Package 'rifi'

July 3, 2025

Title 'rifi' analyses data from rifampicin time series created by microarray or RNAseq

Version 1.13.1

Description 'rifi' analyses data from rifampicin time series created by microarray or RNAseq. 'rifi' is a transcriptome data analysis tool for the holistic identification of transcription and decay associated processes.

The decay constants and the delay of the onset of decay is fitted for each probe/bin. Subsequently, probes/bins of equal properties

are combined into segments by dynamic programming, independent of a existing genome annotation. This allows to detect transcript segments

of different stability or transcriptional events within one annotated gene.

In addition to the classic decay constant/half-

life analysis, 'rifi' detects processing sites, transcription pausing sites, internal transcription start sites in operons, sites of partial transcription termination in operons, identifies areas of likely transcriptional interference by the collision mechanism

and gives an estimate of the transcription velocity.

All data are integrated to give an estimate of continous transcriptional units, i.e. operons. Comprehensive output tables and visualizations of the full genome result and the individual fits for all probes/bins are produced.

Depends R (>= 4.2)

Imports car, cowplot, doMC, parallel, dplyr, egg, foreach, ggplot2, graphics, grDevices, grid, methods, nls2, nnet, rlang, S4Vectors, scales, stats, stringr, SummarizedExperiment, tibble, rtracklayer, reshape2, utils

Suggests DescTools, devtools, knitr, rmarkdown, BiocStyle

VignetteBuilder knitr

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

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

License GPL-3 + file LICENSE

Encoding UTF-8

Roxygen list(markdown = TRUE)

RoxygenNote 7.1.12

Language en-US

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

2 Contents

git_branch devel				
git_last_commit fa604e1				
git_last_commit_date 2025-06-27				
Repository Bioconductor 3.22				
Date/Publication 2025-07-02				
Author Loubna Youssar [aut, ctb], Walja Wanney [aut, ctb], Jens Georg [aut, cre]				

Maintainer Jens Georg <jens.georg@biologie.uni-freiburg.de>

Contents

apply_ancova
apply_event_position
apply_manova
apply_Ttest_delay
apply_t_test
apply_t_test_ti
check_input
dataframe_summary
dataframe_summary_events
dataframe_summary_events_HL_int
dataframe_summary_events_ps_itss
dataframe_summary_events_velocity
dataframe_summary_TI
event_dataframe
example_input_e_coli
example_input_minimal
example_input_synechocystis_6803
finding_PDD
finding_TI
fit_e_coli
fit_minimal
fit_synechocystis_6803
fold_change
fragmentation_e_coli
fragmentation_minimal
fragmentation_synechocystis_6803
fragment_delay
fragment_HL
fragment_inty
fragment_TI
gff3_preprocess
make_df
nls2_fit
penalties_e_coli
penalties_minimal
penalties synechocystis 6803
predict ns itss

apply_ancova 3

	stats_minimal	
	summary_e_coli	73
	summary_minimal	
	TI_fit	
	TUgether	
	wrapper_e_coli	87
	wrapper_minimal	
	wrapper_summary_synecnocystis_6803	88
Index		89

apply_ancova

apply_ancova checks the variances between 2 segments showing either pausing site (ps) or internal starting site (ITSS) independently.

Description

apply_ancova is a statistical test to check if fragments showing ps and ITSS events have significant slope using Ancova test. The function uses ancova test. Ancova is applied when the data contains independent variables, dependent variables and covariant variables. In this case, segments are independent variables, position is the dependent variable and the delay is the covariant.

Usage

apply_ancova(inp)

Arguments

inp SummarizedExperiment: the input data frame with correct format.

4 apply_ancova

Value

the SummarizedExperiment with the columns regarding statistics:

ID: The bin/probe specific ID.position: The bin/probe specific position.strand: The bin/probe specific strand.

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:

String, the factor of TI fragment.

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.

 ${\tt HL_mean_fragment:}$

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_duration:

Integer, the duration between two delay fragments.

event_ps_itss_p_value_Ttest:

p_value of pausing site or iTSS_I.

p_value_slope: Integer, the p_value added to the inp

delay_frg_slope:

Integer, the slope value of the fit through the respective delay fragment

velocity_ratio:

Integer, the ratio value of velocity from 2 delay fragments

apply_event_position 5

Examples

```
data(stats_minimal)
apply_ancova(inp = stats_minimal)
```

%

apply_event_position

apply_event_position

apply_event_position extracts event time duration for pausing site or iTSS

Description

apply_event_position is a short version of apply_Ttest_delay function to extract event time duration for pausing site or iTSS. Its adds a new column with the duration.

Usage

```
apply_event_position(inp)
```

Arguments

inp

SummarizedExperiment: the input data frame with correct format.

Value

The SummarizedExperiment with the columns regarding statistics:

ID: The bin/probe specific ID.

position: The bin/probe specific position. **strand:** The bin/probe specific strand.

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: String, the factor of TI fragment. **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.

6 apply_manova

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.

 $event_duration:$ Integer, the duration between two delay fragments.

event_ps_itss_p_value_Ttest: p_value of pausing site or iTSS_I.

p_value_slope: Integer, the p_value added to the inp.

delay_frg_slope: Integer, the slope value of the fit through the respective delay fragment.

velocity_ratio: Integer, the ratio value of velocity from 2 delay fragments.

event_position: Integer, position of the event added to the input.

Examples

```
data(stats_minimal)
apply_event_position(inp = stats_minimal)
```

apply_manova

%

apply_manova

apply_manova checks if the ratio of hl ratio and intensity ratio is

statistically significant.

Description

apply_manova compares the variance between two fold-changes HL and intensity within the same TU (half-life frgA/half-life frgB/intensity frgA/intensity frgB). HL fragment could cover two intensity fragments therefore this function sets first fragments borders and uses manova_function. Manova checks the variance between 2 segments (independent variables) and two dependents variables (HL and intensity).

Usage

```
apply_manova(inp)
```

Arguments

inp

SummarizedExperiment: the input data frame with correct format.

apply_manova 7

Value

The probe data frame with the columns regarding statistics:

ID: The bin/probe specific ID.

position: The bin/probe specific position.

strand: The bin/probe specific strand.

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: String, the factor of TI fragment.

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_duration: Integer, the duration between two delay fragments.

event ps itss p value Ttest: p value of pausing site or iTSS I.

p_value_slope: Integer, the p_value added to the inp.

delay_frg_slope: Integer, the slope value of the fit through the respective delay fragment.

velocity_ratio: Integer, the ratio value of velocity from 2 delay fragments.

event_position: Integer, position of the event added to the input.

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

FC_fragment_HL: String, the fragments corresponding to HL fold change.

p_value_HL: Integer, the p_value added to the input of 2 HL fragments.

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

FC_fragment_intensity: String, the fragments corresponding to intensity fold change.

p_value_intensity: Integer, the p_value added to the input of 2 intensity fragments.

synthesis_ratio: Integer, the value corresponding to synthesis rate.

8 apply_Ttest_delay

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

FC_HL_intensity: Integer, the value corresponding to HL and intensity fold change.

FC_HL_intensity_fragment: String, the fragments corresponding to intensity and HL fold change.

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.

p_value_Manova: Integer, the p_value added to the input.

Examples

```
data(stats_minimal)
apply_manova(inp = stats_minimal)
```

apply_Ttest_delay

%

apply_Ttest_delay

apply_Ttest_delay checks the significance of the point between 2 segments showing pausing site (ps) and internal starting site (ITSS) independently

Description

apply_Ttest_delay: is a statistical test to check the significance of the point between 2 segments showing pausing site (ps) and internal starting site (ITSS) independently. The function uses t-test. The last point from the first segment and the first point from the second segment are selected and added to the residuals of each model. The sum is subjected to t-test.

Usage

```
apply_Ttest_delay(inp)
```

Arguments

inp

SummarizedExperiment: the input data frame with correct format.

Value

the SummarizedExperiment with the columns regarding statistics:

ID: The bin/probe specific ID.

position: The bin/probe specific position. **strand:** The bin/probe specific strand.

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.

apply_t_test 9

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

TI_termination_factor: String, the factor of TI fragment. **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_duration: Integer, the duration between two delay fragments.event_ps_itss_p_value_Ttest: p_value of pausing site or iTSS_I.

Examples

```
data(stats_minimal)
apply_Ttest_delay(inp = stats_minimal)
```

 ${\tt apply_t_test}$

%

apply_t_test

apply_t_test uses the statistical t_test to check if the fold-change of half -life (HL) fragments and the fold-change intensity fragments respectively are significant.

Description

apply_t_test compares the mean of two neighboring fragments within the same TU to check if the fold-change is significant. Fragments with distance above threshold are not subjected to t-test. Dataframes with less than 3 rows are excluded.

Usage

```
apply_t_test(inp, threshold = 300)
```

10 apply_t_test

Arguments

inp SummarizedExperiment: the input data frame with correct format.

threshold integer: threshold.

Details

The functions used are:

1. fragment_function: checks number of fragments inside TU, less than 2 are excluded otherwise they are gathered for analysis.

2. t_test_function: excludes dataframes with less than 3 rows, makes fold-change and apply t-test, assign fragments names and ratio, add columns with the corresponding p_values.

Value

the SummarizedExperiment with the columns regarding statistics:

ID: The bin/probe specific ID.

position: The bin/probe specific position.

strand: The bin/probe specific strand.

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: String, the factor of TI fragment. **delay_fragment:** The delay fragment the bin belongs to.

 $\textbf{velocity_fragment:} \ \ \text{The velocity value of the respective delay fragment.}$

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.

 $\label{lem:half-life} HL_mean_fragment: \ \ \mbox{The mean half-life value of the respective half-life fragment.}$

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_duration: Integer, the duration between two delay fragments.

event_ps_itss_p_value_Ttest: p_value of pausing site or iTSS_I.

apply_t_test_ti

p_value_slope: Integer, the p_value added to the inp.

delay_frg_slope: Integer, the slope value of the fit through the respective delay fragment.

velocity_ratio: Integer, the ratio value of velocity from 2 delay fragments.

event_position: Integer, position of the event added to the input.

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

FC_fragment_HL: String, the fragments corresponding to HL fold change.

p_value_HL: Integer, the p_value added to the input of 2 HL fragments.

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

FC_fragment_intensity: String, the fragments corresponding to intensity fold change.

p_value_intensity: Integer, the p_value added to the input of 2 intensity fragments.

Examples

```
data(stats_minimal)
apply_t_test(inp = stats_minimal, threshold = 300)
```

apply_t_test_ti

%

apply_t_test_ti

apply_t_test_ti compares the mean of two neighboring TI fragments within the same TU.

Description

apply_t_test_ti uses the statistical t_test to check if two neighboring TI fragments are significant.

Usage

```
apply_t_test_ti(inp)
```

Arguments

inp

SummarizedExperiment: the input data frame with correct format.

Value

the SummarizedExperiment with the columns regarding statistics:

ID: The bin/probe specific ID.

position: The bin/probe specific position.

strand: The bin/probe specific strand.

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.

12 apply_t_test_ti

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: String, the factor of TI fragment. **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_duration: Integer, the duration between two delay fragments.

event_ps_itss_p_value_Ttest: p_value of pausing site or iTSS_I.

p_value_slope: Integer, the p_value added to the inp.

delay_frg_slope: Integer, the slope value of the fit through the respective delay fragment.

velocity_ratio: Integer, the ratio value of velocity from 2 delay fragments.

event_position: Integer, position of the event added to the input.

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

FC_fragment_HL: String, the fragments corresponding to HL fold change.

p value HL: Integer, the p value added to the input of 2 HL fragments.

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

FC_fragment_intensity: String, the fragments corresponding to intensity fold change.

p_value_intensity: Integer, the p_value added to the input of 2 intensity fragments.

synthesis_ratio: Integer, the value corresponding to synthesis rate.

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

FC HL intensity: Integer, the value corresponding to HL and intensity fold change.

FC HL intensity fragment: String, the fragments corresponding to intensity and HL fold change.

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.

p_value_Manova: Integer, the p_value added to the input.

p_value_TI: Integer, the p_value added to the input.

TI_fragments_p_value: String, the fragments subjected to statistical test.

Examples

```
data(stats_minimal)
apply_t_test_ti(inp = stats_minimal)
```

check_input 13

check_input

%

Check_input

Check_input reviews the input given by the user

Description

check_input stops the operation if the input data frame has severe faults. Less severe faults lead to the removal of wrong IDs and a warnings describing the problem. The Summarized Experiment colData must have the columns "timepoint" with the timepoints convertible to numeric and containing the timepoint 0. If replicates are used the column in colData must be called "replicate". The replicate must be convertible to numeric. In the RowRanges, optionally, IDs can be given as character (except ",","|","_"), but need to refer to a unique position/strand combination. Strand information needs to be given. The relative intensity in the assay must be numeric. The relative intensity for the first time point cannot be 0 or NA.

Usage

```
check_input(inp, thrsh = 0)
```

Arguments

inp SummarizedExperiment: the input data frame with correct format.

thrsh numeric: the minimal allowed intensity for time point "0".

Value

the SummarizedExperiment object: checked, and with position, ID and filtration added to the rowRanges.

Examples

```
data(example_input_minimal)
check_input(inp = example_input_minimal, thrsh = 0)
```

dataframe_summary

%

dataframe_summary

dataframe_summary creates two tables relating gene annotation to fragments

14 dataframe_summary

Description

dataframe_summary creates two tables summary of segments and their half-lives. The first output is bin/probe features and the second one is intensity fragment based. The dataframe_summary creates one table with feature_type, gene, locus_tag, position, strand, TU, delay_fragment, HL_fragment, half_life, intensity_fragment, intensity and velocity. The second table is similar to the first one but in compact form. It contains the same columns, the only difference is on position where a start and end position are indicated separately.

Usage

dataframe_summary(data, input)

Arguments

data SummarizedExperiment: the input data frame with correct format.

input dataframe: dataframe from event_dataframe function.

