Package 'STADyUM'

November 13, 2026

Type Package

Title Statistical Transcriptome Analysis under a Dynamic Unified Model

Version 1.3.2

Description STADyUM is a package with functionality for analyzing nascent RNA read counts to infer transcription rates. This includes utilities for processing experimental nascent RNA read counts as well as for simulating PRO-seq data. Rates such as initiation, pause release and landing pad occupancy are estimated from either synthetic or experimental data. There are also options for varying pause sites and including steric hindrance of initiation in the model.

URL https://github.com/rhassett-cshl/STADyUM

BugReports https://github.com/rhassett-cshl/STADyUM

biocViews StatisticalMethod, Transcriptomics, Transcription, Sequencing, Preprocessing

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Encoding UTF-8

LazyData false

Roxygen list(markdown = TRUE)

RoxygenNote 7.3.3

Imports GenomicRanges, IRanges, S4Vectors, methods, tibble, dplyr, ggplot2, progress, GenomeInfoDb, Rcpp, data.table, purrr, rtracklayer, tidyr, rlang, MASS, plyranges, pracma, ggpointdensity, BiocFileCache

LinkingTo Rcpp

Suggests testthat (>= 3.0.0), knitr, rmarkdown, devtools, ggpubr, cowplot

Config/testthat/edition 3

VignetteBuilder knitr

Depends R (>= 4.5.0)

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

git_branch devel

git_last_commit 5006f56

git_last_commit_date 2026-06-22

Repository Bioconductor 3.24

Date/Publication 2026-11-12

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`estimateTranscriptionRates` *Generic function for estimating transcription rates*

Description

Generic function that estimates transcription rates from either simulation data (SimulatePolymerase object) or experimental data (bigwig files and genomic regions).

Usage

```
estimateTranscriptionRates(x, ...)
```

Arguments

x	The input data (either a SimulatePolymerase object or bigwig files)
...	Additional arguments passed to the specific methods

Value

An object containing estimated transcription rates

Examples

```
load(system.file("extdata", "granges_for_read_counting_DLD1_chr21.RData",
package = "STADyUM"))
expRates <- estimateTranscriptionRates(system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_plus_chr21_subset.bw", package = "STADyUM"),
bigwigMinus = system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_minus_chr21_subset.bw", package = "STADyUM"),
    pauseRegions = bw_pause_filtered,
    geneBodyRegions = bw_gb_filtered,
    name = "Control",
    stericHindrance = TRUE,
    omegaScale = 1000
)
```

ExperimentTranscriptionRates-class

Constructor for ExperimentTranscriptionRates object

Description

Class `ExperimentTranscriptionRates` has read counts, pause and gene body genomic region coordinates, steric hindrance and omega scale factor parameters used to estimate the transcription rates as well as the estimated rates

Estimates the transcription rates using Expectation Maximization likelihood formula. Estimates initiation, pause-release rates, average read depth along gene body and pause regions. If steric hindrance is enabled, also estimates the landing pad occupancy. Estimated from experimental data, such as nascent RNA sequencing read counts and genomic coordinates, and contructs an object that holds these rates. Considers models where pause sites are fixed or varied. Considers models where steric hindrance is enabled or disabled.

Accessor for the estimated rates

Accessor for the read counts

Accessor for the pause region coords GRanges-class

Accessor for the gene body regions coords GRanges-class

Accessor for the omega scale used to scale the omega value based on prior studies.

Accessor for the steric hindrance flag. If TRUE, the landing-pad occupancy is inferred in the rates held in this object.

Usage

```
## S4 method for signature 'character'
estimateTranscriptionRates(
  x,
  bigwigMinus,
  pauseRegions,
  geneBodyRegions,
  name,
  stericHindrance = FALSE,
  omegaScale = NULL
)
```

```
## S4 method for signature 'ExperimentTranscriptionRates'
rates(object)

counts(object)

## S4 method for signature 'ExperimentTranscriptionRates'
counts(object)

pauseRegions(object)

## S4 method for signature 'ExperimentTranscriptionRates'
pauseRegions(object)

geneBodyRegions(object)

## S4 method for signature 'ExperimentTranscriptionRates'
geneBodyRegions(object)

omegaScale(object)

## S4 method for signature 'ExperimentTranscriptionRates'
omegaScale(object)

## S4 method for signature 'ExperimentTranscriptionRates'
stericHindrance(object)
```

