Package 'rifiComparative'

December 6, 2024

Title 'rifiComparative' compares the output of rifi from two different conditions.

Version 1.6.0

Description 'rifiComparative' is a continuation of rifi package. It compares two conditions output of rifi using half-life and mRNA at time 0 segments. As an input for the segmentation, the difference between half-life of both condtions and log2FC of the mRNA at time 0 are used. The package provides segmentation, statistics, summary table, fragments visualization and some additional useful plots for further analysis.

Depends R (>= 4.2)

Imports cowplot, doMC, parallel, dplyr, egg, foreach, ggplot2, ggrepel, graphics, grDevices, grid, methods, nnet, rlang, S4Vectors, scales, stats, stringr, tibble, rtracklayer, utils, writexl, DTA, LSD, reshape2, devtools, SummarizedExperiment

Suggests DescTools, knitr, rmarkdown, BiocStyle

VignetteBuilder knitr

biocViews RNASeq, DifferentialExpression, GeneRegulation, Transcriptomics, Microarray, Software

BugReports https://github.com/CyanolabFreiburg/rifiComparative

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Encoding UTF-8

Roxygen list(markdown = TRUE)

RoxygenNote 7.2.3

Language en-US

git_url https://git.bioconductor.org/packages/rifiComparative

git_branch RELEASE_3_20

git_last_commit df3f321

git_last_commit_date 2024-10-29

Repository Bioconductor 3.20

Date/Publication 2024-12-05

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 ${\tt adjusting_HLToInt}$

adjusting_HLToInt Creates one table merging HL and intensity fragments with genome annotation

Description

'adjusting_HLToInt' merges HL and intensity segments adapting the positions to each other and combining to the genome annotation. To make HL and intensity segments comparable, log2FC(HL) is used to generate the data frame instead of distance. The fragments should have a significant p_value from t-test at least from one segmentation, either HL or intensity.

Usage

```
adjusting_HLToInt(data, Strand = c("+", "-"), annotation)
```

Arguments

data frame: data frame combined data by column

Strand string: either "+" or "-"

annotation data frame: data frame from processed gff3 file.

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Details

The functions used are:

1. p_value_function extracts and return the p_values of HL and intensity segments respectively.

- 2. eliminate_outlier_hl eliminates outliers from HL fragments.
- 3. eliminate_outlier_int eliminates outliers from intensity fragments.
- $4. \ \ mean_length_int\ calculates\ the\ mean\ of\ the\ log 2FC (intensity)\ fragments\ adapted\ to\ HL_fragments\ and\ their\ lengths$
- 5. mean_length_hl calculates the mean of log2FC(HL) fragments adapted to the intensity fragments and their lengths.
- 6. calculating_rate calculates decay rate and log2FC(intensity). Both are used to calculate synthesis rate.

Value

The data frame with the corresponding columns:

position: Integer, position of the first fragment

region: String, region annotation covering the fragments

gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragments

strand: Boolean. The bin/probe specific strand (+/-)

fragment_HL: String, HL fragments

fragment_int: String, intensity fragments

position_frg_int: Integer, position of the first fragment and the last position of the last fragment

mean_HL_fragment: Integer, mean of the HL of the fragments involved

mean_int_fragment: Integer, mean of the intensity of the fragments involved

log2FC(decay_rate): Integer, log2FC(decay(condition1)/ decay(condition2))

log2FC(synthesis_rate): Integer, sum of log2FC(decay_rate) and log2FC(intensity)

intensity_FC: Integer, log2FC(mean(intensity(condition1))/mean(intensity(condition2)))

 $\textbf{Log2FC(HL)} + \textbf{Log2FC(int):} \ \ Integer, sum of log2FC(decay_rate) \ and \ log2FC(intensity)$

p_value: String, indicated by "*" means at least one fragment either HL fragment or intensity fragment has a significant p_value

Examples

```
data(stats_df_comb_minimal)
data(annot_g)
df_mean_minimal <- adjusting_HLToInt(data = stats_df_comb_minimal,
annotation = annot_g[[1]])</pre>
```

annot_g

The result of gff3_preprocessing of gff3 file A list containing all necessary information from a gff file for adjusting_HLToInt and visualization.

Description

The result of gff3_preprocessing of gff3 file A list containing all necessary information from a gff file for adjusting_HLToInt and visualization.

Usage

data(annot_g)

Format

A list with 2 items:

data annotation: a data frame with 5853 rows and 6 variables

region: the region from the gff file start: the start of the annotation end: the end of the annotation strand: the strand of the annotation gene: the annotated gene name locus_tag: the annotated locus tag

genome length: a numeric vector containing the length of the genome

Source

https://github.com/CyanolabFreiburg/rifiComparative

data_combined_minimal The result of joining_by_row for inp_s and inp_f example data A data frame containing the output of joining_by_row as a data frame

Description

The result of joining_by_row for inp_s and inp_f example data A data frame containing the output of joining_by_row as a data frame

Usage

```
data(data_combined_minimal)
```

Format

A data frame with 600 rows and 49 variables:

strand: The strand specific

position: The bin/probe specific position

ID: The bin/probe specific ID

FLT: The bin/probe flag for background level **intensity:** The relative intensity at time point 0

probe_TI: An internal value to determine which fitting model is applied

flag: Information on which fitting model is applied
position_segment: The position based segment

delay: The delay value of the bin/probe **half_life:** The half-life of the bin/probe

TI_termination_factor: The termination factor of the bin/probe

delay_fragment: The delay fragment the bin belongs to

velocity_fragment: The velocity value of the respective delay fragmentintercept: The vintercept of fit through the respective delay fragmentslope: The slope of the fit through the respective delay fragment

HL_fragment: The half-life fragment the bin belongs to

HL_mean_fragment: The mean half-life value of the respective half-life fragment

intensity_fragment: The intensity fragment the bin belongs to

intensity_mean_fragment: The mean intensity value of the respective intensity fragment