Value

bin_df: all information regarding bins:

position: Integer, position of the bin/probe on the genome **feature_type:** 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 (+/-) **segment:** String, the bin/probe segment on the genome

TU: String, The overarching transcription unit

delay_fragment: The delay fragment the bin belongs to

delay: Integer, the delay value of the bin/probe

HL_fragment: The half-life fragment the bin belongs to

half_life: The half-life of the bin/probe

intensity_fragment: The intensity fragment the bin belongs to

intensity: The relative intensity at time point 0

flag: String, the flag of the bin/probe, contains information or the distribution

for the #'different fitting models

TI_termination_factor: String, the TI termination factor determined by TI

frag_df: all information regarding fragments:

feature_type: String, region annotation covering the fragments

gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragmentsfirst_position_frg: Integer, the bin/probe specific first positionlast position frg: Integer, the bin/probe specific last position

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

TU: String, The overarching transcription unit **segment:** String, the bin/probe segment on the genome

delay fragment: String, the delay fragment the bin belongs to

HL_fragment: Integer, the half_life fragment of the bin/probe belongs to

TIL_IT agment. Integer, the nan_ine tragment of the onlyprobe belongs to

half_life: Integer, the half-life of the bin/probe

HL_SD: Integer, the half-life standard deviation of the HL fragment, bin/probe based

HL_SE: Integer, the half-life standard error of the HL fragment, bin/probe based

intensity_fragment: Integer, the intensity fragment the bin belongs to **intensity:** Integer, the relative intensity of bin/probe at time point 0

intensity_SD: Integer, the intensity standard deviation of the intensity fragment, bin/probe based

intensity_SE: Integer, the intensity standard error of the intensity fragment, bin/probe based

velocity: The velocity value of the respective delay fragment

Examples

```
data(stats_minimal)
data(res_minimal)
dataframe_summary(data = stats_minimal, input = res_minimal)
```

dataframe_summary_events

0%

dataframe_summary_events

dataframe_summary_events creates one table with all events between the segments

Description

dataframe_summary_events creates one table with the following columns: event, features, p_value, event_position, event_duration, position, region, gene, locus_tag, strand, TU, segment_1, segment_2, length, velocity_ratio, FC_HL, FC_intensity, FC_HL/FC_intensity.

Usage

```
dataframe_summary_events(data, data_annotation)
```

Arguments

data $Summarized Experiment: \ the \ input \ data \ frame \ with \ correct \ format.$ data_annotation

dataframe: dataframe from processed gff3 file.

Value

event: String, event type either pausing site, iTSS_I, iTSS_II, Termination, HL_event, Int_event, HL_Int_event and velocity_change
p_value: Integer, p_value of the event
p_adjusted: Integer, p_value adjusted

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

FC_intensity: Integer, the fold change value of 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: Fold change of half-life/ fold change of intensity

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

velocity_ratio: Integer, ratio of velocity between 2 delay fragments **feature_type:** 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 (+/-)

TU: String, The overarching transcription unit

segment_1: String, the first segment of the event, includes the segment, TU, delay fragment in case of ps or iTSS_I. The rest of the events include HL fragment and intensity fragment

segment_2: String, same description as segment_1 but is the second fragment of the event

event_duration: Integer, the difference (min) between 2 delay fragment when ps or iTSS_I happen

gap_fragments: Integer, length in position (nt), calculated by the difference between the last position of the first fragment and the first position of the second fragment.

features: Integer, number of fragements involved on the event

Examples

```
if(!require(SummarizedExperiment)){
suppressPackageStartupMessages(library(SummarizedExperiment))
}
data(stats_minimal)
dataframe_summary_events(data = stats_minimal,
data_annotation = metadata(stats_minimal)$annot[[1]])
```

 ${\tt dataframe_summary_events_HL_int}$

%

dataframe_summary_events_HL_int

dataframe_summary_events_HL_int creates one table with all events between the segments

Description

The dataframe_summary_events_HL_int creates one table with the following columns: event, features, p_value, event_position, position, region, gene, locus_tag, strand, TU, segment_1, segment_2, length, FC_HL, FC_intensity, FC_HL/FC_intensity.

Usage

```
dataframe_summary_events_HL_int(data, data_annotation)
```

Arguments

data SummarizedExperiment: the input data frame with correct format. data_annotation

dataframe: dataframe from processed gff3 file.

Value

event: String, event type.

p_value: Integer, p_value of the event.p_adjusted: Integer, p_value adjusted.

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

FC_intensity: Integer, the fold change value of 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.

FC_HL_FC_intensity: Fold change of half-life/ fold change of intensity.

feature_type: 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 (+/-).

TU: String, The overarching transcription unit.

segment_1: String, the first segment of the event, includes the segment, TU, delay fragment in case of ps or iTSS_I. The rest of the events include HL fragment and could be extended intensity fragment.

segment_2: String, the second fragment of the two of fragments subjected to analysis.

event_duration: Integer, the difference (min) between 2 delay fragment when ps or iTSS_I happen.

gap_fragments: Integer, length in position (nt), calculated by the difference between the last position of the first fragment and the first position of the second fragment.

features: Integer, number of fragements involved on the event

Examples

```
if(!require(SummarizedExperiment)){
suppressPackageStartupMessages(library(SummarizedExperiment))
}
data(stats_minimal)
dataframe_summary_events_HL_int(data = stats_minimal,
data_annotation = metadata(stats_minimal)$annot[[1]])
```

```
dataframe_summary_events_ps_itss
```

dataframe_summary_events_ps_itss

dataframe_summary_events_ps_itss creates one table with all events between the segments.

Description

The dataframe_summary_events_ps_itss creates one table with the following columns: event, features, p_value, event_position, event_duration, position, region, gene, locus_tag, strand, TU, segment_1, segment_2, length, velocity_ratio.

Usage

```
dataframe_summary_events_ps_itss(data, data_annotation)
```

Arguments

data SummarizedExperiment: the input data frame with correct format.

data_annotation

dataframe: dataframe from processed gff3 file.

Value

event: String, event type.

p_value: Integer, p_value of the event.p_adjusted: Integer, p_value adjusted.

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

velocity_ratio: Integer, the ratio value of velocity from 2 delay fragments.

feature_type: 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 (+/-).

TU: String, The overarching transcription unit.

segment_1: String, the first segment of the event, includes the segment, TU, delay fragment in case of ps or iTSS_I. The rest of the events include HL fragment and could be extended intensity fragment.

segment_2: String, the second fragment of the two of fragments subjected to analysis.

event_duration: Integer, the difference (min) between 2 delay fragment when ps or iTSS_I happen.

gap_fragments: Integer, length in position (nt), calculated by the difference between the last position of the first fragment and the first position of the second fragment.

features: Integer, number of fragements involved on the event

Examples

```
data(stats_minimal)
if(!require(SummarizedExperiment)){
suppressPackageStartupMessages(library(SummarizedExperiment))
}
dataframe_summary_events_ps_itss(data = stats_minimal,
data_annotation = metadata(stats_minimal)$annot[[1]])
```

```
{\tt dataframe\_summary\_events\_velocity}
```

%

dataframe_summary_events_velocity

dataframe_summary_events_velocity creates one table with all events between the segments.

Description

The dataframe_summary_events_velocity creates one table with the following columns: event, features, p_value, event_position, event_duration, position, region, gene, locus_tag, strand, TU, segment_1, segment_2, length, velocity_ratio.

Usage

```
dataframe_summary_events_velocity(data, data_annotation)
```

Arguments

data $Summarized Experiment: \ the \ input \ data \ frame \ with \ correct \ format. \\ data_annotation$

dataframe: dataframe from processed gff3 file.

Value

event: String, event type.

p_value: Integer, p_value of the event.p_adjusted: Integer, p_value adjusted.

event_position: Integer, the position of event, calculated dividing the last position of the first fragment and the first position of the next fragment on 2.

velocity_ratio: Integer, the ratio value of velocity from 2 delay fragments

feature_type: 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 (+/-).

TU: String, The overarching transcription unit.

segment_1: String, the first segment of the event, includes the segment, TU, delay fragment in case of ps or iTSS_I. The rest of the events include HL fragment and could be extended intensity fragment.

segment_2: String, the second fragment of the two of fragments subjected to analysis

event_duration: Integer, the difference (min) between 2 delay fragment when ps or iTSS_I happen.

gap_fragments: Integer, length in position (nt), calculated by the difference between the last position of the first fragment and the first position of the second fragment.

features: Integer, number of fragements involved on the event.

Examples

```
if(!require(SummarizedExperiment)){
suppressPackageStartupMessages(library(SummarizedExperiment))
}
data(stats_minimal)
dataframe_summary_events_velocity(data = stats_minimal,
data_annotation = metadata(stats_minimal)$annot[[1]])
```

%

```
dataframe_summary_TI
```

dataframe_summary_TI

dataframe_summary_TI creates one table with all TI fragments,p_value and the coordinates

Description

The dataframe_summary creates one table with the following columns: event, TI_fragment, TI_factor, TI_fragments_TU, p_value, feature_type, gene, locus_tag, strand, TU, features, event_position, position_1 and position_2.

Usage

```
dataframe_summary_TI(data, input)
```

Arguments

data SummarizedExperiment: the input data frame with correct format.

input dataframe: dataframe from event_dataframe function.

Value

event: String, event type, transcription interference.

TI_fragment: String, the fragment with TI.

TI_termination_factor: String, the factor of TI fragment.

event_dataframe 21

p_value: Integer, p_value of the event.p_adjusted: Integer, p_value adjusted.

feature_type: 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 (+/-).

TU: String, The overarching transcription unit.

Features: Integer, The number of segments within the TI.

event_position: Integer, the position middle between 2 TI fragments.

position_1: String, the first position of TI fragment, if 2 fragments, first position is from the first fragment.

position_2: String, the last position of TI fragment, if 2 fragments, last position is from the second fragment.

WIP

Examples

```
data(stats_minimal)
data(res_minimal)
dataframe_summary_TI(data = stats_minimal, input = res_minimal)
```

event_dataframe

%

event_dataframe

event_dataframe creates a dataframe only with events

Description

event_dataframe creates a dataframe connecting segments, events and the annotation.

Usage

```
event_dataframe(data, data_annotation)
```

Arguments

data dataframe: the probe based data frame.

data_annotation

dataframe: the coordinates are extracted from gff3

22 event_dataframe

Details

The functions used are:

position_function: adds the specific position of ps or iTSS event.

annotation_function_event: adds the events to the annotated genes.

annotation file needs to be supplied. Strand is indicated in case of stranded data The event_dataframe selects columns with statistical features. ID, position, strand and TU columns are required.

Value

A dataframe with unique intensity fragments

feature_type: 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 (+/-)

TU: String, The overarching transcription unit

position: Integer, position of the bin/probe on the genome **segment:** String, the bin/probe segment on the genome

FC_fragment_intensity: String, the fragments subjected to fold change **FC_intensity:** Integer, the fold change value of 2 intensity fragments

p_value_intensity: Integer, p_value of the FC_intensity

FC_fragment_HL: String, the fragments subjected to fold change

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

p_value_HL: Integer, p_value of the FC_HL

FC_HL_FC_intensity_fragment: String, fragments subjected to FC_HL/FC_intensity

FC_HL_FC_intensity: Integer, the value of FC_HL/FC_intensity

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

p_value_manova: Integer, p_value of the event FC_HL/FC_intensity

synthesis_ratio: Integer, the value corresponding to synthesis rate

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

pausing_site: Boolean, presence or absence of pausing_site event (ps)

iTSS_I: Boolean, presence or absence of internal starting site event (iTSS_I)

ps_ts_fragment: String, fragments involved on the event ps or iTSS_I

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

event_duration: Integer, the duration between two delay fragments

delay: Integer, the delay value of the bin/probe

half_life: The half-life of the bin/probe

intensity: The relative intensity at time point 0

delay_frg_slope: Integer, the slope value of the fit through the respective delay fragment

p_value_slope: Integer, the p_value added to the inp

example_input_e_coli 23

Examples

```
if(!require(SummarizedExperiment)){
suppressPackageStartupMessages(library(SummarizedExperiment))
}
data(stats_minimal)
event_dataframe(data = stats_minimal,
data_annotation = metadata(stats_minimal)$annot[[1]])
```

example_input_e_coli

An example SummarizedExperiment from E. coli An example SummarizedExperiment from RNA-seq containing information about the intensities at all time points (assay). Seqnames, IRanges and strand columns (rowRanges) and colData with time point series and replicates.

Description

An example SummarizedExperiment from E. coli An example SummarizedExperiment from RNA-seq containing information about the intensities at all time points (assay). Seqnames, IRanges and strand columns (rowRanges)and colData with time point series and replicates.

Usage

```
data(example_input_e_coli)
```

Format

A assay:

0: relative intensities at 0 min

1: relative intensities at 1 min

10: relative intensities at 10 min

15: relative intensities at 15 min

2: relative intensities at 2 min

20: relative intensities at 20 min

3: relative intensities at 3 min

4: relative intensities at 4 min

5: relative intensities at 5 min

6: relative intensities at 6 min

8: relative intensities at 8 min

Source

https://github.com/CyanolabFreiburg/rifi

example_input_minimal An artificial example SummarizedExperiment An example SummarizedExperiment containing information about the intensities at all time points (assay). Segnames, IRanges and strand columns (rowRanges) and colData with time point series and replicates.

Description

An artificial example SummarizedExperiment An example SummarizedExperiment containing information about the intensities at all time points (assay). Seqnames, IRanges and strand columns (rowRanges) and colData with time point series and replicates.

Usage

```
data(example_input_minimal)
```

Format

An object of class RangedSummarizedExperiment with 4 rows and 33 columns.

Source

https://github.com/CyanolabFreiburg/rifi

example_input_synechocystis_6803

An example input data frame from Synechocystis PCC 6803 A SummarizedExperiment from microarrays data containing information about the intensities at all time points (assay), Seqnames, IRanges and strand columns (rowRanges) and colData with time point series and averaged replicates.

