3) ))
design

# cell types should be assigned to each cell;
# here we load pre-computed cell types:
path_to_DF = file.path(data_dir,"DF_cell_types.txt")
DF_cell_types = read.csv(path_to_DF, sep = "\t", header = TRUE)
matches = match(colnames(sce), DF_cell_types$cell_id)
sce$cell_type = DF_cell_types$cell_type[matches]

# set paths to EC counts and ECs:
path_to_EC_counts = file.path(base_dir,"/alevin/geqc_counts.mtx")
path_to_EC = file.path(base_dir,"/alevin/gene_eqclass.txt.gz")

# load EC counts:
EC_list = load_EC(path_to_EC_counts,
                  path_to_EC,
                  path_to_cell_id,
                  path_to_gene_id,
                  sample_ids)

PB_counts = compute_PB_counts(sce = sce,
                              EC_list = EC_list,
                              design =  design,
                              sample_col_name = "sample",
                              group_col_name = "group",
                              sce_cluster_name = "cell_type",
                              min_cells_per_cluster = 100,
                              min_counts_per_gene_per_group = 20)
```

`DifferentialRegulation` *Discover differentially regulated genes from single-cell RNA-seq data*

Description

`DifferentialRegulation` identified differentially regulated genes between two conditions (e.g., healthy vs. disease or treated vs. untreated) in each cluster of cells. Parameters are inferred via

Markov chain Monte Carlo (MCMC) techniques and a differential testing is performed via a multivariate Wald test on the posterior densities of the group-level USA (Unspliced, Spliced and Ambiguous) counts relative abundance.

Usage

```
DifferentialRegulation(
  PB_counts,
  n_cores = NULL,
  N_MCMC = 2000,
  burn_in = 500,
  undersampling_int = 10,
  traceplot = FALSE
)
```

Arguments

PB_counts	a list, computed via compute_PB_counts
n_cores	the number of cores to parallelize the tasks on. Since parallelization is at the cluster level (each cluster is parallelized on a thread), we suggest setting n_cores to the number of clusters (e.g., cell-types), as set by default if 'n_cores' is not specified.
N_MCMC	the number of iterations for the MCMC algorithm (including burn-in). Min 2*10^3. If our algorithm does not converge (according to Heidelberger and Welch's convergence diagnostic), we automatically double N_MCMC and burn_in, and run it a second time (a message will be printed on screen to inform users).
burn_in	the length of the burn-in; i.e., the initial part of the MCMC chain to be discarded (before convergence is reached). Min 500. If no convergence is reached, the 'burn_in' is automatically increased (up to N_MCMC/2) according to the convergence detected by Heidelberger and Welch's convergence diagnostic. If our algorithm does not converge even after increasing the burn-in, we automatically double N_MCMC and burn_in, and run it a second time (a message will be printed on screen to inform users).
undersampling_int	the undersampling of the latent variables. While model parameters are sampled at each iteration, RNA-seq counts are allocated to their transcript (and spliced/unspliced) version of origin, every 'undersampling_int' iterations. Increasing 'undersampling_int' will decrease the runtime, but may marginally affect performance. In our benchmarks, no differences in performance were observed for values up to 10.
traceplot	a logical value indicating whether to return the posterior chain of "pi_U", for both groups (i.e., the group-level relative abundance of unspliced reads). If TRUE, the posterior chains are stored in 'MCMC_U' object, and can be plotted via 'plot_traceplot' function.

Value

A `list` of 4 `data.frame` objects. 'Differential_results' contains results from differential testing only; 'US_results' has results for the proportion of Spliced and Unspliced counts (Ambiguous counts are allocated 50:50 to Spliced and Unspliced); 'USA_results' includes results for the proportion of Spliced, Unspliced and Ambiguous counts (Ambiguous counts are reported separately from Spliced and Unspliced counts); 'Convergence_results' contains information about convergence of posterior chains. Columns 'Gene_id' and 'Cluster_id' contain the gene and cell-cluster name, while 'p_val', 'p_adj.loc' and 'p_adj.glb' report the raw p-values, locally and globally adjusted p-values, via Benjamini and Hochberg (BH) correction. In locally adjusted p-values ('p_adj.loc') BH correction is applied to each cluster separately, while in globally adjusted p-values ('p_adj.glb') BH correction is performed to the results from all clusters. Columns 'pi' and 'sd' indicate the proportion and standard deviation, respectively, 'S', 'U' and 'A' refer to Spliced, Unspliced and Ambiguous counts, respectively, while 'gr_A' and 'gr_B' refer to group A and B, respectively. For instance, columns 'pi_S-gr_A' and 'sd_S-gr_A' indicate the estimates and standard deviation (sd) for the proportion of Spliced (pi_S) and Unspliced (pi_U) counts in group A, respectively.

Author(s)

Simone Tiberi <simone.tiberi@unibo.it>

See Also

`load_EC`, `load_USA`, codecompute_PB_counts, `plot_pi`, `plot_traceplot`

Examples