TU: The overarching transcription unit

TI_termination_fragment: The TI fragment the bin belongs to

TI_mean_termination_factor: The mean termination factor of the respective TI fragment

seg_ID: The combined ID of the fragment

pausing_site: presence of pausing site indicated by +/-

iTSS_I: presence of iTSS_I indicated by +/-

ps_ts_fragment: The fragments involved in pausing site or iTSS_I
event_ps_itss_p_value_Ttest: p_value of pausing site or iTSS_I

p_value_slope: p_value of the slope

delay_frg_slope: the slope value of the respective delay fragment **velocity_ratio:** Integer, ratio of velocity between 2 delay fragments **event_duration:** Integer, the duration between two delay fragments

event_position: Integer, the position middle between 2 fragments with an event

FC_fragment_HL: Integer, the fold change value of 2 intensity fragments

FC_HL: Integer, the fold change value of 2 HL fragments#'

p_value_HL: p_value of the fold change of HL fragments

FC_intensity: Integer, the fold change value of 2 intensity fragments

FC_fragment_intensity: String, fragments involved in fold change between 2 intensity fragments

p_value_intensity: p_value of the fold change of intensity fragments

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FC_HL_intensity: ratio of fold change between 2 half-life fragments and fold change between 2 intensity fragments

FC_HL_intensity_fragment: fragments involved on ratio of fold change between 2 half-life fragments and fold change between 2 intensity fragments

FC_HL_adapted: Integer, the fold change of half-life/ fold change of intensity, position of the half-life fragment is adapted to intensity fragment

synthesis_ratio: Integer, the value corresponding to synthesis rate

synthesis_ratio_event: String, the event assigned by synthesis rate either Termination or iTSS

p_value_Manova: p_value of the variance between two fold-changes, HL and intensity

p_value_TI: p_value of TI fragment

TI_fragments_p_value: p_value of 2 TI fragments

cdt: The condition assigned to the experiment here cdt2

logFC_int: The bin/probe log2 fold change of intensity at time 0

P.Value: The bin/probe p_value adjusted

Source

https://github.com/CyanolabFreiburg/rifi

df_comb_minimal

The result of joining_by_column for data_combined_minimal example data A data frame containing the output of joining_by_row as a data frame

Description

The result of joining_by_column for data_combined_minimal example data A data frame containing the output of joining_by_row as a data frame

Usage

data(df_comb_minimal)

Format

A data frame with 300 rows and 18 variables:

strand: The strand specific

position: The bin/probe specific position

ID: The bin/probe specific ID

intensity.cdt1: The relative intensity at time point 0 for condition 1

position_segment: The position based segment

half_life.cdt1: The half-life of the bin/probe condition 1

TI_termination_factor.cdt1: The termination factor of the bin/probe condition 1

HL_fragment.cdt1: The half-life fragment the bin belongs to condition 1

intensity_fragment.cdt1: The intensity fragment the bin belongs to condition 1

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TI_termination_fragment.cdt1: The TI fragment the bin belongs to condition 1

logFC_int: The bin/probe log2 fold change of intensity at time 0

P.Value: The bin/probe p_value adjusted

intensity.cdt2: The relative intensity at time point 0 condition 2

half_life.cdt2: The half-life of the bin/probe condition 2

TI termination factor.cdt2: The termination factor of the bin/probe condition 2

HL_fragment.cdt2: The half-life fragment the bin belongs to condition 2 **intensity_fragment.cdt2:** The intensity fragment the bin belongs to condition 2

TI_termination_fragment.cdt2: The TI fragment the bin belongs to condition 2

Source

https://github.com/CyanolabFreiburg/rifiComparative

df_mean_minimal

The result of adjusting_HLToInt for stats_df_comb_minimal and annotation example data A data frame containing the output of adjusting_HLToInt as a data frame

Description

The result of adjusting_HLToInt for stats_df_comb_minimal and annotation example data A data frame containing the output of adjusting_HLToInt as a data frame

Usage

data(df_mean_minimal)

Format

A data frame with 52 rows and 15 variables:

position: The bin/probe specific positionregion: the region from the gff filegene: the annotated gene namelocus_tag: the annotated locus tag

strand: The strand specific

fragment_HL: The half-life fragment the bin belongs
fragment_int: The intensity fragment the bin belongs

position_frg_int: The position of the first fragment and the last position of the last fragment

mean_HL_fragment: The mean half-life value of the respective half-life fragments mean_int_fragment: The mean intensity value of the respective intensity fragments

log2FC(decay_rate): log2FC(decay(condition1)/decay(condition2))

Log2FC(HL)-Log2FC(int): log2FC(decay_rate/intensity)

log2FC(synthesis_rate): log2FC(decay_rate) + log2FC(intensity)

intensity_FC: log2FC(mean(intensity(condition1))/mean(intensity(condition2)))

p_value: indicated by "*" means at least one fragment either HL fragment or intensity fragment has a significant p_value

figures_fun

Source

https://github.com/CyanolabFreiburg/rifiComparative

differential_expression

An example data frame from Synechosystis PCC 6803 differential probes expression obtained from limma package and only interesting variables were selected. The data frame was used entirely.

Description

An example data frame from Synechosystis PCC 6803 differential probes expression obtained from limma package and only interesting variables were selected. The data frame was used entirely.

Usage

data(differential_expression)

Format

A data frame of differential_expression with 55508 rows and 4 variables:

position: The bin/probe specific position

strand: The strand specific

logFC_int: The bin/probe differential expression

P.Value: The bin/probe p_value adjusted

Source

https://github.com/CyanolabFreiburg/rifiComparative

figures_fun

'figures_fun': generates several plots

Description

'figures_fun' plots at one the density of HL, the HL category as histogram, log2FC of decay and synthesis rate and their heatscatter. Scatter plot of HL and volcano plot. The function uses the four output generated previously.

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Usage

```
figures_fun(
  data.1,
  data.2,
  input.1,
  input.2,
  cdt1,
  cdt2,
  y = 30,
  x = 30,
  limits = c(0, 20)
)
```

Arguments

data.1	data frame output of statistic
data.2	data frame joining two outputs from rifi_stats by row
input.1	data frame joining two outputs from rifi_stats by column
input.2	data frame of differential gene expression at time 0
cdt1	string for the first condition
cdt2	string for the second condition
У	integer to break the scaling in scatter plot for y_axis
x	integer to break the scaling in scatter plot for x_axis
limits	vector to limit the scaling in scatter plot for both axis

Details

The functions used are:

plot_decay_synt: plots the changes in RNA decay rates versus the changes in RNA synthesis rates plot_heatscatter: plots the changes in RNA decay rates versus the changes in RNA synthesis rates with density.

plot_volcano: plots statistical significance versus magnitude of change .

plot_histogram: plot a histogram of probe/bin half-life categories from 2 to 20 minutes in both conditions.

plot_density: plots the probe/bin half-life density in both conditions.

plot_scatter: plots of the bin/probe half-life in one condition1 vs. condition2.

extract the object of rifi_statistics from both conditions. The differential gene expression at time 0 needs to be run separately. The columns log2FC, p_value adjusted, position and strand are extracted and saved to a data frame. loading_fun_fig joins the differential gene expression table and the output from rifi statistics into one data frame.