Description

An example input data frame from Synechocystis PCC 6803 A SummarizedExperiment from microarrays data containing information about the intensities at all time points (assay), Seqnames, IRanges and strand columns (rowRanges) and colData with time point series and averaged replicates.

Usage

```
data(example_input_synechocystis_6803)
```

finding_PDD 25

Format

Assay with 3000 rows and 10 variables:

0: relative intensities at 0 min

2: relative intensities at 2 min

4: relative intensities at 4 min

8: relative intensities at 8 min

16: relative intensities at 16 min

32: relative intensities at 32 min

64: relative intensities at 64 min

Source

https://github.com/CyanolabFreiburg/rifi

finding_PDD

%

finding_PDD

finding_PDD Flags potential candidates for post transcription decay

Description

'finding_PDD' uses 'score_fun_linear_PDD' to make groups by the difference to the slope. The slope is further checked for steepness to decide for PDD. 'PDD' is added to the 'flag' column. Post transcription decay is characterized by a strong decrease of intensity by position. The rowRanges need to contain at least 'ID', 'intensity', 'position' and 'position_segment'!

Usage

```
finding_PDD(inp, cores = 1, pen = 2, pen_out = 1, thrsh = 0.001)
```

Arguments

ut.
J

integer: the number of assigned cores for the task cores

numeric: an internal parameter for the dynamic programming. Higher values pen

result in fewer fragments. Advised to be kept at 2. Default is 2.

numeric: an internal parameter for the dynamic programming. Higher values pen_out

result in fewer possible outliers. Advised to be kept at 1. Default is 1.

thrsh numeric: an internal parameter that allows fragments with slopes steeper than

the thrsh to be flagged with 'PDD'. Higher values result in fewer candidates.

Advised to be kept at 0.001. Default is 0.001.

26 finding_TI

Value

The SummarizedExperiment object: with "PDD" added to the flag column.

Examples

```
data(preprocess_minimal)
finding_PDD(inp = preprocess_minimal, cores = 2, pen = 2,
pen_out = 1, thrsh = 0.001)
```

finding_TI

%

finding_TI

finding_TI flags potential candidates for transcription interference

Description

finding_TI uses 'score_fun_ave' to make groups by the mean of "probe_TI". "TI" is added to the "flag" column. TI is characterized by relative intensities at time points later than "0". The rowRanges need to contain at least "ID", "probe_TI" and "position_segment"!

Usage

```
finding_TI(inp, cores, pen = 10, thrsh = 0.5, add = 1000)
```

Arguments

inp	SummarizedExperiment: the input.
cores	integer: the number of assigned cores for the task
pen	numeric: an internal parameter for the dynamic programming. Higher values result in fewer fragments. Advised to be kept at 10. Default is 10.
thrsh	numeric: an internal parameter that allows fragments with a certain amount of IDs with higher relative intensities at time points later than "0" to be flagged as " TI ". Higher values result in fewer candidates0.5 is 25 %, 0 is 50%, 0.5 is 75%. Advised to be kept at 0.5. Default is 0.5.
add	integer: range of nucleotides before and after a potential TI event wherein IDs are fitted with the TI fit.

Value

The SummarizedExperiment object: with "TI" added to the flag column.

Examples

```
data(preprocess_minimal)
finding_TI(inp = preprocess_minimal, cores = 2, pen = 10, thrsh = 0.5,
add = 1000)
```

fit_e_coli 27

fit_e_coli	The result of rifi_fit for E.coli example data A SummarizedExperiment containing the output from rifi_fit as an extension of rowRanges and
	metadata.

Description

The result of rifi_fit for E.coli example data A SummarizedExperiment containing the output from rifi_fit as an extension of rowRanges and metadata.

Usage

```
data(fit_e_coli)
```

Format

Three data frames with 290 rows and 10 variables, 155 rows and 5 variables, and 135 rows and 9 variables are generated. The columns of the first data frame are added to the rowRanges and the rest are added as metadata.

inp: The SummarizedExperiment:

ID: The bin/probe specific ID

position: The bin/probe specific position **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 **postion_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: String, the factor of TI fragment

fit_obj_STD: the fit object for the standard fit:

ID: The bin/probe specific ID

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

inty_S0: The relative intensity at time point 0

intyf: The background value of the fit

fit_obj_TI: the fit object for the TI fit:

delay: The delay value of the bin/probeti_delay: The ti-delay value of the bin/probehalf_life: The half-life of the bin/probeti_value: The ti-value of the bin/probe

TI_termination_factor: String, the factor of TI fragment **synthesis_rate:** The synthesis rate of the bin/probe

 $TI_background:$ The background value of the fit

position: The bin/probe specific position

ID: The bin/probe specific ID

Source

https://github.com/CyanolabFreiburg/rifi

fit_minimal

The artificial result of rifi_fit for artificial example data A Summa-rizedExperiment containing the output from rifi_fit.

Description

The artificial result of rifi_fit for artificial example data A SummarizedExperiment containing the output from rifi_fit.

Usage

```
data(fit_minimal)
```

Format

An object of class RangedSummarizedExperiment with 4 rows and 33 columns.

Source

https://github.com/CyanolabFreiburg/rifi

fit_synechocystis_6803

The result of rifi_fit for Synechocystis 6803 example data A SummarizedExperiment containing the output from rifi_fit as an extension of rowRanges and metadata.

Description

The result of rifi_fit for Synechocystis 6803 example data A SummarizedExperiment containing the output from rifi_fit as an extension of rowRanges and metadata.

Usage

```
data(fit_synechocystis_6803)
```

Format

Three data frames with 3000 rows and 10 variables, 2811 rows and 5 variables, and 189 rows and 9 variable are generated. The columns of the first data frame are added to the rowRanges and the rest are added as metadata.

inp: the SummarizedExperiment:

ID: The bin/probe specific ID

position: The bin/probe specific position **strand:** The bin/probe specific strand

fold_change 29

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 **postion_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: String, the factor of TI fragment

fit_obj_STD: the fit object for the standard fit:

ID: The bin/probe specific ID

delay: The delay value of the bin/probe **half_life:** The half-life of the bin/probe **inty. S0:** The relative intensity at time point.

inty_S0: The relative intensity at time point 0

intyf: The background value of the fit

fit_obj_TI: the fit object for the TI fit:

delay: The delay value of the bin/probeti_delay: The ti-delay value of the bin/probehalf_life: The half-life of the bin/probeti_value: The ti-value of the bin/probe

TI_termination_factor: String, the factor of TI fragment **synthesis_rate:** The synthesis rate of the bin/probe **TI_background:** The background value of the fit

position: The bin/probe specific position

ID: The bin/probe specific ID

Source

https://github.com/CyanolabFreiburg/rifi

fold_change

%

fold_change

fold_change sets a fold-change ratio between the neighboring fragments of Half-life (HL) and intensity

Description

fold_change sets fold change on intensity and fold change HL fragments of two successive fragments. Two intensity fragments could belong to one HL fragment. This function sets first the borders using the position and applies the fold change ratio between the neighboring fragments of HL and those from intensity log2(intensity frgA/intensity frgB/half-life frgA/half-life frgB). All grepped fragments are from the same TU excluding outliers.

Usage

fold_change(inp)

30 fold_change

Arguments

inp SummarizedExperiment: the input data frame with correct format.

Details

The function used is: synthesis_r_Function: assigns events depending on the ratio between HL and intensity of two consecutive fragments. intensity(int) = synthesis rate(k)/decay(deg) (steady state), int1/int2 = k1/deg1*deg2/k2 int1 * (deg1/int2) * deg2 = k1/k2 => synthesis ratio. In case of synthesis ratio is: synthesis ratio > 0 -> New start synthesis ratio < 0 -> Termination

Value

the SummarizedExperiment with the columns regarding statistics:

ID: The bin/probe specific ID.

position: The bin/probe specific position.

strand: The bin/probe specific strand.

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: String, the factor of TI fragment. **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_duration: Integer, the duration between two delay fragments.

event_ps_itss_p_value_Ttest: p_value of pausing site or iTSS_I.

p_value_slope: Integer, the p_value added to the inp.

delay_frg_slope: Integer, the slope value of the fit through the respective delay fragment.

velocity_ratio: Integer, the ratio value of velocity from 2 delay fragments.

fragmentation_e_coli 31

event_position: Integer, position of the event added to the input.

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

FC_fragment_HL: String, the fragments corresponding to HL fold change.

p_value_HL: Integer, the p_value added to the input of 2 HL fragments.

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

FC_fragment_intensity: String, the fragments corresponding to intensity fold change.

p_value_intensity: Integer, the p_value added to the input of 2 intensity fragments.

synthesis_ratio: Integer, the value corresponding to synthesis rate.

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

FC_HL_intensity: Integer, the value corresponding to HL and intensity fold change.

FC_HL_intensity_fragment: String, the fragments corresponding to intensity and HL fold change.

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.

Examples

```
data(stats_minimal)
fold_change(inp = stats_minimal)
```

fragmentation_e_coli

The result of rifi_fragmentation for E.coli example data A SummarizedExperiment containing the output from rifi_fragmentation as an extension of rowRanges

Description

The result of rifi_fragmentation for E.coli example data A SummarizedExperiment containing the output from rifi_fragmentation as an extension of rowRanges

Usage

```
data(fragmentation_e_coli)
```

Format

rowRanges of the SummarizedExperiment with 290 rows and 22 variables:

ID: The bin/probe specific ID

position: The bin/probe specific position

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: String, the factor of TI fragment

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 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

Source

https://github.com/CyanolabFreiburg/rifi

 $\begin{tabular}{lll} Fragmentation_minimal & The result of rifi_fragmentation for artificial example data A Summa-rized Experiment containing the output from rifi_fragmentation as an artificial example data A Summa-rized Experiment containing the output from rifi_fragmentation as an artificial example data A Summa-rized Experiment containing the output from rifi_fragmentation as an artificial example data A Summa-rized Experiment containing the output from rifi_fragmentation as an artificial example data A Summa-rized Experiment containing the output from rifi_fragmentation as an artificial example data A Summa-rized Experiment containing the output from rifi_fragmentation as an artificial example data A Summa-rized Experiment containing the output from rifi_fragmentation as an artificial example data A Summa-rized Experiment containing the output from rifi_fragmentation as an artificial example data A Summa-rized Experiment containing the output from rifi_fragmentation as an artificial example data A Summa-rized Experiment containing the output from rifi_fragmentation as an artificial example data A Summa-rized Experiment containing the output from rifi_fragmentation as an artificial example data A Summa-rized Experiment containing the output from rifi_fragmentation as a summa-rized Experiment containing the output from rifi_fragmentation as a summa-rized Experiment containing the output from rifi_fragmentation and a summa-rized Experiment containing the output from rifi_fragmentation and a summa-rized Experiment containing the output from rifi_fragmentation and a summa-rized Experiment containing the output from rifi_fragmentation and a summa-rized Experiment containing the output from rifi_fragmentation and a summa-rized Experiment containing the output from rifi_fragmentation and a summa-rized Experiment containing the output from rifi_fragment containing the output from rifi_fragment containing the output from rifi_fragment containing the output fragment containing the output fragment containing the outp$

extension of rowRanges and metadata.

Description

The result of rifi_fragmentation for artificial example data A SummarizedExperiment containing the output from rifi_fragmentation as an extension of rowRanges and metadata.

Usage

data(fragmentation_minimal)

Format

An object of class RangedSummarizedExperiment with 24 rows and 33 columns.

Source

https://github.com/CyanolabFreiburg/rifi

fragmentation_synechocystis_6803

The result of rifi_fragmentation for Synechocystis 6803 example data A SummarizedExperiment containing the output from rifi_fragmentation as an extension fo rowRanges

Description

The result of rifi_fragmentation for Synechocystis 6803 example data A SummarizedExperiment containing the output from rifi_fragmentation as an extension fo rowRanges

Usage

data(fragmentation_synechocystis_6803)

Format

rowRanges of the SummarizedExperiment:

ID: The bin/probe specific ID

position: The bin/probe specific position

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: String, the factor of TI fragment **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

Source

https://github.com/CyanolabFreiburg/rifi

34 fragment_delay

fragment_delay %

fragment_delay

fragment_delay performs the delay fragmentation

Description

fragment_delay makes delay_fragments based on position_segments and assigns all gathered information to the SummarizedExperiment object. The columns "delay_fragment", "velocity_fragment", "intercept" and "slope" are added. fragment_delay makes delay_fragments, assigns slopes, which are 1/velocity at the same time, and intercepts for the TU calculation. The function used is: score_fun_linear the input is the SummarizedExperiment object. pen is the penalty for new fragments in the dynamic programming, pen_out is the outlier penalty.

Usage

```
fragment_delay(inp, cores = 1, pen, pen_out)
```

Arguments

inp SummarizedExperiment: the input data frame with correct format.

cores cores: integer: the number of assigned cores for the task.

pen numeric: an internal parameter for the dynamic programming. Higher values

result in fewer fragments. Default is the auto generated value.

pen_out numeric: an internal parameter for the dynamic programming. Higher values

result in fewer allowed outliers. Default is the auto generated value.

Value

the SummarizedExperiment object:

ID: The bin/probe specific ID.

position: The bin/probe specific position.

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.

 $\verb"position_segment":$

The position based segment.

delay: The delay value of the bin/probe.