Arguments

x	The path to a bigwig file from the plus strand recording PRO-seq read counts
bigwigMinus	the path to a bigwig file from the minus strand recording PRO-seq read counts
pauseRegions	a GRanges-class object that must contain a gene_id
geneBodyRegions	a GRanges-class object that must contain a gene_id
name	a character value for the name of the experiment
stericHindrance	a logical value to determine whether to infer landing-pad occupancy or not. Defaults to FALSE.
omegaScale	a numeric value for scaling omega. Defaults to NULL.
object	an ExperimentTranscriptionRates object

Value

an ExperimentTranscriptionRates-class object

a DataFrame with the following columns:

geneId	a character vector of gene IDs
chi	a numeric vector of RNAP density along gene body
betaOrg	a numeric vector
betaAdp	a numeric vector

`fkMean` a numeric vector of the mean position of pause sites

`fkVar` a numeric vector of the variance of pause site positions

`phi` a numeric vector of the landing-pad occupancy

`betaZeta` a numeric vector of the pause-escape rate

`alphaZeta` a numeric vector of the potential initiation rate

`omega` a numeric vector of the effective initiation rate

`omegaZeta` a numeric vector of the effective initiation rate

`expectedPauseSiteCounts`
a numeric vector of the expected pause site counts

`actualPauseSiteCounts`
a numeric vector of the actual pause site counts that are observed

`likelihood` a numeric vector of the likelihood of the model

a `DataFrame` with the following columns:

`geneId` a character vector of gene IDs

`summarizedGbCounts`
a numeric vector of the sum of gene body read counts

`gbLength` a numeric vector of the gene body length

`summarizedPauseCounts`
a numeric vector of the sum of pause region read counts

a GRanges-class object with the pause regions

a GRanges-class object with the gene body regions

a numeric value for the scaling factor for omega

a logical value to determine whether to infer landing-pad occupancy or not

Slots

`counts` a `data.frame` with five columns gene_id, summarizedPauseCounts, pauseLength, summarizedGbCounts, gbLength

`bigwigPlus` a path to bigwig for plus strand recording PRO-seq read counts. This can be generated with the proseq2.0 pipeline.

`bigwigMinus` a path to bigwig for minus strand recording PRO-seq read counts. This can be generated with the proseq2.0 pipeline.

`pauseRegions` a `GRanges-class` object that holds the pause regions coordinates

`geneBodyRegions` a `GRanges-class` object that holds the gene body regions coordinates

`name` a character value for the name of the experiment

`stericHindrance` a logical value indicating whether to infer landing-pad occupancy

`omegaScale` a numeric value for scaling omega, or NULL if steric hindrance is disabled

`rates` a `tbl_df` containing the estimated rates with columns:

- **geneId** Character. Gene identifier
- **chi** Numeric. RNAP density along gene body given as estimate for the gene body elongation rate `[RNAPs/bp]`
- **betaOrg** Numeric. Ratio of gene body RNAP density to pause region RNAP density with fixed pause sites given as an estimate for the pause-escape rate

betaAdp Numeric. Ratio of gene body RNAP density to pause region RNAP density from adapted model which allows pause sites to vary across cells given as an estimate for the pause-escape rate

fkMean Numeric. Mean position of pause sites

fkVar Numeric. Variance of pause site positions

phi Numeric. Landing-pad occupancy (only if steric hindrance is enabled)

betaZeta Numeric. Pause-escape rate (only if steric hindrance is enabled)

alphaZeta Numeric. Potential initiation rate (only if steric hindrance is enabled)

omegaZeta Numeric. Effective initiation rate (only if steric hindrance is enabled)

totalGbRc Numeric. Total gene body read counts

gbLength Numeric. Gene body length

expectedPauseSiteCounts Numeric. Expected pause site counts

actualPauseSiteCounts Numeric. Observed pause site counts

fkSD Numeric. Standard deviation of pause site positions (sqrt of fkVar)

betaGroup Factor. Quantile-based group for betaAdp (Q1–Q5)

fkMeanGroup Factor. Quantile-based group for fkMean (Q1–Q5)

chiGroup Factor. Quantile-based group for chi (Low/Medium/High)

sdGroup Character. Pause site SD classification (Sharp/Broad)