```
# load internal data to the package:
data_dir = system.file("extdata", package = "DifferentialRegulation")

# specify samples ids:
sample_ids = paste0("organoid", c(1:3, 16:18))
# set directories of each sample input data (obtained via alevin-fry):
base_dir = file.path(data_dir, "alevin-fry", sample_ids)
file.exists(base_dir)

# set paths to USA counts, cell id and gene id:
# Note that alevin-fry needs to be run with '--use-mtx' option
# to store counts in a 'quants_mat.mtx' file.
path_to_counts = file.path(base_dir,"/alevin/quants_mat.mtx")
path_to_cell_id = file.path(base_dir,"/alevin/quants_mat_rows.txt")
path_to_gene_id = file.path(base_dir,"/alevin/quants_mat_cols.txt")

# load USA counts:
sce = load_USA(path_to_counts,
               path_to_cell_id,
               path_to_gene_id,
               sample_ids)

# define the design of the study:
design = data.frame(sample = sample_ids,
                    group = c( rep("3 mon", 3), rep("6 mon", 3) ))
```

```
design

# cell types should be assigned to each cell;
# here we load pre-computed cell types:
path_to_DF = file.path(data_dir,"DF_cell_types.txt")
DF_cell_types = read.csv(path_to_DF, sep = "\t", header = TRUE)
matches = match(colnames(sce), DF_cell_types$cell_id)
sce$cell_type = DF_cell_types$cell_type[matches]

# set paths to EC counts and ECs:
path_to_EC_counts = file.path(base_dir,"/alevin/geqc_counts.mtx")
path_to_EC = file.path(base_dir,"/alevin/gene_eqclass.txt.gz")

# load EC counts:
EC_list = load_EC(path_to_EC_counts,
                  path_to_EC,
                  path_to_cell_id,
                  path_to_gene_id,
                  sample_ids)

PB_counts = compute_PB_counts(sce = sce,
                              EC_list = EC_list,
                              design =  design,
                              sample_col_name = "sample",
                              group_col_name = "group",
                              sce_cluster_name = "cell_type",
                              min_cells_per_cluster = 100,
                              min_counts_per_gene_per_group = 20)

# to reduce memory usage, we can remove the EC_list object:
rm(EC_list)

set.seed(1609612)
results = DifferentialRegulation(PB_counts,
                                 n_cores = 2,
                                 traceplot = TRUE)

names(results)

# We visualize differential results:
head(results$Differential_results)

# plot top (i.e., most significant) result:
# plot USA proportions:
plot_pi(results,
        type = "USA",
        gene_id = results$Differential_results$Gene_id[1],
        cluster_id = results$Differential_results$Cluster_id[1])

# plot US proportions:
plot_pi(results,
        type = "US",
        gene_id = results$Differential_results$Gene_id[1],
```

```
        cluster_id = results$Differential_results$Cluster_id[1])

# plot the corresponding traceplot:
plot_traceplot(results,
               gene_id = results$Differential_results$Gene_id[1],
               cluster_id = results$Differential_results$Cluster_id[1])
```

`DifferentialRegulation_bulk` *Discover differentially regulated genes from bulk RNA-seq data*

Description

`DifferentialRegulation_bulk` identified differentially regulated genes between two conditions (e.g., healthy vs. disease or treated vs. untreated) in each cluster of cells. Parameters are inferred via Markov chain Monte Carlo (MCMC) techniques and a differential testing is performed via a multivariate Wald test on the posterior densities of the group-level US (Unspliced, and Spliced) counts relative abundance.

Usage