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

 ${\sf TI_termination_factor}:$

String, the factor of TI fragment.

delay_fragment:

The delay fragment the bin belongs to.

fragment_HL 35

```
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.

Examples

```
data(fragmentation_minimal)
fragment_delay(inp = fragmentation_minimal, cores = 2, pen = 2, pen_out = 1)
```

fragment_HL

%

fragment_HL

fragment_HL performs the half_life fragmentation

Description

fragment_HL makes HL_fragments based on delay_fragments and assigns all gathered information to the SummarizedExperiment object.

Usage

```
fragment_HL(inp, cores = 1, pen, pen_out)
```

Arguments

inp SummarizedExperiment: the input data frame with correct format.

cores integer: the number of assigned cores for the task.

pen numeric: an internal parameter for the dynamic programming. Higher values

result in fewer fragments. Default is the auto generated value.

pen_out numeric: an internal parameter for the dynamic programming. Higher values

result in fewer allowed outliers. Default is the auto generated value.

Details

The columns "HL_fragment" and "HL_mean_fragment" are added.

fragment_HL makes half-life_fragments and assigns the mean of each fragment.

The function used is:

.score_fun_ave.

The input the SummarizedExperiment object.

pen is the penalty for new fragments in the dynamic programming, pen_out is the outlier penalty.

36 fragment_inty

Value

The SummarizedExperiment object:

ID: The bin/probe specific ID

position: The bin/probe specific position

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: String, the factor of TI fragment **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

Examples

```
data(fragmentation_minimal)
fragment_HL(inp = fragmentation_minimal, cores = 2, pen = 2, pen_out = 1)
```

 $fragment_inty$

%

fragment_inty

fragment_inty performs the intensity fragmentation

Description

fragment_inty makes intensity_fragments based on HL_fragments and assigns all gathered information to the SummarizedExperiment object.

Usage

```
fragment_inty(inp, cores = 1, pen, pen_out)
```

fragment_inty 37

Arguments

inp SummarizedExperiment: the input data frame with correct format.

cores cores: integer: the number of assigned cores for the task.

pen numeric: an internal parameter for the dynamic programming. Higher values

result in fewer fragments. Default is the auto generated value.

pen_out numeric: an internal parameter for the dynamic programming. Higher values

result in fewer allowed outliers. Default is the auto generated value.

Details

The columns "intensity_fragment" and "intensity_mean_fragment" are added.

fragment_inty makes intensity_fragments and assigns the mean of each fragment.

The function used is:

.score fun ave.

The input is the SummarizedExperiment object.

pen is the penalty for new fragments in the dynamic programming, pen_out is the outlier penalty.

Value

The SummarizedExperiment object:

ID: The bin/probe specific ID

position: The bin/probe specific position

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:

String, the factor of TI fragment

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

38 fragment_TI

Examples

```
data(fragmentation_minimal)
fragment_inty(inp = fragmentation_minimal, cores = 2, pen = 2, pen_out = 1)
```

fragment_TI

%

fragment_TI

fragment_TI performs the TI fragmentation

Description

fragment_TI makes TI_fragments based on TUs and assigns all gathered information to the SummarizedExperiment object. The columns TI_termination_fragment" and the TI_mean_termination_factor are added.

Usage

```
fragment_TI(inp, cores = 1, pen, pen_out)
```

Arguments

inp SummarizedExperiment: the input data frame with correct format.

cores cores: integer: the number of assigned cores for the task.

pen numeric: an internal parameter for the dynamic programming. Higher values

result in fewer fragments. Default is the auto generated value.

pen_out numeric: an internal parameter for the dynamic programming. Higher values

result in fewer allowed outliers. Default is the auto generated value.

Details

The function used is:

.score_fun_ave.

The input is the SummarizedExperiment object.

pen is the penalty for new fragments in the dynamic programming, pen_out is the outlier penalty.

Value

The SummarizedExperiment object:

ID: The bin/probe specific IDposition: The bin/probe specific positionintensity: 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

gff3_preprocess 39

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:

String, the factor of TI fragment

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

TI_termination_fragment:

The TI fragment the bin belongs to

TI_mean_termination_factor:

The mean termination factor of the respective TI fragment

Examples

```
data(fragmentation_minimal)
fragment_TI(inp = fragmentation_minimal, cores = 2, pen = 2, pen_out = 1)
```

gff3_preprocess

%

gff3_preprocess

gff3_preprocess process gff3 file from database for multiple usage

Description

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

```
gff3_preprocess(path)
```

40 make_df

Arguments

path

path: path to the directory containing the gff3 file.

Details

The resulting dataframe contains region, positions, strand, gene and locus_tag.

Value

A list with 2 items:

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

start: Integer, the start of the annotationend: Integer, the end of the annotationstrand: Boolean, the strand of the annotationgene: String, the annotated gene namelocus_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_e_coli.gff3.gz", package = "rifi"))
)
```

make_df

%

make_df

make_df adds important columns to the SummarizedExperiment object

Description

'make_df' adds to the SummarizedExperiment object with the columns: "intensity", "probe_TI" and "flag".

Usage

```
make_df(inp, cores = 1, bg = 0, rm_FLT = TRUE)
```

Arguments

inp SummarizedExperiment: the (checked) input.
cores integer: the number of assigned cores for the task.

bg numeric: threshold over which the last timepoint has to be fitted with the above

background mode.

rm_FLT logical: remove IDs where all replicates are marked as filtered. Default is

FALSE.

make_pen 41

Details

The replicates are collapsed into their respective means.

"intensity" is the mean intensity from time point 0.

"probe_TI" is a value needed for the distribution for the different fitting models.

"flag" contains information or the distribution for the different fitting models.

Probes that don't reach the background level expression are flagged as "ABG" ("above background"). This is only needed for microarray data and is controlled by the bg parameter. The default for bg = 0, resulting in all probes to be above background (0 is advised for RNAseq data).

Probes where all replicates were filtered in the optional filtration step can be fully removed by rm_FLT = TRUE! If you wish to keep all information in the assay set to FALSE!

Value

the SummarizedExperiment object: with intensity, probe_TI and flag added to the rowRanges.

Examples

make_pen assigns automatically a penalties

Description

'make_pen' calls one of four available penalty functions to automatically assign penalties for the dynamic programming.

```
make_pen(
   inp,
   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
```

42 make_pen

Arguments

inp SummarizedExperiment: the input data frame with correct format.

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. Default is 2.

smpl_min integer: the smaller end of the sampling size. Default is 10.

smpl_max integer: the larger end of the sampling size. Default is 100.

sta_pen numeric: the lower starting penalty. Default is 0.5. end_pen numeric: the higher starting penalty. Default is 4.5.

rez_pen numeric: the number of penalties iterated within the penalty range. Default is 9.

sta_pen_out numeric: the lower starting outlier penalty. Default is 0.5. numeric: the higher starting outlier penalty. Default is 3.5.

rez_pen_out numeric: the number of outlier penalties iterated within the outlier penalty

range. Default is 7.

Details

The four functions to be called are:

- 1. fragment_delay_pen
- 2. fragment_HL_pen
- 3. fragment_inty_pen
- 4. fragment_TI_pen

These functions 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 $n = \text{rez}_\text{pen}$ rows and $n = \text{rez}_\text{pen}_\text{out}$ 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. Returns a penalty object (list of 4 objects) the first being the logbook.

Value

A list with 4 items:

logbook: Interger, the logbook vector containing all penalty information **penalties:** Integer, a vetor with the respective penalty and outlier penalty

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

nls2_fit 43

Examples

```
data(fit_minimal)
make_pen(
  inp = fit_minimal, FUN = rifi:::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
)
```

nls2_fit

%

nls2_fit

nls2_fit estimates decay for each probe or bin

Description

nls2_fit uses nls2 function to fit a probe or bin using intensities of the time series data from different time point. nls2 uses different starting values through expand grid and selects the best fit. Different filters could be applied prior fitting to the model.

Usage

```
nls2_fit(
  inp,
  cores = 1,
  decay = seq(0.01, 0.11, by = 0.02),
  delay = seq(0, 10, by = 0.1),
  k = seq(0.1, 1, 0.2),
  bg = 0.2
)
```

Arguments

inp	SummarizedExperiment: the input with correct format.
cores	integer: the number of assigned cores for the task.
decay	numeric vector: A sequence of starting values for the decay. Default is seq(.08, 0.11, by=.02)
delay	numeric vector: A sequence of starting values for the delay. Default is $seq(0,10, by=.1)$
k	numeric vector: A sequence of starting values for the synthesis rate. Default is $seq(0.1,1,0.2)$
bg	numeric vector: A sequence of starting values. Default is 0.2.

44 penalties_e_coli

Details

To apply nls2_fit function, prior filtration could applied.

1. generic_filter_BG: filter probes with intensities below background using threshold. Those probes are filtered.

- 2. filtration_below_backg: additional functions exclusive to microarrays could be applied. Its very strict to the background (not recommended in usual case).
- 3. filtration_above_backg: selects probes with a very high intensity and above the background (recommended for special transcripts). Probes are flagged with "ABG". Those transcripts are usually related to a specific function in bacteria. This filter selects all probes with the same ID, the mean is applied, the last time point is selected and compared to the threshold.

The model used estimates the delay, decay, intensity of the first time point (synthesis rate/decay) and the background. The coefficients are gathered in vectors with the corresponding IDs. Absence of the fit or a very bad fit are assigned with NA. In case of probes with very high intensities and above the background, the model used makes abstinence of background coefficient. The output of all coefficients is saved in the metadata. The fits are plotted using the function_plot_fit.r through rifi_fit.

Value

the SummarizedExperiment object: with delay and decay added to the rowRanges. The full fit data is saved in the metadata as "fit_STD".

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

Examples

```
data(preprocess_minimal)
nls2_fit(inp = preprocess_minimal, cores = 2)
```

penalties_e_coli

The result of rifi_penalties for E.coli example data. A SummarizedExperiment containing the output from rifi_penalties including the logbook and the four penalty objects as metadata.

Description

The result of rifi_penalties for E.coli example data. A SummarizedExperiment containing the output from rifi_penalties including the logbook and the four penalty objects as metadata.

```
data(penalties_e_coli)
```

penalties_minimal 45

Format

A list with 5 items:

logbook: The logbook vector containing all penalty information

pen_obj_delay: A list with 4 items:

logbook: The logbook vector containing all penalty information

delay_penalties: a vetor with the delay penalty and delay outlier penalty

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

pen_obj_HL: A list with 4 items:

logbook: The logbook vector containing all 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

pen_obj_inty: A list with 4 items:

logbook: The logbook vector containing all penalty information

inty_penalties: a vetor with the intensity penalty and intensity outlier penalty

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

pen_obj_TI: A list with 4 items:

Intersection logbook vector containing all penalty information **TI_penalties:** a vetor with the TI penalty and TI outlier penalty

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

Source

https://github.com/CyanolabFreiburg/rifi

penalties_minimal

The result of rifi_penalties for artificial example data A Summarized-Experiment containing the output from rifi_penalties including the logbook and the four penalty objects as metadata.

Description

The result of rifi_penalties for artificial example data A SummarizedExperiment containing the output from rifi_penalties including the logbook and the four penalty objects as metadata.

Usage

data(penalties_minimal)

Format

An object of class RangedSummarizedExperiment with 24 rows and 33 columns.

Source

https://github.com/CyanolabFreiburg/rifi

penalties_synechocystis_6803

The result of rifi_penalties for Synechocystis 6803 example data. A SummarizedExperiment containing the output from rifi_penalties including the logbook and the four penalty objects as metadata.

Description

The result of rifi_penalties for Synechocystis 6803 example data. A SummarizedExperiment containing the output from rifi_penalties including the logbook and the four penalty objects as metadata.

Usage

```
data(penalties_synechocystis_6803)
```

Format

A list with 5 items:

logbook: The logbook vector containing all penalty information

pen_obj_delay: A list with 4 items:

logbook: The logbook vector containing all penalty information

delay_penalties: a vetor with the delay penalty and delay outlier penalty

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

pen_obj_HL: A list with 4 items:

logbook: The logbook vector containing all 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

pen_obj_inty: A list with 4 items:

logbook: The logbook vector containing all penalty information

inty_penalties: a vetor with the intensity penalty and intensity outlier penalty

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

pen_obj_TI: A list with 4 items:

Intersection logbook vector containing all penalty information **TI_penalties:** a vetor with the TI penalty and TI outlier penalty

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

Source

https://github.com/CyanolabFreiburg/rifi

predict_ps_itss 47

predict_ps_itss

predict_ps_itss predicts pausing sites (ps) and internal starting sites (ITSS) between delay fragments.

Description

predict_ps_itss predicts ps and ITSS within the same TU. Neighboring delay segments are compared to each other by positioning the intercept of the second segment into the first segment using slope and intercept coefficients.

Usage

```
predict_ps_itss(inp, maxDis = 300)
```

Arguments

inp SummarizedExperiment: the input data frame with correct format.

maxDis integer: the maximal distance allowed between two successive fragments.

Details

predict_ps_itss uses 3 steps to identify ps and ITSS:

- 1. select unique TU.
- 2. select from the input dataframe the columns: ID, position, strand, delay. delay fragment, TU and slope coordinates, velocity_fragment and intercept.
- 3. select delay segments in the TU.
- 4. loop into all delay segments and estimate the coordinates of the last point of the first segment using the coefficients of the second segment and vice versa. We get two predicted positions, the difference between them is compared to the threshold.

In case the strand is "-", additional steps are added:

The positions of both segments are ordered from the last position to the first one.

All positions are merged in one column and subtracted from the maximum position. the column is split in 2. The first and second correspond to the positions of the first and second segments respectively.

Both segments are subjected to lm fit and the positions predicted are used on the same way as the opposite strand.

If the difference between the positions predicted is lower than negative threshold, ps is assigned otherwise, and if the difference is higher than the positive threshold, ITSS is assigned.

48 predict_ps_itss

Value

The SummarizedExperiment with the columns regarding statistics:

ID: The bin/probe specific ID.

position: The bin/probe specific position.

strand: The bin/probe specific strand.

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: String, the factor of TI fragment.

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_duration: Integer, the duration between two delay fragments.

Examples

```
data(fragmentation_minimal)
predict_ps_itss(inp = fragmentation_minimal, maxDis = 300)
```

preprocess_e_coli 49

preprocess_e_coli

The result of rifi_preprocess for E.coli example data A SummarizedExperiment containing the output from rifi_penalties including the logbook and the four penalty objects as metadata. A list containing the output from rifi_preprocess, including the inp and the modified input_df.