Examples

```
# Create an ExperimentTranscriptionRates object
load(system.file("extdata", "granges_for_read_counting_DLD1_chr21.RData",
package = "STADyUM"))
expRates <- estimateTranscriptionRates(system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_plus_chr21_subset.bw", package = "STADyUM"),
bigwigMinus = system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_minus_chr21_subset.bw", package = "STADyUM"),
    pauseRegions = bw_pause_filtered,
    geneBodyRegions = bw_gb_filtered,
    name = "Control",
    stericHindrance = TRUE,
    omegaScale = 1000
)
```

`getSdGroupCutoff` *Get SD Group Cutoff*

Description

Computes the midpoint cutoff between the maximum fkSD value among "Sharp" genes and the minimum fkSD value among "Broad" genes for a given fkSD column and its associated sdGroup column. Ported from `get_sdgroup_cutoff` in `1_2_LRT_viz.R`.

Usage

```
getSdGroupCutoff(df, value_col, group_col)
```

Arguments

df	a tbl_df or data.frame containing value_col and group_col
value_col	the name of the column with fkSD values
group_col	the name of the column with "Sharp"/"Broad" group labels

Value

a numeric cutoff value

---

rates *Accessor for estimated rates*

Description

Generic accessor for the estimated rates from any TranscriptionRates object

Usage

```
rates(object)
```

Arguments

object	A TranscriptionRates object

Value

A tibble containing the estimated rates

Examples

```
# Create an ExperimentTranscriptionRates object
load(system.file("extdata",
"granges_for_read_counting_DLD1_chr21.RData", package = "STADyUM"))
expRates <- estimateTranscriptionRates(system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_plus_chr21_subset.bw", package = "STADyUM"),
bigwigMinus = system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_minus_chr21_subset.bw", package = "STADyUM"),
    pauseRegions = bw_pause_filtered,
    geneBodyRegions = bw_gb_filtered,
    name = "Control",
    stericHindrance = TRUE,
    omegaScale = 1000
)
rates(expRates)
```

show,ExperimentTranscriptionRates-method

Show method for ExperimentTranscriptionRates objects

Description

Enhanced show method for ExperimentTranscriptionRates objects that displays summary statistics and provides guidance on data access

Usage

```
## S4 method for signature 'ExperimentTranscriptionRates'
show(object)
```

Arguments

object	An ExperimentTranscriptionRates object

Value

NULL (invisibly)

SimulatePolymerase-class

Constructor for SimulatePolymerase object

Description

Class `SimulatePolymerase` tracks the movement of RNAPs along the DNA templates. It contains the parameters for the simulator as well as the simulator results including the position of RNAPs for the last step, the pause sites, a probability vector containing probability RNAPs move forward or not, and a combined cells data vector containing the total number of RNAPs at each site across all cells

Runs the simulator that tracks the movement of RNAPs along the DNA templates of a large number of cells. It accepts several key user-specified parameters, including the initiation rate, pause-escape rate, a constant or variable elongation rate, the mean and variance of pause sites across cells, as well as the center-to-center spacing constraint between RNAPs, the number of cells being simulated, the gene length, and the total time of transcription. The simulator simply allows each RNAP to move forward or not, in time slices of 1e-4 minutes, according to the specified position-specific rate parameters. It assumes that at most one movement of each RNAP can occur per time slice. The simulator monitors for collisions between adjacent RNAPs, prohibiting one RNAP to advance if it is at the boundary of the allowable distance from the next. After running for specified time, it outputs the position of RNAPs for the last specified number of steps.

Sample read counts from a SimulatePolymerase object. To match our simulated read counts to reality, we need to compute a scaling factor lambda. One way of doing it is computing the read density based on real experiments. For example, we have computed the read density within gene body in *Dukler et al* (2017) for genes with median expression (i.e., 0.0489). If we assume the read counts following a Poisson distribution, we can then sample the read counts with mean equals to the RNAP frequency multiplied by lambda.

Show method for SimulatePolymerase object in human readable format including summary statistics

Accessor for the pause sites numeric vector from a SimulatePolymerase object.