```
DifferentialRegulation_bulk(
  SE,
  EC_list,
  design,
  sample_col_name = "sample",
  group_col_name = "group",
  min_counts_per_transcript_per_group = 10,
  N_MCMC = 2000,
  burn_in = 500,
  min_counts_ECs = 0,
  n_cores = 1,
  undersampling_int = 10,
  traceplot = FALSE
)
```

Arguments

`SE`	a `SummarizedExperiment` object, computed via `load_bulk_US`.
`EC_list`	a `list`, computed via `load_bulk_EC`.
`design`	a `data.frame` indicating the design of the experiment with one row for each sample; 'design' must contain a column with the sample id and one with the group id.
`sample_col_name`	a character ("sample" by default), indicating the column name of the 'design' element which stores the sample id.

`group_col_name` a character ("group" by default), indicating the column name of the 'design' element which stores the group id.

`min_counts_per_transcript_per_group`
minimum number of counts per transcript, across all samples in a group Only genes with at least 'min_counts_per_transcript_per_group' counts in both groups of samples will be analyzed.

`N_MCMC` the number of iterations for the MCMC algorithm (including burn-in). Min 2*10^3. If our algorithm does not converge (according to Heidelberger and Welch's convergence diagnostic), we automatically double N_MCMC and burn_in, and run it a second time (a message will be printed on screen to inform users).

`burn_in` the length of the burn-in; i.e., the initial part of the MCMC chain to be discarded (before convergence is reached). Min 500. If no convergence is reached, the 'burn_in' is automatically increased (up to N_MCMC/2) according to the convergence detected by Heidelberger and Welch's convergence diagnostic. If our algorithm does not converge even after increasing the burn-in, we automatically double N_MCMC and burn_in, and run it a second time (a message will be printed on screen to inform users).

`min_counts_ECs` equivalence classes (ECs) filter. 'min_counts_ECs' indicates the minimum number of counts (across all cells in a cell cluster) for each equivalence class; by default all ECs are considered (min_counts_ECs = 0). ECs with less or equal than 'min_counts_ECs' will be discarded. Increasing 'min_counts_ECs' will marginally decrease computational cost computational at the cost of a marginal loss in performance.

`n_cores` the number of cores to parallelize the tasks on. Note that only a minor part of the function runs in parallel (i.e., the prior for the dispersion), while most of the function does not (e.g., the MCMC).

`undersampling_int`
the undersampling of the latent variables. While model parameters are sampled at each iteration, RNA-seq counts are allocated to their transcript (and spliced/unspliced) version of origin, every 'undersampling_int' iterations. Increasing 'undersampling_int' will decrease the runtime, but may marginally affect performance. In our benchmarks, no differences in performance were observed for values up to 10.

`traceplot` a logical value indicating whether to return the posterior chain of "pi_U", for both groups (i.e., the group-level relative abundance of unspliced reads). If TRUE, the posterior chains are stored in 'MCMC_U' object, and can be plotted via 'plot_bulk_traceplot' function.

Value

A `list` of `data.frame` objects. 'Differential_results' contains results from differential testing only; 'Convergence_results' contains information about convergence of posterior chains; 'MCMC_U' (only if traceplot is TRUE) contains the posterior chains for 'pi_U' in both groups. Columns 'Gene_id' and 'Cluster_id' contain the gene and cell-cluster name, while 'p_val', 'p_adj.loc' and 'p_adj.glb' report the raw p-values, locally and globally adjusted p-values, via Benjamini and Hochberg (BH) correction. In locally adjusted p-values ('p_adj.loc') BH correction is applied to each cluster separately, while in globally adjusted p-values ('p_adj.glb') BH correction is performed

to the results from all clusters. Columns 'pi' and 'sd' indicate the proportion and standard deviation, respectively, 'S', 'U' and 'A' refer to Spliced, Unspliced and Ambiguous counts, respectively, while 'gr_A' and 'gr_B' refer to group A and B, respectively. For instance, columns 'pi_S-gr_A' and 'sd_S-gr_A' indicate the estimates and standard deviation (sd) for the proportion of Spliced (pi_S) and Unspliced (pi_U) counts in group A, respectively.

Author(s)

Simone Tiberi <simone.tiberi@unibo.it>

See Also

load_bulk_EC, load_bulk_US, plot_pi, plot_bulk_traceplot

Examples

```
# load internal data to the package:
data_dir = system.file("extdata", package = "DifferentialRegulation")

# specify samples ids:
sample_ids = paste0("sample", seq_len(6))

# US estimates:
quant_files = file.path(data_dir, "salmon", sample_ids, "quant.sf")
file.exists(quant_files)

# Equivalence classes:
equiv_classes_files = file.path(data_dir, "salmon", sample_ids, "aux_info/eq_classes.txt.gz")
file.exists(equiv_classes_files)

