# Package 'rifi'

July 4, 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

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# 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.

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#### 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.

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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.

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**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.

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**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)
```

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### **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.

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**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

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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

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# 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

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**FC\_HL\_intensity:** ratio of fold change between 2 half-life fragments and fold change between 2 intensity fragments

**FC\_HL\_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

%

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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

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**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**

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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

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**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

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**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

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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
)
```

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#### 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

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