Description

The result of rifi_preprocess for E.coli example data A SummarizedExperiment containing the output from rifi_penalties including the logbook and the four penalty objects as metadata. A list containing the output from rifi_preprocess, including the inp and the modified input_df.

Usage

```
data(preprocess_e_coli)
```

Format

A SummarizedExperiment:

inp: the SummarizedExperiment:

ID: The bin/probe specific ID

position: The bin/probe specific position

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 **postion_segment:** The position based segment

fit_obj_TI: the fit object for the TI fit:

0: relative intensities at 0 min

1: relative intensities at 1 min

10: relative intensities at 10 min

15: relative intensities at 15 min

2: relative intensities at 2 min

20: relative intensities at 20 min

3: relative intensities at 3 min

4: relative intensities at 4 min

5: relative intensities at 5 min

6: relative intensities at 6 min

8: relative intensities at 8 min

ID: The bin/probe specific ID

position: The bin/probe specific position

filtration: indicator wether the replicate is filtered or not

Source

https://github.com/CyanolabFreiburg/rifi

preprocess_minimal

The result of rifi_preprocess for artificial example data A Summarized-Experiment containing the output from rifi_preprocess

Description

The result of rifi_preprocess for artificial example data A SummarizedExperiment containing the output from rifi_preprocess

Usage

```
data(preprocess_minimal)
```

Format

An object of class RangedSummarizedExperiment with 4 rows and 33 columns.

Source

https://github.com/CyanolabFreiburg/rifi

preprocess_synechocystis_6803

The result of rifi_preprocess for Synechocystis 6803 example data is a A SummarizedExperiment containing the output of rifi_preprocess as an extention to rowRanges

Description

The result of rifi_preprocess for Synechocystis 6803 example data is a A SummarizedExperiment containing the output of rifi_preprocess as an extention to rowRanges

Usage

```
data(preprocess_synechocystis_6803)
```

Format

A SummarizedExperiment:

inp: the SummarizedExperiment:

ID: The bin/probe specific ID

position: The bin/probe specific position **strand:** The bin/probe specific strand

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 **postion_segment:** The position based segment

fit_obj_TI: the fit object for the TI fit:

res_minimal 51

0: relative intensities at 0 min

2: relative intensities at 2 min

4: relative intensities at 4 min

8: relative intensities at 8 min

16: relative intensities at 16 min

32: relative intensities at 32 min

64: relative intensities at 64 min

ID: The bin/probe specific ID

position: The bin/probe specific position

filtration: indicator wether the replicate is filtered or not

Source

https://github.com/CyanolabFreiburg/rifi

res_minimal

The result of event_dataframe for E.coli artificial example. A data frame combining the processed genome annotation and a SummarizedExperiment data from rifi_stats. The dataframe is

Description

The result of event_dataframe for E.coli artificial example. A data frame combining the processed genome annotation and a SummarizedExperiment data from rifi_stats. The dataframe is

Usage

data(res_minimal)

Format

A list with 2 items:

region: the region from the gff file

gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragments

strand: the strand of the annotation **TU:** The overarching transcription unit **position:** The bin/probe specific position

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

FC_intensity: Integer, the fold change value of 2 intensity fragments
p_value_intensity: p_value of the fold change of intensity fragments
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_HL_intensity_fragment: fragments involved on ratio of fold change between 2 half-life fragments and fold change between 2 intensity fragments

52 rifi_fit

FC_HL_intensity: 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

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

synthesis_ratio: Integer, the value corresponding to synthesis rate

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

pausing_site: presence of pausing site indicated by +/-

iTSS_I: presence of iTSS_I indicated by +/-

event_ps_itss_p_value_Ttest: p_value of pausing site or iTSS_I

ps_ts_fragment: The fragments involved in pausing site or iTSS_I

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

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

p_value_slope: p_value of the slopedelay: The delay value of the bin/probehalf_life: The half-life of the bin/probe

intensity: The relative intensity at time point 0

Source

https://github.com/CyanolabFreiburg/rifi

rifi_fit

rifi_fit

%

rifi_fit wraps conveniently all fitting steps

Description

rifi_fit wraps the functions:

- 1. nls2_fit
- 2. TI_fit
- 3. plot_nls2_function
- 4. plot_singleProbe_function

rifi_fit 53

Usage

```
rifi_fit(
  inp,
  cores = 1,
  viz = FALSE,
  restr = 0.2,
  decay = seq(0.08, 0.11, by = 0.02),
  delay = seq(0, 10, by = 0.1),
  k = seq(0.1, 1, 0.2),
  bg = 0.2,
  TI_k = seq(0, 1, by = 0.5),
  TI_{decay} = c(0.05, 0.1, 0.2, 0.5, 0.6),
  TI = seq(0, 1, by = 0.5),
  TI_{delay} = seq(0, 2, by = 0.5),
  TI_rest_delay = seq(0, 2, by = 0.5),
  TI_bg = 0
)
```

Arguments

inp	SummarizedExperiment: the input with correct format.
cores	integer: the number of assigned cores for the task.
viz	logical: whether to visualize the output.
restr	numeric: a parameter that restricts the freedom of the fit to avoid wrong TI-term_factors, ranges from $0\ to\ 0.2$
decay	numeric vector: A sequence of starting values for the decay. Default is seq(.08, 0.11, by=.02)
delay	numeric vector: A sequence of starting values for the delay. Default is $seq(0,10, by=.1)$
k	numeric vector: A sequence of starting values for the synthesis rate. Default is $\sec(0.1,1,0.2)$
bg	numeric vector: A sequence of starting values. Default is 0.2.
TI_k	numeric vector: A sequence of starting values for the synthesis rate. Default is $seq(0, 1, by = 0.5)$.
TI_decay	numeric vector: A sequence of starting values for the decay. Default is $c(0.05, 0.1, 0.2, 0.5, 0.6)$.
TI	numeric vector: A sequence of starting values for the TI. Default is $seq(0, 1, by = 0.5)$.
TI_delay	numeric vector: A sequence of starting values for the delay. Default is $seq(0, 2, by = 0.5)$.
TI_rest_delay	numeric vector: A sequence of starting values. Default is $seq(0, 2, by = 0.5)$.
TI_bg	numeric vector: A sequence of starting values. Default is 0.

Value

the SummarizedExperiment object: with delay, decay and TI_{total} and

54 rifi_fragmentation

See Also

```
nls2_fit
TI_fit
plot_nls2
plot_singleProbe
```

Examples

```
data(preprocess_minimal)
rifi_fit(
  inp = preprocess_minimal,
  cores = 1, viz = FALSE, restr = 0.1,
  decay = seq(.08, 0.11, by = .02),
  delay = seq(0, 10, by = .1), k = seq(0.1, 1, 0.2), bg = 0.2,
  TI_k = seq(0, 1, by = 0.5), TI_decay = c(0.05, 0.1, 0.2, 0.5, 0.6),
  TI = seq(0, 1, by = 0.5), TI_delay = seq(0, 2, by = 0.5),
  TI_rest_delay = seq(0, 2, by = 0.5), TI_bg = 0
)
```

rifi_fragmentation

%

rifi_fragmentation

rifi_fragmentation wraps conveniently all fragmentation steps

Description

rifi_fragmentation is wrapper of the following functions:

- 1. fragment_delay
- 2. fragment_HL
- 3. fragment_inty
- 4. TUgether
- 5. fragment_TI

```
rifi_fragmentation(
   inp,
   cores = 1,
   pen_delay = NULL,
   pen_out_delay = NULL,
   pen_HL = NULL,
   pen_out_HL = NULL,
   pen_inty = NULL,
   pen_out_inty = NULL,
```

rifi_fragmentation 55

```
pen_TU = NULL,
pen_TI = NULL,
pen_out_TI = NULL
)
```

Arguments

SummarizedExperiment: the input data frame with correct format. inp integer: the number of assigned cores for the task. cores numeric: an internal parameter for the dynamic programming. Higher values pen_delay result in fewer fragments. Default is the auto generated value. numeric: an internal parameter for the dynamic programming. Higher values pen_out_delay result in fewer allowed outliers. Default is the auto generated value. pen_HL numeric: an internal parameter for the dynamic programming. Higher values result in fewer fragments. Default is the auto generated value. pen_out_HL numeric: an internal parameter for the dynamic programming. Higher values result in fewer allowed outliers. Default is the auto generated value. numeric: an internal parameter for the dynamic programming. Higher values pen_inty result in fewer fragments. Default is the auto generated value. numeric: an internal parameter for the dynamic programming. Higher values pen_out_inty result in fewer allowed outliers. Default is the auto generated value. numeric: an internal parameter for the dynamic programming. Higher values pen_TU result in fewer fragments. Default -0.75. pen_TI numeric: an internal parameter for the dynamic programming. Higher values result in fewer fragments. Default is the auto generated value.

Value

pen_out_TI

the SummarizedExperiment object: with delay_fragment, HL_fragment, intensity_fragment, TI_termination_fragment and TU, and the respective values added to the rowRanges.

numeric: an internal parameter for the dynamic programming. Higher values

result in fewer allowed outliers. Default is the auto generated value.

See Also

```
fragment_delay
fragment_HL
fragment_inty
TUgether
fragment_TI
```

Examples

```
data(penalties_minimal)
rifi_fragmentation(inp = penalties_minimal, cores = 2)
```

rifi_penalties

```
rifi_penalties %
```

rifi_penalties

rifi_penalties wraps conveniently all penalty steps

Description

rifi_penalties wraps the functions:

```
1. make_pen,
```

```
2. viz_pen_obj
```

Usage

```
rifi_penalties(
 inp,
 details = FALSE,
 viz = FALSE,
  top_i = 25,
 cores = 1,
 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 = 4.5,
 rez_pen_out = 9
)
```

Arguments

inp	SummarizedExperiment: the input data frame with correct format.
details	logical: whether to return the penalty objects or just the logbook.
viz	logical: whether to visualize the output or not. Default is FALSE
top_i	integer: the number of top results visualized. Default is all.
cores	integer: the number of assigned cores for the task.
dpt	integer: the number of times a full iteration cycle is repeated with a more narrow range based on the previous cycle. Default is 2.
smpl_min	integer: the smaller end of the sampling size. Default is 10.
smpl_max	integer: the larger end of the sampling size. Default is 100.
sta_pen	numeric: the lower starting penalty. Default is 0.5.
end_pen	numeric: the higher starting penalty. Default is 4.5.
rez_pen	numeric: the number of penalties iterated within the penalty range. Default is 9.

rifi_preprocess 57

```
sta_pen_out numeric: the lower starting outlier penalty. Default is 0.5.

end_pen_out numeric: the higher starting outlier penalty. Default is 3.5.

rez_pen_out numeric: the number of outlier penalties iterated within the outlier penalty range. Default is 7.
```

Value

The SummarizedExperiment object: with the penalties in the logbook added to the metadata. Also adds logbook_details if details is TRUE, and plots the penalties if viz is TRUE.

See Also

```
make_pen
viz_pen_obj
```

Examples

```
data(fit_minimal)
rifi_penalties(
  inp = fit_minimal, details = FALSE, viz = FALSE,
  top_i = 25, cores = 2, 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 = 4.5, rez_pen_out = 9
)
```

rifi_preprocess

%

rifi_preprocess

rifi_preprocess wraps conveniently all pre-processing steps

Description

rifi_preprocess wraps the functions:

- 1. check_input
- 2. make_df
- 3. function_seg
- 4. finding_PDD
- 5. finding_TI

58 rifi_preprocess

Usage

```
rifi_preprocess(
  inp,
  cores,
  FUN_filter = function(x) {
                                   FALSE },
  bg = 0,
  rm_FLT = FALSE,
  thrsh\_check = 0,
  dista = 300,
  run_PDD = FALSE,
  pen_PDD = 2,
 pen_out_PDD = 1,
  thrsh_PDD = 0.001,
  pen_TI = 10,
  thrsh TI = 0.5.
  add = 1000
)
```

Arguments

inp	SummarizedExperiment:	the input.

cores integer: the number of assigned cores for the task.

FUN_filter function: A function of x, returning a logical. x is the numeric vector of the

intensity from all time points for a specific replicate.

bg numeric: threshold over which the last time point has to be to be fitted with the

above background mode.

rm_FLT logical: remove IDs where all replicates are marked as filtered by the back-

ground check. Default is FALSE.

thrsh_check numeric: the minimal allowed intensity for time point "0". Advised to be kept

at 0! Default is 0.

dista integer: the amount of nucleotides defining the gap. Default is 300.

run_PDD logical: running the PDD flag function

pen_PDD numeric: an internal parameter for the dynamic programming. Higher values

result in fewer fragments. Advised to be kept at 2. Default is 2.

result in fewer possible outliers. Advised to be kept at 1. Default is 1.

thrsh_PDD numeric: an internal parameter that allows fragments with slopes steeper than

the threshold to be flagged with "PDD". Higher values result in fewer candidates

. Advised to be kept at 0.001. Default is 0.001.

pen_TI numeric: an internal parameter for the dynamic programming. Higher values

result in fewer fragments. Advised to be kept at 10. Default is 10.

thrsh_TI numeric: an internal parameter that allows fragments with a certain amount of

IDs with higher relative intensities at time points later than "0" to be flagged as "TI". Higher values result in fewer candidates. -0.5 is 25 %, 0 is 50%, 0.5 is

75%. Advised to be kept at 0.5. Default is 0.5.

add integer: range of nucleotides before a potential TI event where in IDs are fitted

with the TI fit.

rifi_stats 59

Details

rifi_preprocess allows for the optional integration of filter functions. Filter functions mark replicates with TRUE. Those are then not considered in the fit! FUN_filter is a general filter usually to exclude probes with low expression or "bad" patterns.

Value

The SummarizedExperiment object: checked, and with position, ID, intensity, probe_TI, position_segment, flag and filtration added to the rowRanges.