# load EC:
EC_list = load_bulk_EC(path_to_eq_classes = equiv_classes_files,
                       n_cores = 2)

# load US estimated counts:
SE = load_bulk_US(quant_files,
                  sample_ids)

# define the design of the study:
group_names = rep(c("A", "B"), each = 3)
design = data.frame(sample = sample_ids,
                    group = group_names)
design

set.seed(1609612)
results = DifferentialRegulation_bulk(SE = SE,
                                      EC_list = EC_list,
                                      design = design,
                                      n_cores = 2,
                                      traceplot = TRUE)

names(results)
```

```
# We visualize differential results:
head(results$Differential_results)

# plot top (i.e., most significant) result:
# plot USA proportions:
plot_bulk_pi(results,
             transcript_id = results$Differential_results$Transcript_id[1])

# plot the corresponding traceplot:
plot_bulk_traceplot(results,
                    transcript_id = results$Differential_results$Transcript_id[1])
```

`load_bulk_EC` *Create a list containing the equivalence classes objects for the bulk RNA-seq data*

Description

`load_bulk_EC` imports the bulk equivalence classes (computed by salmon), and stores them into a list.

Usage

```
load_bulk_EC(path_to_eq_classes = NULL, n_cores = NULL)
```

Arguments

`path_to_eq_classes`	a vector of length equals to the number of samples: each element indicates the path to the equivalence classes counts of the respective sample (i.e., aux_info/eq_classes.txt.gz file).
`n_cores`	the number of cores to parallelize the tasks on. Since parallelization is at the sample level (each sample is parallelized on a thread), we suggest setting n_cores to the number of sample, as set by default if 'n_cores' is not specified.

Value

A `list` object.

Author(s)

Simone Tiberi <simone.tiberi@unibo.it>

See Also

`load_bulk_US`, `DifferentialRegulation_bulk`

Examples

```
# load internal data to the package:
data_dir = system.file("extdata", package = "DifferentialRegulation")

# specify samples ids:
sample_ids = paste0("sample", seq_len(6))

# Equivalence classes:
equiv_classes_files = file.path(data_dir, "salmon", sample_ids, "aux_info/eq_classes.txt.gz")
file.exists(equiv_classes_files)

# load EC:
EC_list = load_bulk_EC(path_to_eq_classes = equiv_classes_files,
                       n_cores = 2)
```

`load_bulk_US` *Creates a SummarizedExperiment containing the estimated US counts for the bulk RNA-seq data*

Description

`load_bulk_US` imports the bulk estimated US (Unspliced, and Spliced) counts (computed by salmon), and stores them into a `SummarizedExperiment` object.

Usage

```
load_bulk_US(path_to_quant_files, sample_ids)
```

Arguments

`path_to_quant_files`	a vector of length equals to the number of samples: each element indicates the path to the US estimated count matrix of the respective sample (i.e., quant.sf file).
`sample_ids`	a vector of length equals to the number of samples, indicating the names of the respective samples in 'path_to_quant_files'.

Value

A `SummarizedExperiment` object.

Author(s)

Simone Tiberi <simone.tiberi@unibo.it>

See Also

`load_bulk_EC`, `DifferentialRegulation_bulk`

Examples

```
# load internal data to the package:
data_dir = system.file("extdata", package = "DifferentialRegulation")

# specify samples ids:
sample_ids = paste0("sample", seq_len(6))

# US estimates:
quant_files = file.path(data_dir, "salmon", sample_ids, "quant.sf")
file.exists(quant_files)

# load US estimated counts:
SE = load_bulk_US(quant_files,
                  sample_ids)
```

`load_EC` *Create a list containing the equivalence classes objects for the single-cell RNA-seq data*

Description

`load_EC` imports the single-cell equivalence classes (computed by alevin-fry), and stores them into a list.

Usage

```
load_EC(
  path_to_EC_counts,
  path_to_EC,
  path_to_cell_id,
  path_to_gene_id,
  sample_ids
)
```

Arguments

`path_to_EC_counts`
: a vector of length equals to the number of samples: each element indicates the path to the equivalence classes counts of the respective sample (i.e., geqc_counts.mtx file).

`path_to_EC`
: a vector of length equals to the number of samples: each element indicates the path to the equivalence classes of the respective sample (i.e., gene_eqclass.txt.gz file).

`path_to_cell_id`
: a vector of length equals to the number of samples: each element indicates the path to the cell ids of the respective sample (i.e., quants_mat_rows.txt file).

path_to_gene_id
: a vector of length equals to the number of samples: each element indicates the path to the gene ids of the respective sample (i.e., quants_mat_cols.txt file).

sample_ids
: a vector of length equals to the number of samples: each element indicates the name of the sample.

Value

A `list` object.

Author(s)

Simone Tiberi <simone.tiberi@unibo.it>

See Also

load_USA, DifferentialRegulation

Examples