Value

several plots

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Examples

```
data(data_combined_minimal)
data(df_comb_minimal)
data(differential_expression)
data(df_mean_minimal)
figures_fun(data.1 = df_mean_minimal, data.2 = data_combined_minimal,
input.1 = df_comb_minimal, input.2 = differential_expression, cdt1 = "sc",
cdt2 = "fe")
```

fragmentation

fragmentation: Conveniently wraps all fragmentation steps

Description

fragmentation fragments the half-life and intensity into segments using the penalties output.

Usage

```
fragmentation(data, pen_HL, pen_int, cores = 2)
```

Arguments

data frame: data frame combined data by column

pen_HL list: list of the penalties set optimal for the fragmentation for half-life

pen_int list: list of the penalties set optimal for the fragmentation for intensity

cores integer: the number of assigned cores for the task. It needs to be increased in

case of big data.

Value

Two data frames with half-life and intensity fragments and the mean of the coefficient fragment based.

Examples

```
data(penalties_df)
data(pen_HL)
data(pen_int)
df_comb_minimal <- fragmentation(data = penalties_df, pen_HL,
pen_int)</pre>
```

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fragment_int	The result of fragmentation for df_comb_minimal example data A data
	frame containing the output of fragmentation as a data frame

Description

The result of fragmentation for df_comb_minimal example data A data frame containing the output of fragmentation as a data frame

Usage

```
data(fragment_int)
```

Format

A data frame with 500 rows and 24 variables:

strand: The strand specific

position: The bin/probe specific position

ID: The bin/probe specific ID

intensity.cdt1: The relative intensity at time point 0 for condition 1

position segment: The position based segment

half life.cdt1: The half-life of the bin/probe condition 1

TI_termination_factor.cdt1: The termination factor of the bin/probe condition 1

HL_fragment.cdt1: The half-life fragment the bin belongs to condition 1

intensity_fragment.cdt1: The intensity fragment the bin belongs to condition 1TI_termination_fragment.cdt1: The TI fragment the bin belongs to condition 1

logFC_int: The bin/probe log2 fold change of intensity at time 0

P.Value: The bin/probe p_value adjusted

intensity.cdt2: The relative intensity at time point 0 condition 2

half_life.cdt2: The half-life of the bin/probe condition 2

TI_termination_factor.cdt2: The termination factor of the bin/probe condition 2

HL_fragment.cdt2: The half-life fragment the bin belongs to condition 2

intensity_fragment.cdt2: The intensity fragment the bin belongs to condition 2

TI_termination_fragment.cdt2: The TI fragment the bin belongs to condition 2

distance HL: The bin/probe difference of half-life from both conditions

distance_int: The bin/probe log2 fold change of intensity at time 0

HL_comb_fragment: The half-life fragment the bin belongs to both conditions

HL_mean_comb_fragment: The half-life mean of the fragment the bin belongs to both conditions

intensity_comb_fragment: The intensity fragment the bin belongs to both conditions

intensity_mean_comb_fragment: The intensity mean of the fragment the bin belongs to both conditions

Source

https://github.com/CyanolabFreiburg/rifiComparative

gff3_preprocess

gff3_preprocess processes gff3 file from database

Description

gff3_preprocess processes the gff3 file extracting gene names and locus_tag from all coding regions (CDS). UTRs/ncRNA/asRNA if available, are also extracted. The resulting dataframe contains region, positions, strand, gene and locus_tag.

Usage

```
gff3_preprocess(path)
```

Arguments

path

path: path to the directory containing the gff3 file.

Value

A list with 2 items:

data annotation: region: String, the region from the gff file

start: Integer, the start of the annotation **end:** Integer, the end of the annotation

strand: Boolean, the strand of the annotation **gene:** String, the annotated gene name **locus_tag:** String, the annotated locus tag

genome length: a numeric vector containing the length of the genome

Examples

```
gff3_preprocess(
path = gzfile(system.file("extdata", "gff_synechocystis_6803.gff.gz",
package = "rifiComparative"))
)
```

inp_f

The result of loading_fun for stats_se_cdt2 example data Two data frame containing the output of loading_fun as second element of a list.

Description

The result of loading_fun for stats_se_cdt2 example data Two data frame containing the output of loading_fun as second element of a list.

Usage

```
data(inp_f)
```

 $inp_{_}f$

Format

A data frame with 500 rows and 49 variables:

strand: The strand specific

position: The bin/probe specific position

ID: The bin/probe specific ID

FLT: The bin/probe flag for background level **intensity:** The relative intensity at time point 0

probe_TI: An internal value to determine which fitting model is applied

flag: Information on which fitting model is applied
position_segment: The position based segment

delay: The delay value of the bin/probe **half_life:** The half-life of the bin/probe

TI_termination_factor: The termination factor of the bin/probe

delay_fragment: The delay fragment the bin belongs to

velocity_fragment: The velocity value of the respective delay fragmentintercept: The vintercept of fit through the respective delay fragmentslope: The slope of the fit through the respective delay fragment

HL_fragment: The half-life fragment the bin belongs to

HL_mean_fragment: The mean half-life value of the respective half-life fragment

intensity_fragment: The intensity fragment the bin belongs to

intensity_mean_fragment: The mean intensity value of the respective intensity fragment