See Also

```
check_input
make_df
segment_pos
finding_PDD
finding_TI
```

Examples

```
data(example_input_minimal)
rifi_preprocess(
  inp = example_input_minimal, cores = 2, bg = 100, rm_FLT = FALSE,
  thrsh_check = 0, dista = 300, run_PDD = FALSE
  )
```

rifi_stats

rifi_stats wraps all statistical prediction steps conveniently

Description

rifi_stats wraps the functions:

- 1. predict_ps_itss
- 2. apply_Ttest_delay
- 3. apply_ancova
- 4. apply_event_position
- 5. apply_t_test
- 6. fold_change
- 7. apply_manova
- 8. apply_t_test_ti
- 9. gff3_preprocess

```
rifi_stats(inp, dista = 300, path)
```

60 rifi_summary

Arguments

inp SummarizedExperiment: the input data frame with correct format.

dista integer: the maximal distance allowed between two successive fragments. De-

fault is the auto generated value.

path path: to the directory containing the gff3 file.

Value

The SummarizedExperiment object: 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_event, p_value_Manova, p_value_TI, TI_fragments_p_value

See Also

```
predict_ps_itss
apply_Ttest_delay
apply_ancova
apply_event_position
apply_t_test
fold_change
apply_manova
apply_t_test_ti
gff3_preprocess
```

Examples

```
data(fragmentation_minimal)
rifi_stats(inp = fragmentation_minimal, dista = 300,
path = gzfile(system.file("extdata", "gff_e_coli.gff3.gz",
package = "rifi")))
```

rifi_summary

%

rifi_summary

rifi_summary wraps conveniently and summarize all rifi outputs

rifi_summary 61

Description

rifi_summary wraps the functions:

- 1. event_dataframe
- 2. dataframe_summary
- 3. dataframe_summary_events
- 4. dataframe_summary_events_HL_int
- 5. dataframe_summary_events_ps_itss
- 6. dataframe_summary_events_velocity
- 7. dataframe_summary_TI

Usage

```
rifi_summary(inp, data_annotation = metadata(inp)$annot[[1]])
```

Arguments

```
inp SummarizedExperiment: the input data frame with correct format. data_annotation dataframe: gff3 dataframe after processing.
```

Value

WIP

See Also

```
event_dataframe
dataframe_summary
dataframe_summary_events
dataframe_summary_events_HL_int
dataframe_summary_events_ps_itss
dataframe_summary_events_velocity
dataframe_summary_TI
```

Examples

```
data(stats_minimal)
if(!require(SummarizedExperiment)){
suppressPackageStartupMessages(library(SummarizedExperiment))
}
rifi_summary(inp = stats_minimal, data_annotation =
metadata(stats_minimal)$annot[[1]])
```

```
rifi_visualization %
```

rifi_visualization

rifi_visualization plots all the data with fragments and events from both strands

Description

rifi_visualization plots the whole genome with genes, transcription units (TUs), delay, half-life (HL), intensity fragments, features, events, velocity, annotation, coverage if available.

```
rifi_visualization(
 data,
 genomeLength,
 annot,
 coverage = 0,
 chr_fwd = NA,
 chr_rev = NA,
 region = c("CDS", "asRNA", "5'UTR", "ncRNA", "3'UTR", "tRNA"),
 color_region = c("grey0", "red", "blue", "orange", "yellow", "green", "white",
    "darkseagreen1", "grey50", "black"),
 color_text.1 = "grey0",
 color_text.2 = "black",
 color_TU = "blue",
 Alpha = 0.5,
  size_tu = 1.6,
  size_locusTag = 1.6,
  size\_gene = 1.6,
 Limit = 10,
  shape = 22,
  col_outiler = "grey50",
  col_coverage = "grey",
  shape_outlier = 13,
 limit_intensity = NA,
  face = "bold",
  tick_length = 0.3,
 arrow.color = "darkseagreen1",
 minVelocity = 3000,
 medianVelocity = 6000,
 col_above20 = "#00FFFF",
  fontface = "plain",
  shape_above20 = 14,
  col_outlierabove10 = "darkorchid",
  shape_outlierabove10 = 5,
  axis_text_y_size = 3,
  axis_title_y_size = 6,
```

```
TI_threshold = 1.1,
termination_threshold = -0.5,
iTSS_threshold = 0.5,
p_value_int = 0.05,
p_value_event = 0.05,
p_value_hl = 0.05,
p_value_TI = 0.05,
p_value_manova = 0.05,
event_duration_ps = 1,
event_duration_itss = -1,
HL_threshold_1 = log_2(1.5),
HL_{threshold_2} = -log_2(1.5),
vel_threshold = 200,
HL_threshold_color = "black",
vel_threshold_color = "grey52",
ps_color = "orange",
iTSS_I_color = "blue"
```

Arguments

data SummarizedExperiment: the input data frame with correct format.

genomeLength integer: genome length output of gff3_preprocess function and element of meta-

data of SummarizedExperiment.

annot dataframe: the annotation file, output of gff3_preprocess function and element

of metadata of SummarizedExperiment.

coverage integer: in case the coverage is available.

chr_fwd string object: coverage of the forward strand.
chr_rev string object: coverage of the reverse strand.

region dataframe: gff3 features of the genome.

color_region string vector: vector of colors.

color_text.1 string: TU color text
color_text.2 string: genes color text

color_TU string. TU color

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. col_outiler string: outlier color.

col_coverage integer: color for coverage plot. shape_outlier integer: value for outlier shape.

limit_intensity

integer: intensity limit if applicable.

face string: label font.

tick_length integer: value for ticks. arrow.color string: arrows color. minVelocity integer: threshold to fix the minimum of velocity. medianVelocity integer: threshold to fix the maximum of velocity. col_above20 string: color for probes/bin above value 20. fontface integer: font type shape_above20 integer: shape for probes/bins above value 20. col_outlierabove10 string: color for probes/bin outliers between 10 and 20, shape_outlierabove10 integer: shape for probes/bin outliers between 10 and 20, axis_text_y_size integer: text size for y-axis. axis_title_y_size integer: title size for y-axis. TI_threshold integer: threshold for TI between two fragments in case the TI termination factor drops from the first segment to the second, default 1.1. If threshold is reached a line is drawn to seperates the two TI segments. termination_threshold integer: threshold for termination to plot, default .8. iTSS_threshold integer: threshold for iTSS_II selected to plot, default 1.2. integer: p_value of intensity fragments fold-change to plot, default 0.05. p_value_int integer: p_value of t-test from pausing site and iTSS_I events to plot, default p_value_event 0.05. p_value_hl integer: p_value of half_life fragments fold-change to plot, default 0.05. p_value_TI integer: p_value of TI fragments selected to be plotted, default 0.05. p_value_manova integer: p_value of manova test fragments to plot, default 0.05. event_duration_ps integer: threshold for pausing sites selected to plot, default -2. event_duration_itss integer: threshold for iTSS_I selected to plot, default 2. HL_threshold_1 integer: threshold for log2FC(HL) selected to plot, default log2(1.5). log2FC(HL) >= log2(1.5) are indicated by black color. If p_value $<= p_value_hl$ (default 0.05), log2FC(HL) is indicated by HL* otherwise HL. HL_threshold_2 integer: threshold for log2FC(HL) selected to plot, default -log2(1.5). log2FC(HL) <= -log2(1.5) are indicated by green color. If p_value <= p_value_hl (default 0.05), log2FC(HL) is indicated by HL* otherwise HL. In case of p_value is significant and the log2FC(HL) is between -log2FC(1.5) and log2FC(1.5), FC is assigned by green color and HL*. integer: threshold for velocity ratio selected to plot, default 200. vel threshold HL_threshold_color string: color for HL fold change plot. vel_threshold_color string: color for velocity ratio plot. ps_color string: color for pausing site plot. string: color for iTSS_I plot. iTSS_I_color

Details

rifi_visualization uses several functions to plot the genes including as-RNA and ncRNA and TUs as segments. The function plots delay, HL and intensity fragments with statistical t-test between the neighboring fragment, significant t-test is assigned with ''. t-test and Manova statistical test are also depicted as ''.

The functions used are:

- 1. annotation_plot: plots the corresponding annotation.
- 2. positive_strand_function: plots delay, HL, intensity and events of positive strand.
- 3. negative_strand_function: plots delay, HL, intensity and events of negative strand.
- 4. empty_data_positive: plots empty boxes in case no data is available for positive strand.
- 5. empty_data_negative: plots empty boxes in case no data is available for negative strand.
- 6. strand_selection: check if data is stranded and arrange by position.
- 7. splitGenome function: splits the genome into fragments.
- 8. indice_function: assign a new column to the data to distinguish between fragments, outliers from delay or HL or intensity.
- 9. TU annotation: designs the segments border for the genes and TUs annotation
- 10. 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.
- 11. label_log2_function: used to add log scale to intensity values.
- 12. label_square_function: used to add square scale to coverage values.
- 13. coverage function: this function is used only in case of coverage is available.
- 14. 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.
- 15. outlier_plot: plot the outliers with half-life between 10 and 30 on the maximum of the yaxis.
- 16. add_genomeBorders: when the annotated genes are on the borders, they can not be plotted, therefore the region was split in 2 adding the row corresponding to the split part to the next annotation (i + 1) except for the first page.
- 17. my_arrow: creates an arrow for the annotation.
- 18. arrange_byGroup: selects the last row for each segment and add 40 nucleotides in case of negative strand for a nice plot.
- 19. regr: plots the predicted delay from linear regression if the data is on negative strand.
- 20. meanPosition: assign a mean position for the plot.
- 21. delay_mean: adds a column in case of velocity is NA or equal to 60. The mean of the delay is calculated outliers.
- 22. my_segment_T: plots terminals and pausing sites labels.
- 23. my segment NS: plots internal starting sites 'iTSS'.
- 24. min_value: returns minimum value for event plots in intensity plot.
- 25. velocity_fun: function for velocity plot.
- 26. limit_function: for values above 10 or 20 in delay and hl. Limit of the axis is set differently. y-axis limit is applied only if we have more than 3 values above 10 and lower or equal to 20. An exception is added in case a dataframe has less than 3 rows and 1 or more values are above 10, the rest of the values above 20 are adjusted to 20 on "secondaryAxis" function.

66 rifi_wrapper

27. empty_boxes: used only in case the dataframe from the positive strand is not empty, the TU are annotated.

- 28. function_TU_arrow: used to avoid plotting arrows when a TU is split into two pages.
- 29. terminal_plot_lm: draws a linear regression line when terminal outliers have an intensity above a certain threshold and are consecutive. Usually are smallRNA (ncRNA, asRNA).
- 30. slope_function: replaces slope lower than 0.0009 to 0.
- 31. velo_function: replaces infinite velocity with NA.
- 32. plot the coverage of RNA_seq in exponential phase growth

Value

The visualization plot.

Examples

```
data(stats_minimal)
if(!require(SummarizedExperiment)){
suppressPackageStartupMessages(library(SummarizedExperiment))
}
rifi_visualization(data = stats_minimal,
genomeLength = metadata(stats_minimal)$annot[[2]],
annot = metadata(stats_minimal)$annot[[1]])
```

rifi_wrapper

%

rifi_wrapper

rifi_wrapper wraps conveniently all functions included on rifi workflow

Description

rifi_wrapper wraps the functions:

- 1. rifi_preprocess
- 2. rifi_fit
- 3. rifi_penalties
- 4. rifi_fragmentation
- 5. rifi_stats
- 6. rifi_summary
- 7. rifi_visualization.

```
rifi_wrapper(inp, cores, path, bg, restr)
```

segment_pos 67

Arguments

inp data frame: the input data frame with correct format.

cores integer: the number of assigned cores for the task.

path path: path to an annotation file in gff format.

bg numeric: threshold over which the last time point has to be to be fitted with the

above background mode.

restr numeric: a parameter that restricts the freedom of the fit to avoid wrong TI-

term_factors, ranges from 0 to 0.2

Value

All intermediate objects

See Also

```
rifi_preprocess
rifi_fit
rifi_penalties
rifi_fragmentation
rifi_stats
rifi_summary
rifi_visualization
```

Examples

```
data(example_input_minimal)
rifi_wrapper(inp = example_input_minimal, cores = 2, path =
gzfile(system.file("extdata", "gff_e_coli.gff3.gz", package = "rifi")),
bg = 0, restr = 0.01)
```

segment_pos %

 $segment_pos$

segment_pos divides all IDs by position into position_segments

Description

segment_pos adds the column "position_segment" to the rowRanges. To reduce run time, the data is divided by regions of no expression larger than "dist" nucleotides.

```
segment_pos(inp, dista = 300)
```

68 stats_e_coli

Arguments

inp SummarizedExperiment: the input.

dista integer: the amount of nucleotides defining the gap. Default is 300.