```
# load internal data to the package:
data_dir = system.file("extdata", package = "DifferentialRegulation")

# specify samples ids:
sample_ids = paste0("organoid", c(1:3, 16:18))
# set directories of each sample input data (obtained via alevin-fry):
base_dir = file.path(data_dir, "alevin-fry", sample_ids)
file.exists(base_dir)

# set paths to USA counts, cell id, gene id, EC counts and ECs:
# Note that alevin-fry needs to be run with `--use-mtx` option
# to store counts in a `quants_mat.mtx` file.
path_to_cell_id = file.path(base_dir,"/alevin/quants_mat_rows.txt")
path_to_gene_id = file.path(base_dir,"/alevin/quants_mat_cols.txt")
path_to_EC_counts = file.path(base_dir,"/alevin/geqc_counts.mtx")
path_to_EC = file.path(base_dir,"/alevin/gene_eqclass.txt.gz")

# load EC counts:
EC_list = load_EC(path_to_EC_counts,
                  path_to_EC,
                  path_to_cell_id,
                  path_to_gene_id,
                  sample_ids)
```

load_USA *Creates a SingleCellExperiment containing the estimated USA counts for the single-cell RNA-seq data*

Description

load_USA imports the single-cell estimated USA (Unspliced, Spliced and Ambiguous) counts (computed by alevin-fry), and stores them into a SingleCellExperiment object.

Usage

```
load_USA(path_to_counts, path_to_cell_id, path_to_gene_id, sample_ids)
```

Arguments

path_to_counts	a vector of length equals to the number of samples: each element indicates the path to the USA estimated count matrix of the respective sample (i.e., quants_mat.mtx file).
path_to_cell_id	a vector of length equals to the number of samples: each element indicates the path to the cell ids of the respective sample (i.e., quants_mat_rows.txt file).
path_to_gene_id	a vector of length equals to the number of samples: each element indicates the path to the gene ids of the respective sample (i.e., quants_mat_cols.txt file).
sample_ids	a vector of length equals to the number of samples: each element indicates the name of the sample.

Value

A SingleCellExperiment object.

Author(s)

Simone Tiberi <simone.tiberi@unibo.it>

See Also

load_EC, DifferentialRegulation

Examples

```
# load internal data to the package:
data_dir = system.file("extdata", package = "DifferentialRegulation")

# specify samples ids:
sample_ids = paste0("organoid", c(1:3, 16:18))
# set directories of each sample input data (obtained via alevin-fry):
base_dir = file.path(data_dir, "alevin-fry", sample_ids)
```

```
file.exists(base_dir)

# set paths to USA counts, cell id and gene id:
# Note that alevin-fry needs to be run with `--use-mtx` option
# to store counts in a `quants_mat.mtx` file.
path_to_counts = file.path(base_dir,"/alevin/quants_mat.mtx")
path_to_cell_id = file.path(base_dir,"/alevin/quants_mat_rows.txt")
path_to_gene_id = file.path(base_dir,"/alevin/quants_mat_cols.txt")

# load USA counts:
sce = load_USA(path_to_counts,
               path_to_cell_id,
               path_to_gene_id,
               sample_ids)
```

---

plot_bulk_pi *Plot the estimated proportions of US counts in each group - bulk RNA-seq data*

Description

plot_bulk_pi plots the posterior means of the proportions of US counts, in each group. If 'CI' is TRUE, a profile Wald type confidence interval will also be added; the level of the confidence interval is specified by 'CI_level'.

Usage

```
plot_bulk_pi(results, transcript_id, CI = TRUE, CI_level = 0.95)
```

Arguments

results	a list of data.frame objects, computed via DifferentialRegulation.
transcript_id	a character, indicating the transcript to plot.
CI	a logical ('TRUE' by default), indicating whether to plot a profile Wald type confidence interval around the estimated proportions.
CI_level	a numeric between 0 and 1, indicating the level of the confidence interval.

Value

A ggplot object.

Author(s)

Simone Tiberi <simone.tiberi@unibo.it>

See Also

DifferentialRegulation_bulk

Examples