TU: The overarching transcription unit

TI_termination_fragment: The TI fragment the bin belongs to

TI_mean_termination_factor: The mean termination factor of the respective TI fragment

seg_ID: The combined ID of the fragment

pausing_site: presence of pausing site indicated by +/-

iTSS_I: presence of iTSS_I indicated by +/-

ps_ts_fragment: The fragments involved in pausing site or iTSS_I
event_ps_itss_p_value_Ttest: p_value of pausing site or iTSS_I

p_value_slope: p_value of the slope

delay_frg_slope: the slope value of the respective delay fragment **velocity_ratio:** Integer, ratio of velocity between 2 delay fragments **event_duration:** Integer, the duration between two delay fragments

event position: Integer, the position middle between 2 fragments with an event

FC_HL: Integer, the fold change value of 2 HL fragments

FC_fragment_HL: Integer, the fold change value of 2 intensity fragments

 $\boldsymbol{p}_\boldsymbol{value}_\boldsymbol{HL}\boldsymbol{:}\;p_\boldsymbol{value}$ of the fold change of HL fragments

FC_intensity: Integer, the fold change value of 2 intensity fragments

FC_fragment_intensity: String, fragments involved in fold change between 2 intensity fragments

p_value_intensity: p_value of the fold change of intensity fragments

inp_s

FC_HL_intensity: ratio of fold change between 2 half-life fragments and fold change between 2 intensity fragments

FC_HL_intensity_fragment: fragments involved on ratio of fold change between 2 half-life fragments and fold change between 2 intensity fragments

FC_HL_adapted: Integer, the fold change of half-life/ fold change of intensity, position of the half-life fragment is adapted to intensity fragment

synthesis_ratio: Integer, the value corresponding to synthesis rate

synthesis_ratio_event: String, the event assigned by synthesis rate either Termination or iTSS

p_value_Manova: p_value of the variance between two fold-changes, HL and intensity

p_value_TI: p_value of TI fragment

TI_fragments_p_value: p_value of 2 TI fragments

cdt: The condition assigned to the experiment here cdt2

logFC_int: The bin/probe log2 fold change of intensity at time 0

P.Value: The bin/probe p_value adjusted

Source

https://github.com/CyanolabFreiburg/rifiComparative

inp_s

The result of loading_fun for stats_se_cdt1 example data Two data frame containing the output of loading_fun as first element of a list.

Description

The result of loading_fun for stats_se_cdt1 example data Two data frame containing the output of loading_fun as first element of a list.

Usage

data(inp_s)

Format

A data frame with 500 rows and 49 variables:

strand: The strand specific

position: The bin/probe specific position

ID: The bin/probe specific ID

FLT: The bin/probe flag for background level **intensity:** The relative intensity at time point 0

probe_TI: An internal value to determine which fitting model is applied

flag: Information on which fitting model is applied **position_segment:** The position based segment

delay: The delay value of the bin/probe **half_life:** The half-life of the bin/probe

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TI_termination_factor: The termination factor of the bin/probe

delay_fragment: The delay fragment the bin belongs to

velocity_fragment: The velocity value of the respective delay fragment **intercept:** The vintercept of fit through the respective delay fragment **slope:** The slope of the fit through the respective delay fragment

HL_fragment: The half-life fragment the bin belongs to

HL_mean_fragment: The mean half-life value of the respective half-life fragment

intensity_fragment: The intensity fragment the bin belongs to

intensity_mean_fragment: The mean intensity value of the respective intensity fragment

TU: The overarching transcription unit

TI_termination_fragment: The TI fragment the bin belongs to

TI_mean_termination_factor: The mean termination factor of the respective TI fragment

seg_ID: The combined ID of the fragment

pausing_site: presence of pausing site indicated by +/-

iTSS_I: presence of iTSS_I indicated by +/-

ps_ts_fragment: The fragments involved in pausing site or iTSS_I **event_ps_itss_p_value_Ttest:** p_value of pausing site or iTSS_I

p_value_slope: p_value of the slope

delay_frg_slope: the slope value of the respective delay fragment **velocity_ratio:** Integer, ratio of velocity between 2 delay fragments **event_duration:** Integer, the duration between two delay fragments

event_position: Integer, the position middle between 2 fragments with an event

FC_HL: Integer, the fold change value of 2 HL fragments

FC fragment HL: Integer, the fold change value of 2 intensity fragments

p_value_HL: p_value of the fold change of HL fragments

FC intensity: Integer, the fold change value of 2 intensity fragments

FC_fragment_intensity: String, fragments involved in fold change between 2 intensity fragments

p_value_intensity: p_value of the fold change of intensity fragments

FC_HL_intensity: ratio of fold change between 2 half-life fragments and fold change between 2 intensity fragments

FC_HL_intensity_fragment: fragments involved on ratio of fold change between 2 half-life fragments and fold change between 2 intensity fragments

FC_HL_adapted: Integer, the fold change of half-life/ fold change of intensity, position of the half-life fragment is adapted to intensity fragment

synthesis_ratio: Integer, the value corresponding to synthesis rate

synthesis_ratio_event: String, the event assigned by synthesis rate either Termination or iTSS

p_value_Manova: p_value of the variance between two fold-changes, HL and intensity

p_value_TI: p_value of TI fragment

TI_fragments_p_value: p_value of 2 TI fragments **cdt:** The condition assigned to the experiment here cdt1

logFC_int: The bin/probe log2 fold change of intensity at time 0

P.Value: The bin/probe p value adjusted

Source

https://github.com/CyanolabFreiburg/rifiComparative

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joining_data_column joining_data_column joins two data frames by column

Description

'joining_data_column': joins two data frames from different conditions by column.

Usage

```
joining_data_column(data)
```

Arguments

data

data frame with joined columns from both conditions

Value

The data frame with joined columns from both conditions with the corresponding columns: strand, position, ID, intensity.cdt1, position_segment, half_life.cdt1, TI_termination_factor.cdt1", HL_fragment.cdt1, intensity_fragment.cdt1, TI_termination_fragment.cdt1, logFC_int, P.Value, intensity.cdt2, half_life.cdt2, TI_termination_factor.cdt2, HL_fragment.cdt2, intensity_fragment.cdt2, TI_termination_fragment.cdt2.

cdt1: first condition, cdt2: second condition.

Examples

```
data(data_combined_minimal)
df_comb_minimal <- joining_data_column(data = data_combined_minimal)</pre>
```

joining_data_row

joining_data_row joins two data frames by row

Description

joining_data_row joins two data frames from different conditions by row.