Accessor for the probability numeric matrix from a SimulatePolymerase object. The matrix has dimensions (sites x cells) where each element represents the transition probability for a specific site and cell.

Accessor for the combined cells data numeric vector from a SimulatePolymerase object.

Accessor for the position matrices from a SimulatePolymerase object.

Accessor for the final position matrix from a SimulatePolymerase object. This represents the last state of the simulation.

Accessor for the simulation parameters from a SimulatePolymerase object.

Accessor for the read counts numeric vector sampled from a SimulatePolymerase object.

Get the position matrix for a specific time point from a SimulatePolymerase object.

Get all available time points from a SimulatePolymerase object.

Get the combined cells data vector for a specific time point from a SimulatePolymerase object. This function calculates the total number of polymerases at each site across all cells for the specified time point, similar to the final combinedCellsData but for intermediate time points.

Plot the distribution of pause sites across the gene as a histogram.

Plot position matrices as a ggplot2 heatmap showing polymerase positions across all cells at specific time points. Each cell in the heatmap represents whether a polymerase is present (1) or absent (0) at a specific site in a specific cell. By default, shows the final position matrix. Plots for pause region which is from site 1 to user-defined kMax.

Create a ggplot2 PCA plot of the polymerase position matrix, showing the first two principal components for each cell. Useful for exploring heterogeneity and clustering among cells. Useful for exploring the site occupancy patterns for each cell.

Plot combined cells data as a ggplot2 plot. For SimulatePolymerase objects, can plot data from specific time points by calculating combined cells data from position matrices.

Usage

```
simulatePolymerase(
  k = 50,
  ksd = 25,
  kMin = 17,
  kMax = 200,
  geneLen = 1950,
  alpha = 1,
  beta = 1,
  zeta = 2000,
  zetaSd = 1000,
  zetaMin = 1500,
  zetaMax = 2500,
  zetaVec = NULL,
  cellNum = 1000,
  polSize = 33,
  addSpace = 17,
  time = 1,
  timesToRecord = NULL
```

```
)

sampleReadCounts(object, readDensity = 0.0489)

## S4 method for signature 'SimulatePolymerase'
sampleReadCounts(object, readDensity = 0.0489)

## S4 method for signature 'SimulatePolymerase'
show(object)

pauseSites(object)

## S4 method for signature 'SimulatePolymerase'
pauseSites(object)

siteProbabilities(object)

## S4 method for signature 'SimulatePolymerase'
siteProbabilities(object)

combinedCellsData(object)

## S4 method for signature 'SimulatePolymerase'
combinedCellsData(object)

positionMatrices(object)

## S4 method for signature 'SimulatePolymerase'
positionMatrices(object)

finalPositionMatrix(object)

## S4 method for signature 'SimulatePolymerase'
finalPositionMatrix(object)

parameters(object)

## S4 method for signature 'SimulatePolymerase'
parameters(object)

readCounts(object)

## S4 method for signature 'SimulatePolymerase'
readCounts(object)

getPositionMatrixAtTime(object, timePoint)

## S4 method for signature 'SimulatePolymerase'
getPositionMatrixAtTime(object, timePoint)

getAvailableTimePoints(object)
```

```
## S4 method for signature 'SimulatePolymerase'
getAvailableTimePoints(object)

getCombinedCellsDataAtTime(object, timePoint = NULL)

## S4 method for signature 'SimulatePolymerase'
getCombinedCellsDataAtTime(object, timePoint = NULL)

plotPauseSites(object, file = NULL, width = 8, height = 6)

## S4 method for signature 'SimulatePolymerase'
plotPauseSites(object, file = NULL, width = 8, height = 6)

plotPositionHeatmap(
  object,
  timePoint = NULL,
  maxCells = NULL,
  file = NULL,
  width = 10,
  height = 8,
  dpi = 300
)

## S4 method for signature 'SimulatePolymerase'
plotPositionHeatmap(
  object,
  timePoint = NULL,
  maxCells = NULL,
  file = NULL,
  width = 10,
  height = 8,
  dpi = 300
)

plotPolymerasePCA(
  object,
  timePoint = NULL,
  file = NULL,
  width = 8,
  height = 6,
  dpi = 300
)

## S4 method for signature 'SimulatePolymerase'
plotPolymerasePCA(
  object,
  timePoint = NULL,
  file = NULL,
  width = 8,
  height = 6,
  dpi = 300
)
```

```
plotCombinedCells(
  object,
  start = NULL,
  end = NULL,
  timePoint = NULL,
  file = NULL
)

## S4 method for signature 'SimulatePolymerase'
plotCombinedCells(
  object,
  start = NULL,
  end = NULL,
  timePoint = NULL,
  file = NULL
)
```