```
# see the example of DifferentialRegulation_bulk function:
help(DifferentialRegulation_bulk)
```

`plot_bulk_traceplot` *Traceplot of the posterior chain of the proportion (i.e., pi) of unspliced (U) counts in each group - bulk RNA-seq data*

Description

`plot_bulk_traceplot` plots the traceplot of the posterior chain of the proportion (i.e., pi) of unspliced (U) counts in each group. The vertical grey dashed line indicates the burn-in (the iterations on the left side of the burn-in are discarded in posterior analyses).

Usage

```
plot_bulk_traceplot(results, transcript_id)
```

Arguments

`results`	a `list` of `data.frame` objects, computed via `DifferentialRegulation` (single-cell RNA-seq data), or `DifferentialRegulation_bulk` (bulk RNA-seq data).
`transcript_id`	a character, indicating the transcript to plot.

Value

A `gtable` object.

Author(s)

Simone Tiberi <simone.tiberi@unibo.it>

See Also

`DifferentialRegulation_bulk`

Examples

```
# see the example of DifferentialRegulation_bulk function:
help(DifferentialRegulation_bulk)
```

plot_pi *Plot the estimated proportions of US or USA counts in each group - single-cell RNA-seq data*

Description

plot_pi plots the posterior means of the proportions of US (if 'type' = 'US') or USA (if 'type' = 'USA') counts, in each group. If 'CI' is TRUE, a profile Wald type confidence interval will also be added; the level of the confidence interval is specified by 'CI_level'.

Usage

```
plot_pi(results, gene_id, cluster_id, type = "USA", CI = TRUE, CI_level = 0.95)
```

Arguments

results	a list of data.frame objects, computed via DifferentialRegulation.
gene_id	a character, indicating the gene to plot.
cluster_id	a character, indicating the cell cluster to plot.
type	a character (either 'SU' or 'SUA'). If "SU", it plots the proportion of Spliced and Unspliced counts (Ambiguous counts are assigned 50:50 to Spliced and Unspliced counts). If "SUA" (default), it plots the proportion of Spliced, Unspliced and Ambiguous counts (Ambiguous counts are kept separately). Note that, although US reads are easier to interpret, USA reads more closely reflect what is being compared between conditions.
CI	a logical ('TRUE' by default), indicating whether to plot a profile Wald type confidence interval around the estimated proportions.
CI_level	a numeric between 0 and 1, indicating the level of the confidence interval.

Value

A ggplot object.

Author(s)

Simone Tiberi <simone.tiberi@unibo.it>

See Also

DifferentialRegulation

Examples

```
# see the example of DifferentialRegulation function:
help(DifferentialRegulation)
```

plot_traceplot *Traceplot of the posterior chain of the proportion (i.e., pi) of unspliced (U) counts in each group - single-cell RNA-seq data*

Description

plot_traceplot plots the traceplot of the posterior chain of the proportion (i.e., pi) of unspliced (U) counts in each group. The vertical grey dashed line indicates the burn-in (the iterations on the left side of the burn-in are discarded in posterior analyses).

Usage

```
plot_traceplot(results, gene_id, cluster_id)
```

Arguments

results	a list of data.frame objects, computed via DifferentialRegulation (single-cell RNA-seq data), or DifferentialRegulation_bulk (bulk RNA-seq data).
gene_id	a character, indicating the gene to plot.
cluster_id	a character, indicating the cell cluster to plot.

Value

A gtable object.

Author(s)

Simone Tiberi <simone.tiberi@unibo.it>

See Also

DifferentialRegulation

Examples

```
# see the example of DifferentialRegulation function:
help(DifferentialRegulation)
```

Index

∗ **package**
DifferentialRegulation-package, 2

compute_PB_counts, 3, *6, 7*

data.frame, *3*, *9*, *17–20*
DifferentialRegulation, *3, 4*, 5, *15–20*
DifferentialRegulation-package, 2
DifferentialRegulation_bulk, 9, *12, 13*, *17, 18*, *20*

load_bulk_EC, *9*, *11*, 12, *13*
load_bulk_US, *9*, *11, 12*, 13
load_EC, *3, 4*, *7*, 14, *16*
load_USA, *3, 4*, *7*, *15*, 16

plot_bulk_pi, 17
plot_bulk_traceplot, *11*, 18
plot_pi, *4*, *7*, *11*, 19
plot_traceplot, *7*, 20