Usage

```
joining_data_row(input1, input2)
```

Arguments

input1 data frame from SummarizedExperiment output of rifi_stats from rifi package

of the first condition.

input2 data frame from SummarizedExperiment output of rifi_stats from rifi package

of the second condition.

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Value

The data frame with joined rows from both conditions with the corresponding columns: ID with position, strand, intensity, probe_TI, flag, position_segment, delay, half_life, TI_termination_factor, delay_fragment, velocity_fragment, intercept, slope, HL_fragment, HL_mean_fragment, intensity_fragment, intensity_mean_fragment, TU, TI_termination_fragment, TI_mean_termination_factor, seg_ID, pausing_site, iTSS_I, ps_ts_fragment, event_ps_itss_p_value_Ttest, p_value_slope, delay_frg_slope, velocity_ratio, event_duration, event_position, FC_HL, FC_fragment_HL, p_value_HL, FC_intensity, FC_fragment_intensity, p_value_intensity, FC_HL_intensity, FC_HL_intensity_fragment, FC_HL_adapted, synthesis_ratio, synthesis_ratio_event, p_value_Manova, p_value_TI, cdt (condition), logFC_int (log2FC(intensity)), P.Value of log2FC(intensity)

Examples

```
data(inp_s)
data(inp_f)
data_combined_minimal <-
joining_data_row(input1 = inp_s, input2 = inp_f)</pre>
```

loading_fun

loading_fun loads the data of all conditions

Description

loading_fun extract outputs from rifi_stats of all conditions and join each data to the differential expression table. The differential gene expression at time 0 needs to be run separately. The columns log2FC, p_value adjusted, position and strand are extracted and saved to a data frame. loading_fun joins the differential gene expression table and the output from rifi statistics into one data frame.

Usage

```
loading_fun(data1, data2, data3)
```

Arguments

data1	data frame from rifi_stats of one condition
data2	data frame from rifi_stats of other condition
data3	data frame from differential gene expression at time 0

Value

A list of two data frames with joined columns from differential expression and output of rifi_stats with the corresponding columns: ID with position, strand, intensity, probe_TI, flag, position_segment, delay, half_life, TI_termination_factor, delay_fragment, velocity_fragment, intercept, slope, HL_fragment, HL_mean_fragment, intensity_fragment, intensity_mean_fragment, TU, TI_termination_fragment, TI_mean_termination_factor, seg_ID, pausing_site, iTSS_I, ps_ts_fragment, event_ps_itss_p_value_Ttest, p_value_slope, delay_frg_slope, velocity_ratio, event_duration, event_position, FC_HL, FC_fragment_HL, p_value_HL, FC_intensity, FC_fragment_intensity, p_value_intensity, FC_HL_intensity, FC_HL_intensity_fragment, FC_HL_adapted, synthesis_ratio, synthesis_ratio_event, p_value_Manova, p_value_TI, cdt (condition), logFC_int (log2FC(intensity)), P.Value of log2FC(intensity).

18 make_pen

Examples

```
data(stats_se_cdt1)
data(stats_se_cdt2)
data(differential_expression)
inp_s <-
loading_fun(stats_se_cdt1, stats_se_cdt2, differential_expression)[[1]]
inp_f <-
loading_fun(stats_se_cdt1, stats_se_cdt2, differential_expression)[[2]]</pre>
```

make_pen

make_pen assigns automatically penalties

Description

make_pen calls one of four available penalty functions to automatically assign penalties for the dynamic programming. The two functions to be called are:

- 1. fragment_HL_pen
- 2. fragment_inty_pen

Usage

```
make_pen(
   probe,
   FUN,
   cores = 1,
   logs,
   dpt = 1,
   smpl_min = 10,
   smpl_max = 100,
   sta_pen = 0.5,
   end_pen = 4.5,
   rez_pen = 9,
   sta_pen_out = 0.5,
   end_pen_out = 3.5,
   rez_pen_out = 7
```

Arguments

probe	data frame: data frame combined data by column
FUN	function: one of the four bottom level functions (see details)
cores	integer: the number of assigned cores for the task
logs	numeric vector: the logbook vector.
dpt	integer: the number of times a full iteration cycle is repeated with a more narrow range based on the previous cycle.
smpl_min	integer: the smaller end of the sampling size.
smpl_max	integer: the larger end of the sampling size.

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```
sta_pen numeric: the lower starting penalty.

rez_pen numeric: the higher starting penalty.

rez_pen numeric: the number of penalties iterated within the penalty range.

sta_pen_out numeric: the lower starting outlier penalty.

end_pen_out numeric: the higher starting outlier penalty.

rez_pen_out numeric: the number of outlier penalties iterated within the outlier penalty range.
```

Details

The two functions called return the amount of statistically correct and statistically wrong splits at a specific pair of penalties. 'make_pen' iterates over many penalty pairs and picks the most suitable pair based on the difference between wrong and correct splits. The sample size, penalty range and resolution as well as the number of cycles can be customized. The primary start parameters create a matrix with n = rez_pen rows and n = rez_pen_out columns with values between sta_pen/sta_pen_out and end_pen/end_pen_out. The best penalty pair is picked. If dept is bigger than 1 the same process is repeated with a new matrix of the same size based on the result of the previous cycle. Only position segments with length within the sample size range are considered for the penalties to increase run time.

Value

A list with 4 items:

logbook: The logbook vector containing all penalty informationpenalties: a vector with the respective penalty and outlier penaltycorrect: a matrix of the correct splitswrong: a matrix of the incorrect splits

Examples

```
data(df_comb_minimal)

df_comb_minimal$distance_HL <- df_comb_minimal$half_life.cdt1 -

df_comb_minimal$half_life.cdt2

df_comb_minimal$distance_int <- df_comb_minimal$logFC_int

pen_HL <- make_pen(
    probe = df_comb_minimal, FUN = rifiComparative:::fragment_HL_pen,
    cores = 2, logs = as.numeric(rep(NA, 8)), dpt = 1, smpl_min = 10,
    smpl_max = 50, sta_pen = 0.5, end_pen = 4.5, rez_pen = 9, sta_pen_out = 0.5,
    end_pen_out = 3.5, rez_pen_out = 7
)

pen_int <- make_pen(
    probe = df_comb_minimal, FUN = rifiComparative:::fragment_inty_pen,
    cores = 2, logs = as.numeric(rep(NA, 8)), dpt = 1, smpl_min = 10,
    smpl_max = 50, sta_pen = 0.5, end_pen = 4.5, rez_pen = 9, sta_pen_out = 0.5,
    end_pen_out = 3.5, rez_pen_out = 7
)</pre>
```

20 penalties

penalties	penalties wraps conveniently all penalty steps	
-----------	--	--

Description

penalties finds the best set of penalties for half-life and intensity fragmentation using dynamic programming. The segmentation of the HL uses the difference between 2 conditions and the segmentation of the intensity uses the log2FC.

Usage