Arguments

k	an integer value for the mean of pause sites across cells.
ksd	a numeric value for the standard deviation of pause sites across cells.
kMin	an integer value for the upper bound of pause sites allowed.
kMax	an integer value for the lower bound of pause sites allowed.
geneLen	an integer value for the length of the gene.
alpha	a numeric value for the initiation rate.
beta	a numeric value for the pause release rate.
zeta	a numeric value for the mean elongation rate across sites.
zetaSd	a numeric value for the standard deviation of pause sites across sites.
zetaMin	a numeric value for the minimum elongation rate.
zetaMax	a numeric value for the maximum elongation rate.
zetaVec	a character value for the path to the zetaVec file.
cellNum	an integer value for the number of cells to simulate.
polSize	an integer value for the polymerase II size.
addSpace	an integer value for the additional space in addition to RNAP size.
time	a numeric value for the time to simulate.
timesToRecord	a numeric vector of specific time points to record position matrices for, or NULL to record no extra position matrices. Final position matrix is always recorded. Default is NULL.
object	A SimulatePolymerase-class object
readDensity	A numeric value for the read density within gene body in *Dukler et al.* (2017) for genes with median expression (i.e., 0.0489).
timePoint	Optional numeric value specifying the time point being plotted (used for automatic title generation and documentation)
file	Optional file path to save the plot to
width	Plot width in inches

height	Plot height in inches
maxCells	Maximum number of cells to display (for performance with large datasets). If NULL, shows all cells.
dpi	Plot resolution in DPI
start	Integer, starting position for plotting (default: NULL, uses full range)
end	Integer, ending position for plotting (default: NULL, uses full range)

Value

a SimulatePolymerase object

The SimulatePolymerase object with read counts sampled given the readDensity parameter used

A list containing all simulation parameters including k, ksd, kMin, kMax, geneLen, alpha, beta, zeta, zetaSd, zetaMin, zetaMax, zetaVec, cellNum, polSize, addSpace, time, and stepsToRecord

A 2D matrix of polymerase positions for the specified time point

A numeric vector of available time points

An integer vector representing the total number of polymerases at each site across all cells for the specified time point

A ggplot object showing the distribution of pause sites

A ggplot object showing heatmap of polymerase positions

A ggplot object showing the PCA plot of cells

A ggplot object

Slots

k an integer value for the mean of pause sites across cells.

ksd a numeric value for the standard deviation of pause sites across cells.

kMin an integer value for the upper bound of pause sites allowed.

kMax an integer value for the lower bound of pause sites allowed.

geneLen an integer value for the length of the gene.

alpha a numeric value for the initiation rate.

beta a numeric value for the pause release rate.

zeta a numeric value for the mean elongation rate across sites.

zetaSd a numeric value for the standard deviation of pause sites across sites.

zetaMin a numeric value for the minimum elongation rate.

zetaMax a numeric value for the maximum elongation rate.

zetaVec a character value for the path to the zetaVec file.

cellNum an integer value for the number of cells to simulate.

polSize an integer value for the polymerase II size.

addSpace an integer value for the additional space in addition to RNAP size.

time a numeric value for the time to simulate.

timesToRecord a numeric vector of specific time points to record position matrices for, or NULL to record no extra position matrices. Final position matrix is always recorded. Default is NULL.

pauseSites a numeric vector of pause sites

`siteProbabilities` a numeric matrix representing the probability that the polymerase move forward or not at each site for each cell (sites x cells)

`combinedCellsData` an integer vector representing the total number of RNAPs at each site across all cells

`positionMatrices` a list of position matrices

`finalPositionMatrix` a matrix representing the final position matrix

`readCounts` a numeric vector for read counts per nucleotide

Note

The `simulatePolymerase` function can take a few minutes to run for large numbers of cells (> 1000) and long simulation times (> 200).

