## Package 'rifiComparative'

April 1, 2025

**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 anaylsis.

**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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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	data frame: data frame combined data by column
Strand	string: either "+" or "-"
annotation	data frame: data frame from processed gff3 file.

## 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 log2FC(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)))

**Log2FC(HL)+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

## 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 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\_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

- **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

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 an-
	notation example data A data frame containing the output of adjust-
	ing_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 position

**region:** the region from the gff file

gene: the annotated gene name

**locus\_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

#### 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.

## figures\_fun

## 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
х	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

## 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	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

## 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 1 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 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

#### 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"))
)
```

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 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 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

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

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.

#### loading\_fun

#### 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).

## 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.
<pre>smpl_max</pre>	integer: the larger end of the sampling size.

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#### make\_pen

sta_pen	numeric: the lower starting penalty.
end_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 information

penalties: a vector with the respective penalty and outlier penalty

correct: a matrix of the correct splits

wrong: 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>
```

penalties

## 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>
```

penalties\_df

#### 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 1 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 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

#### 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 information **int\_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

#### rifiComparative

#### 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

data	dataframe: the probe based dataframe with joined columns.
data_c	dataframe: the probe based dataframe with joined rows.
genomeLength	integer: genome length output of gff3_preprocess function.
annot	dataframe: the annotation file.
condition	string: assigned as cdt1 (condition 1) and cdt2 (condition2), it could be adapted to any name.
Strand	string: either ("+" or "-").
region	dataframe: gff3 features of the genome.
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
color_text.2	string: genes color text

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#### rifi\_visualization\_comparison

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

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#### 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 1 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 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

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 position end: The bin/probe end position width: The bin/probe length 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 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#' **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)

#### Format

A rowRanges of SummarizedExperiment with 500 rows and 50 variables:

seqnames: The sequence name chromosome start: The bin/probe start position end: The bin/probe end position width: The bin/probe length 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 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

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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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