```
penalties(data, cores = 2)
```

Arguments

data data frame with the joined columns from differential expression and output of

rifi_stats.

cores integer: the number of assigned cores for the task. It needs to be increased in

case of big data.

Details

```
The function uses 4 functions: score_fun_ave.r make_pen.r fragment_HL_pen.r fragment_inty_pen.r
```

Value

A list of data frame and penalties, The first element is data frame with 2 more variables, second and third are HL and intensity penalties respectively.

Examples

```
data(df_comb_minimal)
penalties_df <- penalties(df_comb_minimal)[[1]]
pen_HL <- penalties(df_comb_minimal)[[2]]
pen_int <- penalties(df_comb_minimal)[[3]]</pre>
```

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	The result of penalties for df_comb_minimal example data A data frame containing the output of penalties as a data frame
--	--

Description

The result of penalties for df_comb_minimal example data A data frame containing the output of penalties as a data frame

Usage

```
data(penalties_df)
```

Format

A data frame with 300 rows and 20 variables:

strand: The strand specific

position: The bin/probe specific position

ID: The bin/probe specific ID

intensity.cdt1: The relative intensity at time point 0 for condition 1

position_segment: The position based segment

half_life.cdt1: The half-life of the bin/probe condition 1

TI_termination_factor.cdt1: The termination factor of the bin/probe condition 1

HL_fragment.cdt1: The half-life fragment the bin belongs to condition 1

intensity_fragment.cdt1: The intensity fragment the bin belongs to condition 1TI_termination_fragment.cdt1: The TI fragment the bin belongs to condition 1

logFC_int: The bin/probe log2 fold change of intensity at time 0

P.Value: The bin/probe p_value adjusted

intensity.cdt2: The relative intensity at time point 0 condition 2

half_life.cdt2: The half-life of the bin/probe condition 2

TI_termination_factor.cdt2: The termination factor of the bin/probe condition 2

HL_fragment.cdt2: The half-life fragment the bin belongs to condition 2

intensity_fragment.cdt2: The intensity fragment the bin belongs to condition 2TI_termination_fragment.cdt2: The TI fragment the bin belongs to condition 2

distance_HL: The bin/probe difference of half-life from both conditions

distance_int: The bin/probe log2 fold change of intensity at time 0

Source

https://github.com/CyanolabFreiburg/rifiComparative

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pen_HL	The result of penalties for df_comb_minimal example data. A list
	containing the output from penalties including the logbook and two penalty objects.

Description

The result of penalties for df_comb_minimal example data. A list containing the output from penalties including the logbook and two penalty objects.

Usage

```
data(pen_HL)
```

Format

A list with 5 items:

pen_obj_HL: A list with 4 items:

logbook: The logbook vector containing half-life penalty information **HL_penalties:** a vetor with the half-life penalty and half-life outlier penalty

correct: a matrix of the correct splits **wrong:** a matrix of the incorrect splits

Source

https://github.com/CyanolabFreiburg/rifi

pen_int The result of penalties for df_comb_minimal example data. A list containing the output from penalties including the logbook and two penalty objects.

Description

The result of penalties for df_comb_minimal example data. A list containing the output from penalties including the logbook and two penalty objects.

Usage

```
data(pen_int)
```

Format

A list with 5 items:

pen_int: A list with 4 items:

logbook: The logbook vector containing intensity penalty informationint_penalties: a vector with the intensity penalty and intensity outlier penalty

correct: a matrix of the correct splits **wrong:** a matrix of the incorrect splits

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Source

https://github.com/CyanolabFreiburg/rifi

rifiComparative

%

rifiComparative

rifiComparative a successor package of rifi. It compares 2 rifi outputs from 2 different conditions of the same organism.

Description

rifiComparative was developed to compare 2 rifi outputs from 2 conditions. The rifi output may differ significantly from 2 conditions. The complexity of the segments number, position, length and the events make the comparison between 2 conditions nearly impossible. rifiComparative uses a simple strategy to generate single segments for both conditions, extract the features and make them comparable.

Details

Five major steps ate described in rifiComparative:

- 1. Joining data
- 2. penalties
- 3. fragmentation
- 4. statistics
- 5. visualization

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rifi_visualization_comparison

rifi_visualization_comparison plots the segments and genome annotation

Description

rifi_visualization_comparison plots the genome annotation, half-life difference (HL), log2FC(intensity) fragments. It uses several functions to plot TUs and genes including small-RNAs. Additionally it plots the statistical t-test between the neighboring fragment, significant p-values from t-test are assigned with '*'.

Usage

```
rifi_visualization_comparison(
 data,
  data_c,
  genomeLength = annot_g[[2]],
 annot = annot_g[[1]],
  condition = c("cdt1", "cdt2"),
  Strand = c("+", "-"),
  region = c("CDS", "asRNA", "5'UTR", "ncRNA", "3'UTR", "tRNA"),
 color_region = c("grey0", "red", "blue", "orange", "yellow", "green", "white",
    "darkseagreen1", "grey50", "black"),
  color_TU = c("cyan", "yellow", "orange"),
  scaling_TU = c(0, 3.4, 6.6),
  color_text.1 = "grey0"
  color_text.2 = "black",
 Alpha = 0.5,
  size_tu = 1.6,
  size_locusTag = 1.6,
  size\_gene = 1.6,
 Limit = 10,
  shape = 22,
  face = "bold"
  tick_length = 0.3,
  arrow.color = "darkseagreen1",
  col_above20 = "#00FFFF",
  fontface = "plain",
  shape_above20 = 14,
 axis_text_y_size = 3,
 axis_title_y_size = 6,
  iTSS_threshold = 1.2,
 p_value_manova = 0.05,
  termination_threshold = 0.8
)
```

Arguments

color_text.2

string: genes color text