Examples

```
# Create a SimulatePolymerase object
sim <- simulatePolymerase(
    k = 50, ksd = 25, kMin = 17, kMax = 200, geneLen = 1950,
    alpha = 1, beta = 1, zeta = 2000, zetaSd = 1000, zetaMin = 1500,
    zetaMax = 2500, zetaVec = NULL, cellNum = 100, polSize = 33,
    addSpace = 17, time = 1, timesToRecord = NULL
)
# Create a SimulatePolymerase object with specific time points recorded
sim2 <- simulatePolymerase(
    k = 50, ksd = 25, kMin = 17, kMax = 200, geneLen = 1950,
    alpha = 1, beta = 1, zeta = 2000, zetaSd = 1000, zetaMin = 1500, zetaMax
= 2500,
    zetaVec = NULL, cellNum = 1000, polSize = 33, addSpace = 17, time = 1,
    timesToRecord = c(0.5, 1.0)
)
# Create a SimulatePolymerase object
sim <- simulatePolymerase(
    k = 50, ksd = 25, kMin = 17, kMax = 200, geneLen = 1950,
    alpha = 1, beta = 1, zeta = 2000, zetaSd = 1000, zetaMin = 1500,
    zetaMax = 2500, zetaVec = NULL, cellNum = 100, polSize = 33,
    addSpace = 17, time = 1, timesToRecord = NULL
)
# Sample read counts per nucleotide
sim <- sampleReadCounts(sim)
# Print the read counts per nucleotide
print(readCounts(sim))
# Create a SimulatePolymerase object
sim <- simulatePolymerase(
    k = 50, ksd = 25, kMin = 17, kMax = 200, geneLen = 1950,
    alpha = 1, beta = 1, zeta = 2000, zetaSd = 1000, zetaMin = 1500,
    zetaMax = 2500, zetaVec = NULL, cellNum = 100, polSize = 33,
    addSpace = 17, time = 1, timesToRecord = c(0.5, 1.0))
# Plot final position matrix
plotPositionHeatmap(sim, file="position_heatmap.png")
# Plot position matrix at time 0.5
plotPositionHeatmap(sim, timePoint = 0.5, file="position_heatmap.png")
# Plot position matrix with maxCells = 100
plotPositionHeatmap(sim, timePoint = 0.5, maxCells = 100,
file="position_heatmap.png")
# Create a SimulatePolymerase object
```

```
sim <- simulatePolymerase(
    k = 50, ksd = 25, kMin = 17, kMax = 200, geneLen = 1950,
    alpha = 1, beta = 1, zeta = 2000, zetaSd = 1000, zetaMin = 1500,
    zetaMax = 2500, zetaVec = NULL, cellNum = 100, polSize = 33,
    addSpace = 17, time = 1, timesToRecord = c(0.5, 1.0))
# Plot final combined cells data
plotCombinedCells(sim, file="combined_cells.png")
# Plot specific time point data
plotCombinedCells(sim, timePoint = 0.5, file="combined_cells.png")
# Plot with custom range and title
plotCombinedCells(sim, timePoint = 0.5, start = 100, end = 500,
file="combined_cells.png")
```

`SimulationTranscriptionRates-class`
Constructor for SimulationTranscriptionRates object

Description

Class containing the simulated data, such as the nascent RNA sequencing reads sampled from the simulated polymerase movement in the `SimulatePolymerase` object. It also contains the estimated average read depths along the gene body and pause regions, given fixed or varied pause sites, as well as the landing pad occupancy estimate. These can be under a model with or without steric hindrance.

Estimates transcription rates from a SimulatePolymerase object using Expectation Maximization likelihood formula.

Show method for SimulationTranscriptionRates object in human readable format including summary statistics

Accessor for the SimulatePolymerase object from a SimulationTranscriptionRates object.

Accessor for the steric hindrance flag from a SimulationTranscriptionRates object.

Accessor for the tibble containing the estimated rates from a SimulationTranscriptionRates object.