Value

The SummarizedExperiment object:

ID: The bin/probe specific IDposition: The bin/probe specific positionintensity: 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

Examples

```
data(preprocess_minimal)
segment_pos(inp = preprocess_minimal, dista = 300)
```

stats_e_coli The result of rifi_stats for E.coli example data A SummarizedExperi-

ment containing the output from rifi_stats

Description

The result of rifi_stats for E.coli example data A SummarizedExperiment containing the output from rifi_stats

Usage

```
data(stats_e_coli)
```

Format

A SummarizedExperiment:

ID: The bin/probe specific ID

position: The bin/probe specific position **strand:** The bin/probe specific strand

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

stats_e_coli 69

TI_termination_factor: String, the factor of TI fragment **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

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

70 stats_minimal

i	The result of rifi_stats for artificial example data A SummarizedExperiment containing the output of rifi_stats as an extention to rowRanges and metadata (gff file processed, see gff file documentation)
	(db J

Description

The result of rifi_stats for artificial example data A SummarizedExperiment containing the output of rifi_stats as an extention to rowRanges and metadata (gff file processed, see gff file documentation)

Usage

```
data(stats_minimal)
```

Format

A rowRanges of SummarizedExperiment with 24 rows and 45 variables:

ID: The bin/probe specific ID

position: The bin/probe specific position

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: String, the factor of TI fragment **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 fragment

 $\boldsymbol{slope:}\,$ 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

Source

https://github.com/CyanolabFreiburg/rifi

stats_synechocystis_6803

The result of rifi_stats for Synechocystis 6803 example data A SummarizedExperiment containing the output of rifi_stats as an extention to rowRanges

Description

The result of rifi_stats for Synechocystis 6803 example data A SummarizedExperiment containing the output of rifi_stats as an extention to rowRanges

Usage

data(stats_synechocystis_6803)

Format

The rowRanges of SummarizedExperiment:

ID: The bin/probe specific ID

position: The bin/probe specific position

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 appliedposition_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: String, the factor of TI fragment **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

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

summary_e_coli 73

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/rifi

summary_e_coli

The result of rifi_summary for E.coli example data A Summarized-Experiment containing the output of rifi_stats as an extention to rowRanges

Description

The result of rifi_summary for E.coli example data A SummarizedExperiment containing the output of rifi_stats as an extention to rowRanges

Usage

data(summary_e_coli)

Format

The rowRanges of SummarizedExperiment:

bin_df: all information regarding bins:

ID: The bin/probe specific ID

feature_type: String, region annotation covering the fragments

gene: String, gene annotation covering the fragments

locus tag: String, locus tag annotation covering the fragments

position: The bin/probe specific position **strand:** The bin/probe specific strand

segment: The segment the bin/probe belongs to

TU: The overarching transcription unit

delay_fragment: The delay fragment the bin/probe belongs to

delay: The delay of the bin/probe

HL_fragment: The half-life fragment the bin/probe belongs to

half_life: The half-life of the bin/probe

intensity_fragment: The intensity fragment the bin/probe belongs to

intensity: The relative intensity at time point 0

74 summary_e_coli

flag: The flag of the bin/probe(TI, PDD)

TI_termination_factor: String, the factor of TI fragment

frag_df: all information regarding fragments:

feature type: String, region annotation covering the fragments

gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragmentsfirst_position_frg: The first position of the fragment on the genomelast_position_frg: The last position of the fragment on the genome

strand: The bin/probe specific strand **TU:** The overarching transcription unit

segment: The segment the fragment belongs todelay_fragment: The delay fragment of the fragmentHL_fragment: The half-life fragment of the fragment

half_life: The half-life mean of the fragment

HL_SD: The half-life standard deviation of the fragmentHL_SE: The half-life standard error of the fragment

intensity_fragment: The intensity_fragment of the fragment

intensity: The relative intensity at time point 0

intensity_SD: The intensity standard deviation of the fragmentintensity_SE: The intensity standard error of the fragmentvelocity: The velocity value of the respective delay fragment

event df: all information regarding events:

event: String, event type

p_value: Integer, p_value of the eventp_adjusted: Integer, p_value adjusted

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

FC intensity: Fold change of intensity

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

FC_HL_FC_intensity: Fold change of half-life/ fold change of intensity

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

velocity_ratio: Integer, ratio of velocity between 2 delay fragments **feature_type:** String, region annotation covering the fragments

gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragments

strand: The bin/probe specific strand **TU:** The overarching transcription unit

segment_1: String, the first fragment of the two of fragments subjected to analysissegment_2: String, the second fragment of the two of fragments subjected to analysis

event_duration: Integer, the duration between two delay fragments **gap_fragments:** Integer, the distance between two delay fragments **features:** Integer, number of fragements involved on the event

events_HL_int_df: all information regarding events related to half-life and intensity:

event: String, event type

p_value: Integer, p_value of the event

summary_e_coli 75

p adjusted: Integer, p value adjusted **FC_HL:** Integer, the fold change value of 2 HL fragments **FC_intensity:** Integer, the fold change value of 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 FC_HL_FC_intensity: Fold change of half-life/ fold change of intensity event_position: Integer, the position middle between 2 fragments with an event feature_type: String, region annotation covering the fragments gene: String, gene annotation covering the fragments locus tag: String, locus tag annotation covering the fragments **strand:** The bin/probe specific strand TU: The overarching transcription unit **segment 1:** String, the first fragment of the two of fragments subjected to analysis segment 2: String, the second fragment of the two of fragments subjected to analysis event_duration: Integer, the duration between two delay fragments gap fragments: Integer, the distance between two delay fragments features: Integer, number of fragements involved on the event events_ps_itss_df: all information regarding events related to pausing sites and iTSS_I: event: String, event type **p_value:** Integer, p_value of the event p_adjusted: Integer, p_value adjusted event_position: Integer, the position middle between 2 fragments with an event **velocity ratio:** Integer, ratio of velocity between 2 delay 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 **feature_type:** String, region annotation covering the fragments gene: String, gene annotation covering the fragments locus_tag: String, locus_tag annotation covering the fragments strand: The bin/probe specific strand **TU:** The overarching transcription unit **segment_1:** String, the first fragment of the two of fragments subjected to analysis segment_2: String, the second fragment of the two of fragments subjected to analysis event duration: Integer, the duration between two delay fragments gap_fragments: Integer, the distance between two delay fragments features: Integer, number of fragements involved on the event events velocity df: all information regarding events related to velocity: event: String, event type **p_value:** Integer, p_value of the event p_adjusted: Integer, p_value adjusted **event_position:** Integer, the position middle between 2 fragments with an event **velocity_ratio:** Integer, ratio of velocity between 2 delay fragments feature_type: String, region annotation covering the fragments gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragments

strand: The bin/probe specific strand

TU: The overarching transcription unit

segment_1: String, the first fragment of the two of fragments subjected to analysissegment_2: String, the second fragment of the two of fragments subjected to analysis

event_duration: Integer, the duration between two delay fragments **gap_fragments:** Integer, the distance between two delay fragments **features:** Integer, number of fragements involved on the event

TI_df: all information regarding TI:

event: String, event type

TI_fragment: String, the fragment with TI

TI_termination_factor: String, the factor of TI fragment

p_value: Integer, p_value of the eventp_adjusted: Integer, p_value adjusted

feature_type: String, region annotation covering the fragments

gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragments

strand: The bin/probe specific strand **TU:** The overarching transcription unit

features: Integer, number of fragements involved on the event

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

position_1: the first position of TI fragment, if 2 fragments, first position is from the first fragment

position_2: the last position of TI fragment, if 2 fragments, last position is from the second fragment.

Source

https://github.com/CyanolabFreiburg/rifi

summary_minimal

The result of rifi_summary for artificial example data A Summarized-Experiment with the output from rifi_summary as metadata

Description

The result of rifi_summary for artificial example data A SummarizedExperiment with the output from rifi_summary as metadata

Usage

data(summary_minimal)

Format

A list of 7 data frames with 290 rows and 11 variables, 36 rows and 11 variables, 57 rows and 18 variables, and 8 rows and 14 variables:

bin_df: all information regarding bins:

ID: The bin/probe specific ID

feature_type: String, region annotation covering the fragments

gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragments

position: The bin/probe specific position **strand:** The bin/probe specific strand

segment: The segment the bin/probe belongs to

TU: The overarching transcription unit

delay_fragment: The delay fragment the bin/probe belongs to

delay: The delay of the bin/probe

HL_fragment: The half-life fragment the bin/probe belongs to

half_life: The half-life of the bin/probe

intensity_fragment: The intensity fragment the bin/probe belongs to

intensity: The relative intensity at time point 0 **flag:** The flag of the bin/probe(TI, PDD)

TI_termination_factor: String, the factor of TI fragment

frag_df: all information regarding fragments:

feature_type: String, region annotation covering the fragments

gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragmentsfirst_position_frg: The first position of the fragment on the genomelast_position_frg: The last position of the fragment on the genome

strand: The bin/probe specific strand **TU:** The overarching transcription unit

segment: The segment the fragment belongs to

delay_fragment: The delay fragment of the fragment **HL_fragment:** The half-life fragment of the fragment

half_life: The half-life mean of the fragment

HL_SD: The half-life standard deviation of the fragment **HL SE:** The half-life standard error of the fragment

intensity_fragment: The intensity_fragment of the fragment

intensity: The relative intensity at time point 0

intensity_SD: The intensity standard deviation of the fragmentintensity_SE: The intensity standard error of the fragmentvelocity: The velocity value of the respective delay fragment

event df: all information regarding events:

event: String, event type

p_value: Integer, p_value of the eventp adjusted: Integer, p value adjusted

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

FC_intensity: Fold change of intensity

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

FC_HL_FC_intensity: Fold change of half-life/ fold change of intensity

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

velocity_ratio: Integer, ratio of velocity between 2 delay fragments

feature type: String, region annotation covering the fragments gene: String, gene annotation covering the fragments locus_tag: String, locus_tag annotation covering the fragments strand: The bin/probe specific strand TU: The overarching transcription unit **segment_1:** String, the first fragment of the two of fragments subjected to analysis segment_2: String, the second fragment of the two of fragments subjected to analysis event_duration: Integer, the duration between two delay fragments gap fragments: Integer, the distance between two delay fragments features: Integer, number of fragements involved on the event events HL int df: all information regarding events related to half-life and intensity: event: String, event type **p_value:** Integer, p_value of the event p adjusted: Integer, p value adjusted FC_HL: Integer, the fold change value of 2 HL fragments FC_intensity: Integer, the fold change value of 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 FC_HL_FC_intensity: Fold change of half-life/ fold change of intensity event_position: Integer, the position middle between 2 fragments with an event feature_type: String, region annotation covering the fragments gene: String, gene annotation covering the fragments locus_tag: String, locus_tag annotation covering the fragments strand: The bin/probe specific strand TU: The overarching transcription unit **segment 1:** String, the first fragment of the two of fragments subjected to analysis **segment_2:** String, the second fragment of the two of fragments subjected to analysis event_duration: Integer, the duration between two delay fragments gap fragments: Integer, the distance between two delay fragments features: Integer, number of fragements involved on the event events_ps_itss_df: all information regarding events related to pausing sites and iTSS_I: event: String, event type **p_value:** Integer, p_value of the event **p_adjusted:** Integer, p_value adjusted event_position: Integer, the position middle between 2 fragments with an event **velocity_ratio:** Integer, ratio of velocity between 2 delay 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 **feature_type:** String, region annotation covering the fragments gene: String, gene annotation covering the fragments **locus_tag:** String, locus_tag annotation covering the fragments strand: The bin/probe specific strand TU: The overarching transcription unit segment_1: String, the first fragment of the two of fragments subjected to analysis segment_2: String, the second fragment of the two of fragments subjected to analysis

event_duration: Integer, the duration between two delay fragmentsgap_fragments: Integer, the distance between two delay fragmentsfeatures: Integer, number of fragements involved on the event

events_velocity_df: all information regarding events related to velocity:

event: String, event type

p_value: Integer, p_value of the eventp_adjusted: Integer, p_value adjusted

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

velocity_ratio: Integer, ratio of velocity between 2 delay fragments **feature_type:** String, region annotation covering the fragments

gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragments

strand: The bin/probe specific strand **TU:** The overarching transcription unit

segment_1: String, the first fragment of the two of fragments subjected to analysissegment_2: String, the second fragment of the two of fragments subjected to analysis

event_duration: Integer, the duration between two delay fragments **gap_fragments:** Integer, the distance between two delay fragments **features:** Integer, number of fragements involved on the event

TI_df: all information regarding TI:

event: String, event type

TI_fragment: String, the fragment with TI

TI_termination_factor: String, the factor of TI fragment

p_value: Integer, p_value of the eventp_adjusted: Integer, p_value adjusted

feature_type: String, region annotation covering the fragments

gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragments

strand: The bin/probe specific strand **TU:** The overarching transcription unit

features: Integer, number of fragements involved on the event

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

position_1: the first position of TI fragment, if 2 fragments, first position is from the first fragment

position_2: the last position of TI fragment, if 2 fragments, last position is from the second fragment.

Source

https://github.com/CyanolabFreiburg/rifi

summary_synechocystis_6803

The result of rifi_summary for Synechocystis 6803 example data A list containing the output from rifi_summary, including the fragment based data frame, bin based data frame, event data frame and the TI dataframe.

Description

The result of rifi_summary for Synechocystis 6803 example data A list containing the output from rifi_summary, including the fragment based data frame, bin based data frame, event data frame and the TI dataframe.