```
dataframe: the probe based dataframe with joined columns.
data
                  dataframe: the probe based dataframe with joined rows.
data_c
                  integer: genome length output of gff3_preprocess function.
genomeLength
annot
                  dataframe: the annotation file.
                  string: assigned as cdt1 (condition 1) and cdt2 (condition2), it could be adapted
condition
                  to any name.
Strand
                  string: either ("+" or "-").
                  dataframe: gff3 features of the genome.
region
color_region
                  string vector: vector of colors.
color_TU
                  string. TU color
scaling_TU
                  vector: values to adjusted termination and iTSSs to TUs.
color_text.1
                  string: TU color text
```

Alpha integer: color transparency degree.

size_tu integer: TU size

size_locusTag integer: locus_tag size

size_gene integer: font size for gene annotation.

Limit integer: value for y-axis limit.

shape integer: value for shape.

face string: label font.

tick_length integer: value for ticks.
arrow.color string: arrows color.

col_above20 string: color for probes/bin above value 20.

fontface integer: font type

shape_above20 integer: shape for probes/bins above value 20.

axis_text_y_size

integer: text size for y-axis.

axis_title_y_size

integer: title size for y-axis.

iTSS_threshold integer: threshold for iTSS_II selected to plot, default 1.2.

p_value_manova integer: p value of manova test fragments to plot, default 0.05.

termination_threshold

integer: threshold for termination to plot, default .8.

Details

The functions used are:

strand_selection: plots HL, intensity fragments from both strands.

splitGenome_function: splits the genome into fragments. annotation_plot_comp: plots the corresponding annotation.

indice_function: assign a new column to the data to distinguish between fragments, outliers from delay or HL or intensity.

empty_data_positive: plots empty boxes in case no data is available for positive strand empty_data_negative: plots empty boxes in case no data is available for negative strand

TU annotation: designs the segments border for the genes and TUs annotation.

gene_annot_function: it requires gff3 file, returns a dataframe adjusting each fragment according to its annotation. It allows as well the plot of genes and TUs shared into two pages.

secondaryAxis: adjusts the half-life or delay to 20 in case of the dataframe row numbers is equal to 1 and the half-life or delay exceed the limit, they are plotted with different shape and color.

my_arrow: creates an arrow for the annotation.

arrange_byGroup: selects the last row for each segment and add 40 nucleotides in case of negative strand for a nice plot.

my_segment_T: plots terminals and pausing sites labels.

Value

The plot.

Examples

```
data(data_combined_minimal)
data(stats_df_comb_minimal)
data(annot_g)
rifi_visualization_comparison(
    data = data_combined_minimal,
    data_c = stats_df_comb_minimal,
    genomeLength = annot_g[[2]],
    annot = annot_g[[1]]
)
```

statistics

statistics check segments significance using statistical test

Description

statistics uses t-test to check HL and intensity segments significance. The function returns the data frame with p_value and p_value adjusted. The function used is t_test_function.

Usage

```
statistics(data)
```

Arguments

data

data frame: data frame output of fragmentation

Value

A list of two data frames, the first one contains all segments with p_value and p_value adjusted. The second one removes the duplicated segments from intensity and could be saved as an excel file.

Examples

```
data(fragment_int)
stats_df_comb_minimal <- statistics(data= fragment_int)[[1]]
df_comb_uniq_minimal <- statistics(data= fragment_int)[[2]]</pre>
```

stats_df_comb_minimal The result of statistics for fragment_int example data A data frame containing the output of statistics as a data frame

Description

The result of statistics for fragment_int example data A data frame containing the output of statistics as a data frame

stats_df_comb_minimal 27

Usage

```
data(stats_df_comb_minimal)
```

Format

A data frame with 500 rows and 26 variables:

strand: The strand specific

position: The bin/probe specific position

ID: The bin/probe specific ID

intensity.cdt1: The relative intensity at time point 0 for condition 1

position_segment: The position based segment

half life.cdt1: The half-life of the bin/probe condition 1

TI_termination_factor.cdt1: The termination factor of the bin/probe condition 1

HL fragment.cdt1: The half-life fragment the bin belongs to condition 1

intensity_fragment.cdt1: The intensity fragment the bin belongs to condition 1TI termination fragment.cdt1: The TI fragment the bin belongs to condition 1

logFC_int: The bin/probe log2 fold change of intensity at time 0

P.Value: The bin/probe p value adjusted

intensity.cdt2: The relative intensity at time point 0 condition 2

half_life.cdt2: The half-life of the bin/probe condition 2

TI_termination_factor.cdt2: The termination factor of the bin/probe condition 2

HL_fragment.cdt2: The half-life fragment the bin belongs to condition 2

intensity_fragment.cdt2: The intensity fragment the bin belongs to condition 2

TI_termination_fragment.cdt2: The TI fragment the bin belongs to condition 2

distance HL: The bin/probe difference of half-life from both conditions

distance_int: The bin/probe log2 fold change of intensity at time 0

HL_comb_fragment: The half-life fragment the bin belongs to both conditions

HL_mean_comb_fragment: The half-life mean of the fragment the bin belongs to both conditions

intensity_comb_fragment: The intensity fragment the bin belongs to both conditions

intensity_mean_comb_fragment: The intensity mean of the fragment the bin belongs to both conditions

p_value_distance_HL: The p_value adjusted of the half-life fragment the bin belongs to both conditions

p_value_distance_intensity: The p_value adjusted of the intensity fragment the bin belongs to both conditions

Source

https://github.com/CyanolabFreiburg/rifiComparative

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stats_se_cdt1 An example SummarizedExperiment from Synechosystis PCC 6803 first condition obtained from rifi_statistics and used as input for rifiComparative

Description

An example SummarizedExperiment from Synechosystis PCC 6803 first condition obtained from rifi_statistics and used as input for rifiComparative

Usage