Usage

```
## S4 method for signature 'SimulatePolymerase'
estimateTranscriptionRates(x, name, stericHindrance = FALSE)

## S4 method for signature 'SimulationTranscriptionRates'
show(object)

simpol(object)

## S4 method for signature 'SimulationTranscriptionRates'
simpol(object)

## S4 method for signature 'SimulationTranscriptionRates'
stericHindrance(object)

## S4 method for signature 'SimulationTranscriptionRates'
rates(object)
```

Arguments

x	a SimulatePolymerase object
name	a string providing information about the simulation
stericHindrance	a logical value to determine whether to infer landing-pad occupancy or not. Defaults to FALSE.
object	a SimulationTranscriptionRates object

Value

a SimulationTranscriptionRates object

Slots

simpol a SimulatePolymerase object

name a character value for the name of the experiment

stericHindrance a logical value to determine whether to infer landing-pad occupancy or not. Defaults to FALSE.

rates a tbl_df containing the estimated rates with columns:

trial Numeric. Trial number, each trial samples 5000 cells

chi Numeric. RNAP density along gene body given as estimate for the gene body elongation rate [RNAPs/bp]

betaOrg Numeric. Ratio of gene body RNAP density to pause region RNAP density with fixed pause sites given as an estimate for the pause-escape rate

betaAdp Numeric. Ratio of gene body RNAP density to pause region RNAP density from adapted model which allows pause sites to vary across cells given as an estimate for the pause-escape rate

phi Numeric. Landing-pad occupancy representing the fraction of time in the simulation that the landing pad is occupied by RNA polymerase. The landing pad, also known as the initiation site, is the region where the RNA polymerase binds to the DNA and begins transcription. It is the polSize + addSpace length (only applicable if steric hindrance is enabled)

fk list. Likelihood of pausing at each pause region position

fkMean Numeric. Mean position of pause sites

fkVar Numeric. Variance of pause site positions

totalTssRc Numeric. Total RNAP read counts in the TSS region (across all cells)

totalGbRc Numeric. Total RNAP read counts in the gene body region (across all cells)

totalLandingRc Numeric. Total RNAP read counts in the landing pad region (across all cells)

avgRcPerCell Numeric. Average number of RNAPs per cell for gene body and TSS regions

avgTssRcPerCell Numeric. Average number of RNAPs per cell for pause regions (across all cells)

avgLandingRcPerCell Numeric. Average number of RNAPs per cell for landing pad regions (across all cells)

actualPauseSiteCounts list. Simulated pause site counts for each cell

expectedPauseSiteCounts list. Expected pause site counts for each cell estimated from the EM algorithm

expectationMaximizationStatus character. Status of the expectation maximization algorithm. "normal": converged normally, "single_site": converged to single pause site, "max_iterations": reached maximum iterations without convergence. Note max # of iterations is 500.

likelihood a numeric vector of the likelihood of the model

Examples

```
# Create a SimulatePolymerase object
sim <- simulatePolymerase(
    k = 50, ksd = 25, kMin = 17, kMax = 200, geneLen = 1950,
    alpha = 1, beta = 1, zeta = 2000, zetaSd = 1000, zetaMin = 1500,
    zetaMax = 2500, zetaVec = NULL, cellNum = 100, polSize = 33,
    addSpace = 17, time = 1)
# Estimate transcription rates
estRates <- estimateTranscriptionRates(sim, name="sim_beta1_k50")
# Print the estimated rates
print(estRates)
```

---

stericHindrance *Accessor for steric hindrance flag*

Description

Generic accessor for the steric hindrance flag from any TranscriptionRates object

Usage

```
stericHindrance(object)
```

Arguments

object	A TranscriptionRates object

Value

A logical value indicating whether steric hindrance was modeled

Examples

```
# Create an ExperimentTranscriptionRates object
load(system.file("extdata", "granges_for_read_counting_DLD1_chr21.RData",
package = "STADyUM"))
expRates <- estimateTranscriptionRates(system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_plus_chr21_subset.bw", package = "STADyUM"),
bigwigMinus = system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_minus_chr21_subset.bw", package = "STADyUM"),
    pauseRegions = bw_pause_filtered,
    geneBodyRegions = bw_gb_filtered,
    name = "Control",
    stericHindrance = TRUE,
    omegaScale = 1000
)
```

```
stericHindrance(expRates)
```

`TranscriptionRates-class` *Base class for TranscriptionRates objects*

Description

Virtual base class that defines the common interface for transcription rate objects. Both `ExperimentTranscriptionRate` and `SimulationTranscriptionRates` inherit from this class.