Usage

data(summary_synechocystis_6803)

Format

A list of 4 data frames with 3000 rows and 11 variables, 297 rows and 11 variables, 486 rows and 18 variables, and 10 rows and 14 variables:

bin_df: all information regarding bins:

ID: The bin/probe specific ID

feature_type: String, region annotation covering the fragments

gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragments

position: The bin/probe specific position **strand:** The bin/probe specific strand

segment: The segment the bin/probe belongs to

TU: The overarching transcription unit

delay fragment: The delay fragment the bin/probe belongs to

delay: The delay of the bin/probe

HL_fragment: The half-life fragment the bin/probe belongs to

half_life: The half-life of the bin/probe

intensity_fragment: The intensity fragment the bin/probe belongs to

intensity: The relative intensity at time point 0 **flag:** The flag of the bin/probe(TI, PDD)

TI_termination_factor: String, the factor of TI fragment

frag_df: all information regarding fragments:

feature type: String, region annotation covering the fragments

gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragmentsfirst_position_frg: The first position of the fragment on the genomelast_position_frg: The last position of the fragment on the genome

strand: The bin/probe specific strand **TU:** The overarching transcription unit

segment: The segment the fragment belongs todelay_fragment: The delay fragment of the fragment

HL_fragment: The half-life fragment of the fragment

half_life: The half-life mean of the fragment

HL_SD: The half-life standard deviation of the fragmentHL_SE: The half-life standard error of the fragment

intensity_fragment: The intensity_fragment of the fragment

intensity: The relative intensity at time point 0

intensity_SD: The intensity standard deviation of the fragmentintensity_SE: The intensity standard error of the fragmentvelocity: The velocity value of the respective delay fragment

event_df: all information regarding events:

event: String, event type

p_value: Integer, p_value of the eventp_adjusted: Integer, p_value adjusted

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

FC_intensity: Fold change of intensity

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

FC_HL_FC_intensity: Fold change of half-life/ fold change of intensity

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

velocity_ratio: Integer, ratio of velocity between 2 delay fragments **feature_type:** String, region annotation covering the fragments

gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragments

strand: The bin/probe specific strand **TU:** The overarching transcription unit

segment_1: String, the first fragment of the two of fragments subjected to analysissegment_2: String, the second fragment of the two of fragments subjected to analysis

event_duration: Integer, the duration between two delay fragments **gap_fragments:** Integer, the distance between two delay fragments **features:** Integer, number of fragements involved on the event

events_HL_int_df: all information regarding events related to half-life and intensity:

event: String, event type

p_value: Integer, p_value of the eventp_adjusted: Integer, p_value adjusted

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

FC_intensity: Integer, the fold change value of 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

FC_HL_FC_intensity: Fold change of half-life/ fold change of intensity

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

feature_type: String, region annotation covering the fragments

gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragments

strand: The bin/probe specific strand **TU:** The overarching transcription unit segment_1: String, the first fragment of the two of fragments subjected to analysis segment_2: String, the second fragment of the two of fragments subjected to analysis event duration: Integer, the duration between two delay fragments gap_fragments: Integer, the distance between two delay fragments features: Integer, number of fragements involved on the event events ps itss df: all information regarding events related to pausing sites and iTSS I: event: String, event type p_value: Integer, p_value of the event **p_adjusted:** Integer, p_value adjusted event_position: Integer, the position middle between 2 fragments with an event velocity_ratio: Integer, ratio of velocity between 2 delay 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 **feature_type:** String, region annotation covering the fragments gene: String, gene annotation covering the fragments locus_tag: String, locus_tag annotation covering the fragments strand: The bin/probe specific strand **TU:** The overarching transcription unit **segment_1:** String, the first fragment of the two of fragments subjected to analysis segment_2: String, the second fragment of the two of fragments subjected to analysis event_duration: Integer, the duration between two delay fragments gap fragments: Integer, the distance between two delay fragments features: Integer, number of fragements involved on the event events_velocity_df: all information regarding events related to velocity: event: String, event type **p_value:** Integer, p_value of the event p_adjusted: Integer, p_value adjusted event_position: Integer, the position middle between 2 fragments with an event velocity_ratio: Integer, ratio of velocity between 2 delay fragments feature_type: String, region annotation covering the fragments gene: String, gene annotation covering the fragments locus tag: String, locus tag annotation covering the fragments strand: The bin/probe specific strand **TU:** The overarching transcription unit segment_1: String, the first fragment of the two of fragments subjected to analysis segment_2: String, the second fragment of the two of fragments subjected to analysis event_duration: Integer, the duration between two delay fragments gap fragments: Integer, the distance between two delay fragments features: Integer, number of fragements involved on the event **TI_df:** all information regarding TI:

event: String, event type

TI_fragment: String, the fragment with TI

TI_termination_factor: String, the factor of TI fragment

TI_fit

p_value: Integer, p_value of the eventp_adjusted: Integer, p_value adjusted

feature_type: String, region annotation covering the fragments

gene: String, gene annotation covering the fragments

locus_tag: String, locus_tag annotation covering the fragments

strand: The bin/probe specific strand **TU:** The overarching transcription unit

features: Integer, number of fragements involved on the event

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

position_1: the first position of TI fragment, if 2 fragments, first position is from the first fragment

position_2: the last position of TI fragment, if 2 fragments, last position is from the second fragment.

Source

https://github.com/CyanolabFreiburg/rifi

TI_fit

%

====

TI_fit

TI_fit estimates transcription interference and termination factor using nls function for probe or bin flagged as "TI".

Description

TI_fit uses nls2 function to fit the flagged probes or bins with "TI" found using finding_TI.r. It estimates the transcription interference level (referred later to TI) as well as the transcription factor fitting the probes/bins with nls function looping into several starting values.

Usage

```
TI_fit(
  inp,
  cores = 1,
  restr = 0.2,
  k = seq(0, 1, by = 0.5),
  decay = c(0.05, 0.1, 0.2, 0.5, 0.6),
  ti = seq(0, 1, by = 0.5),
  ti_delay = seq(0, 2, by = 0.5),
  rest_delay = seq(0, 2, by = 0.5),
  bg = 0
)
```

84 *TI_fit*

Arguments

inp	SummarizedExperiment: the input with correct format.
cores	integer: the number of assigned cores for the task.
restr	numeric: a parameter that restricts the freedom of the fit to avoid wrong TI-term_factors, ranges from $0\ {\rm to}\ 0.2.$
k	numeric vector: A sequence of starting values for the synthesis rate. Default is $seq(0, 1, by = 0.5)$.
decay	numeric vector: A sequence of starting values for the decay Default is $c(0.05, 0.1, 0.2, 0.5, 0.6)$.
ti	numeric vector: A sequence of starting values for the delay. Default is $seq(0, 1, by = 0.5)$.
ti_delay	numeric vector: A sequence of starting values for the delay. Default is $seq(0, 2, by = 0.5)$.
rest_delay	numeric vector: A sequence of starting values. Default is $seq(0, 2, by = 0.5)$.
bg	numeric vector: A sequence of starting values. Default is 0.

Details

To determine TI and termination factor, TI_fit function is applied to the flagged probes and to the probes localized 1000 nucleotides upstream. Before applying TI_fit function, some probes/bins are filtered out if they are below the background using generic_filter_BG. The model loops into a dataframe containing sequences of starting values and the coefficients are extracted from the fit with the lowest residuals. When many residuals are equal to 0, the lowest residual can not be determined and the coefficients extracted could be wrong. Therefore, a second filter was developed. First we loop into all starting values, we collect nls objects and the corresponding residuals. They are sorted and residuals non equal to 0 are collected in a vector. If the first residuals are not equal to 0, 20 % of the best residuals are collected in tmp_r_min vector and the minimum termination factor is selected. In case the first residuals are equal to 0 then values between 0 to 20% of the values collected in tmp_r_min vector are gathered. The minimum termination factor coefficient is determined and saved. The coefficients are gathered in res vector and saved as an object.

Value

the SummarizedExperiment object: with delay, decay and TI_termination_factor added to the rowRanges. The full fit data is saved in the metadata as "fit_TI".

Examples

```
data(preprocess_minimal)
TI_fit(inp = preprocess_minimal, cores=2, restr=0.01)
```

TUgether 85

TUgether %

TUgether

TUgether combines delay fragments into TUs

Description

TUgether combines delay fragments into TUs. The column "TU" is added. It uses score fun_increasing on the start and end points of delay_fragments.

Usage

```
TUgether(inp, cores = 1, pen = -0.75)
```

Arguments

inp SummarizedExperiment: the input data frame with correct format.

cores cores: integer: the number of assigned cores for the task.

pen numeric: an internal parameter for the dynamic programming. Higher values

result in fewer fragments. Default -0.75.

Details

The function used is: .score_fun_increasing

The input is the SummarizedExperiment object. pen is the penalty for new fragments in the dynamic programming. Since high scores are aimed, pen is negative.

Value

The SummarizedExperiment with the columns regarding the TU:

ID: The bin/probe specific ID.

position: The bin/probe specific position.

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: String, the factor of TI fragment. **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.

86 viz_pen_obj

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.

Examples

```
data(fragmentation_minimal)
TUgether(inp = fragmentation_minimal, cores = 2, pen = -0.75)
```

viz_pen_obj

%

viz_pen_obj

_pen_obj

Description

viz_pen_obj provides an optional visualization of any penalty object created by make_pen. the function aan be customized to show only the $n = top_i$ top results.

viz_pen_obj visualizes penalty objects

Usage

```
viz_pen_obj(obj, top_i = nrow(obj[[3]][[1]]) * ncol(obj[[3]][[1]]))
```

Arguments

obj object: penalty object(make_pen output)

top_i integer: the number of top results visualized. Default is all.

Value

A visualization of the penalty object

Examples

```
data(penalties_e_coli)
viz_pen_obj(penalties_e_coli$pen_obj_delay,25)
```

wrapper_e_coli 87

wrapper_e_coli

The result of rifi_wrapper for E.coli example data A list of SummarizedExperiment containing the output of rifi_wrapper. The list contains 6 elements of SummarizedExperiment output of rifi_preprocess, rifi_fit, rifi_penalties, rifi_fragmentation, rifi_stats and rifi_summary. The plot is generated from rifi_visualization. for more detail, please refer to each function separately.

Description

The result of rifi_wrapper for E.coli example data A list of SummarizedExperiment containing the output of rifi_wrapper. The list contains 6 elements of SummarizedExperiment output of rifi_preprocess, rifi_fit, rifi_penalties, rifi_fragmentation, rifi_stats and rifi_summary. The plot is generated from rifi_visualization. for more detail, please refer to each function separately.

Usage

```
data(wrapper_e_coli)
```

Format

An object of class list of length 6.

Source

https://github.com/CyanolabFreiburg/rifi

wrapper_minimal

The result of rifi_wrapper for E.coli artificial example. A list of SummarizedExperiment containing the output of rifi_wrapper. The list contains 6 elements of SummarizedExperiment output of rifi_preprocess, rifi_fit, rifi_penalties, rifi_fragmentation, rifi_stats and rifi_summary. The plot is generated from rifi_visualization. for more detail, please refer to each function separately.

Description

The result of rifi_wrapper for E.coli artificial example. A list of SummarizedExperiment containing the output of rifi_wrapper. The list contains 6 elements of SummarizedExperiment output of rifi_preprocess, rifi_fit, rifi_penalties, rifi_fragmentation, rifi_stats and rifi_summary. The plot is generated from rifi_visualization. for more detail, please refer to each function separately.

Usage

```
data(wrapper_minimal)
```

Format

An object of class list of length 6.

Source

https://github.com/CyanolabFreiburg/rifi

wrapper_summary_synechocystis_6803

The result of rifi_wrapper for summary_synechocystis_6803 example data A list of SummarizedExperiment containing the output of rifi_wrapper. The list contains 6 elements of SummarizedExperiment output of rifi_preprocess, rifi_fit, rifi_penalties, rifi_fragmentation, rifi_stats and rifi_summary. The plot is generated from rifi_visualization. for more detail, please refer to each function separately.

Description

The result of rifi_wrapper for summary_synechocystis_6803 example data A list of SummarizedExperiment containing the output of rifi_wrapper. The list contains 6 elements of SummarizedExperiment output of rifi_preprocess, rifi_fit, rifi_penalties, rifi_fragmentation, rifi_stats and rifi_summary. The plot is generated from rifi_visualization. for more detail, please refer to each function separately.

Usage

data(wrapper_summary_synechocystis_6803)

Format

An object of class list of length 6.

Source

https://github.com/CyanolabFreiburg/rifi

Index

```
* datasets
                                                dataframe_summary_TI, 20
    example_input_e_coli, 23
                                                event_dataframe, 21
    example_input_minimal, 24
                                                example_input_e_coli, 23
    example_input_synechocystis_6803,
                                                example_input_minimal, 24
                                                example_input_synechocystis_6803, 24
    fit_e_coli, 27
    fit_minimal, 28
                                                finding_PDD, 25
    fit_synechocystis_6803, 28
                                                finding_TI, 26
    fragmentation_e_coli, 31
                                                fit_e_coli, 27
    fragmentation_minimal, 32
                                                fit_minimal, 28
    fragmentation_synechocystis_6803,
                                                fit_synechocystis_6803, 28
                                                fold_change, 29
    penalties_e_coli, 44
                                                fragment_delay, 34
    penalties_minimal, 45
                                                fragment_HL, 35
    penalties_synechocystis_6803,46
                                                fragment_inty, 36
    preprocess_e_coli, 49
                                                fragment_TI, 38
    preprocess_minimal, 50
                                                fragmentation_e_coli, 31
    preprocess_synechocystis_6803, 50
                                                fragmentation_minimal, 32
    res_minimal, 51
                                                fragmentation_synechocystis_6803, 33
    stats_e_coli, 68
    stats_minimal, 70
                                                gff3_preprocess, 39
    stats_synechocystis_6803,71
    summary_e_coli, 73
                                                make_df, 40
    summary_minimal, 76
                                                make_pen, 41
    summary_synechocystis_6803,80
    wrapper_e_coli, 87
                                                nls2_fit, 43
    wrapper_minimal, 87
                                                penalties_e_coli, 44
    wrapper_summary_synechocystis_6803,
                                                penalties_minimal, 45
                                                penalties_synechocystis_6803, 46
apply_ancova, 3
                                                predict_ps_itss, 47
apply_event_position, 5
                                                preprocess_e_coli, 49
apply_manova, 6
                                                preprocess_minimal, 50
apply_t_test, 9
                                                preprocess_synechocystis_6803, 50
apply_t_test_ti, 11
                                                res_minimal, 51
apply_Ttest_delay, 8
                                                rifi_fit, 52
                                                rifi_fragmentation, 54
check_input, 13
                                                rifi_penalties, 56
dataframe_summary, 13
                                                rifi_preprocess, 57
dataframe_summary_events, 15
                                                rifi_stats, 59
dataframe_summary_events_HL_int, 16
                                                rifi_summary, 60
dataframe_summary_events_ps_itss, 18
                                                rifi_visualization, 62
dataframe_summary_events_velocity, 19
                                                rifi_wrapper, 66
```

90 INDEX

```
segment_pos, 67
stats_e_coli, 68
stats_minimal, 70
stats_synechocystis_6803, 71
summary_e_coli, 73
summary_minimal, 76
summary_synechocystis_6803, 80

TI_fit, 83
TUgether, 85
viz_pen_obj, 86
wrapper_e_coli, 87
wrapper_minimal, 87
wrapper_summary_synechocystis_6803, 88
```