```
data(stats_se_cdt1)
```

Format

A rowRanges of SummarizedExperiment with 500 rows and 50 variables:

seqnames: The sequence name chromosome

start: The bin/probe start positionend: The bin/probe end positionwidth: The bin/probe lengthstrand: The strand specific

position: The bin/probe specific position

ID: The bin/probe specific ID

FLT: The bin/probe flag for background level **intensity:** The relative intensity at time point 0

probe_TI: An internal value to determine which fitting model is applied

flag: Information on which fitting model is applied **position_segment:** The position based segment

delay: The delay value of the bin/probe **half_life:** The half-life of the bin/probe

TI_termination_factor: The termination factor of the bin/probe

delay_fragment: The delay fragment the bin belongs to

velocity_fragment: The velocity value of the respective delay fragmentintercept: The vintercept of fit through the respective delay fragmentslope: The slope of the fit through the respective delay fragment

HL_fragment: The half-life fragment the bin belongs to

HL_mean_fragment: The mean half-life value of the respective half-life fragment

intensity_fragment: The intensity fragment the bin belongs to

intensity_mean_fragment: The mean intensity value of the respective intensity fragment

TU: The overarching transcription unit

TI_termination_fragment: The TI fragment the bin belongs to

TI_mean_termination_factor: The mean termination factor of the respective TI fragment

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seg_ID: The combined ID of the fragment

pausing_site: presence of pausing site indicated by +/-

iTSS_I: presence of iTSS_I indicated by +/-

ps_ts_fragment: The fragments involved in pausing site or iTSS_I **event_ps_itss_p_value_Ttest:** p_value of pausing site or iTSS_I#' **delay frg slope:** the slope value of the respective delay fragment

p_value_slope: p_value of the slope

velocity_ratio: Integer, ratio of velocity between 2 delay fragments **event_duration:** Integer, the duration between two delay fragments

event_position: Integer, the position middle between 2 fragments with an event

FC_HL: Integer, the fold change value of 2 HL fragments

FC_fragment_HL: Integer, the fold change value of 2 intensity fragments

p value HL: p value of the fold change of HL fragments

FC_intensity: Integer, the fold change value of 2 intensity fragments

FC_fragment_intensity: String, fragments involved in fold change between 2 intensity fragments

p_value_intensity: p_value of the fold change of intensity fragments

FC_HL_intensity: ratio of fold change between 2 half-life fragments and fold change between 2 intensity fragments

FC_HL_intensity_fragment: fragments involved on ratio of fold change between 2 half-life fragments and fold change between 2 intensity fragments

FC_HL_adapted: Integer, the fold change of half-life/ fold change of intensity, position of the half-life fragment is adapted to intensity fragment

synthesis ratio: Integer, the value corresponding to synthesis rate

synthesis_ratio_event: String, the event assigned by synthesis rate either Termination or iTSS

p_value_Manova: p_value of the variance between two fold-changes, HL and intensity

p_value_TI: p_value of TI fragment

TI_fragments_p_value: p_value of 2 TI fragments

Source

https://github.com/CyanolabFreiburg/rifiComparative

stats_se_cdt2

An example SummarizedExperiment from Synechosystis PCC 6803 second condition obtained from rifi_statistics and used as input for rifiComparative

Description

An example SummarizedExperiment from Synechosystis PCC 6803 second condition obtained from rifi_statistics and used as input for rifiComparative

Usage

data(stats_se_cdt2)

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Format

A rowRanges of SummarizedExperiment with 500 rows and 50 variables:

seqnames: The sequence name chromosome

start: The bin/probe start positionend: The bin/probe end positionwidth: The bin/probe lengthstrand: The strand specific

position: The bin/probe specific position

ID: The bin/probe specific ID

FLT: The bin/probe flag for background level **intensity:** The relative intensity at time point 0

probe_TI: An internal value to determine which fitting model is applied

flag: Information on which fitting model is applied
position_segment: The position based segment

delay: The delay value of the bin/probe **half_life:** The half-life of the bin/probe

TI_termination_factor: The termination factor of the bin/probe

delay_fragment: The delay fragment the bin belongs to

velocity_fragment: The velocity value of the respective delay fragmentintercept: The vintercept of fit through the respective delay fragmentslope: The slope of the fit through the respective delay fragment

HL_fragment: The half-life fragment the bin belongs to

HL_mean_fragment: The mean half-life value of the respective half-life fragment

intensity_fragment: The intensity fragment the bin belongs to

intensity_mean_fragment: The mean intensity value of the respective intensity fragment

TU: The overarching transcription unit

TI_termination_fragment: The TI fragment the bin belongs to

TI_mean_termination_factor: The mean termination factor of the respective TI fragment

 $\mathbf{seg_ID}$: The combined ID of the fragment

pausing_site: presence of pausing site indicated by +/-

iTSS_I: presence of iTSS_I indicated by +/-

ps_ts_fragment: The fragments involved in pausing site or iTSS_I
event_ps_itss_p_value_Ttest: p_value of pausing site or iTSS_I

p_value_slope: p_value of the slope

delay_frg_slope: the slope value of the respective delay fragment **velocity_ratio:** Integer, ratio of velocity between 2 delay fragments **event_duration:** Integer, the duration between two delay fragments

event_position: Integer, the position middle between 2 fragments with an event

FC_HL: Integer, the fold change value of 2 HL fragments

FC_fragment_HL: Integer, the fold change value of 2 intensity fragments

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p_value_HL: p_value of the fold change of HL fragments

FC_intensity: Integer, the fold change value of 2 intensity fragments

FC_fragment_intensity: String, fragments involved in fold change between 2 intensity fragments

p_value_intensity: p_value of the fold change of intensity fragments

FC_HL_intensity: ratio of fold change between 2 half-life fragments and fold change between 2 intensity fragments

FC_HL_intensity_fragment: fragments involved on ratio of fold change between 2 half-life fragments and fold change between 2 intensity fragments

FC_HL_adapted: Integer, the fold change of half-life/ fold change of intensity, position of the half-life fragment is adapted to intensity fragment

synthesis_ratio: Integer, the value corresponding to synthesis rate

synthesis_ratio_event: String, the event assigned by synthesis rate either Termination or iTSS

p_value_Manova: p_value of the variance between two fold-changes, HL and intensity

p_value_TI: p_value of TI fragment

TI_fragments_p_value: p_value of 2 TI fragments

Source

https://github.com/CyanolabFreiburg/rifiComparative

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