Creates a histogram plot showing the distribution of observed mean pause site positions across all genes. This visualization helps identify the range and shape of pause site positions

Creates a scatter plot comparing actual pause site counts against expected pause site counts from the EM algorithm. It is comparing the number of polymerase at the pause site in the simulated/experimental data against the number of polymerase at the pause site expected by the model. This visualization assesses the goodness-of-fit of the pause site model by showing how well the model predictions align with the actual data. A perfect fit would show all points on the diagonal line (y=x). The R^2 value is calculated and displayed on the plot to quantify the model fit quality. This plot is useful for validating the accuracy of the pause site estimation and identifying any systematic biases in the model predictions.

Creates a density plot showing the distribution of gene body RNAP density (chi) across all genes. This visualization helps identify the range and shape of RNA polymerase density in gene bodies, which can reveal patterns in transcriptional activity.

Plot a scatter plot with gene body RNAP density on the x-axis and beta (ratio of gene body RNAP density to pause region RNAP density) on the y-axis. Fits a linear model to the data and plots the line. Can plot beta for either the adapted model or the single pause site model.

Creates a scatter plot colored by local point density for any two columns in the rates tibble, with optional log10 transformation on either axis. When a discrete `color_var` is supplied the points are colored by that variable instead of by density.

Plot a contour map with mean pause site position on the x-axis and pause site variance on the y-axis.

Usage

```
plotMeanPauseDistrib(object, file = NULL, width = 8, height = 6, dpi = 300)

## S4 method for signature 'TranscriptionRates'
plotMeanPauseDistrib(object, file = NULL, width = 8, height = 6, dpi = 300)

plotExpectedVsActualPauseSiteCounts(
  object,
  file = NULL,
  width = 8,
  height = 6,
  dpi = 300
)

## S4 method for signature 'TranscriptionRates'
plotExpectedVsActualPauseSiteCounts(
```

```
  object,
  file = NULL,
  width = 8,
  height = 6,
  dpi = 300
)

plotChiDistrib(object, file = NULL, width = 8, height = 6, dpi = 300)

## S4 method for signature 'TranscriptionRates'
plotChiDistrib(object, file = NULL, width = 8, height = 6, dpi = 300)

plotBetaVsChi(object, label_x, label_y, xlim = NULL, ylim = NULL, file = NULL)

## S4 method for signature 'TranscriptionRates'
plotBetaVsChi(object, label_x, label_y, xlim = NULL, ylim = NULL, file = NULL)

plotScatterDensity(
  object,
  xvar,
  yvar,
  xlab = NULL,
  ylab = NULL,
  log_x = FALSE,
  log_y = FALSE,
  color_var = NULL,
  color_values = NULL,
  color_lab = NULL,
  xlim = NULL,
  ylim = NULL,
  file = NULL,
  width = 3.5,
  height = 3,
  dpi = 300
)

## S4 method for signature 'TranscriptionRates'
plotScatterDensity(
  object,
  xvar,
  yvar,
  xlab = NULL,
  ylab = NULL,
  log_x = FALSE,
  log_y = FALSE,
  color_var = NULL,
  color_values = NULL,
  color_lab = NULL,
  xlim = NULL,
  ylim = NULL,
  file = NULL,
  width = 3.5,
```

```
  height = 3,
  dpi = 300
)

plotPauseSiteContourMap(object, file = NULL, width = 8, height = 6, dpi = 300)

## S4 method for signature 'TranscriptionRates'
plotPauseSiteContourMap(object, file = NULL, width = 8, height = 6, dpi = 300)
```

Arguments

object	an TranscriptionRates object
file	the path to a file to save the plot to
width	the width of the plot in inches
height	the height of the plot in inches
dpi	the resolution of the plot in dpi
label_x	the x-axis label
label_y	the y-axis label
xlim	optional numeric(2); forwarded to coord_cartesian
ylim	optional numeric(2); forwarded to coord_cartesian
xvar	character; column name in rates(object) for the x-axis
yvar	character; column name in rates(object) for the y-axis
xlab	x-axis label (string or expression); defaults to xvar with a log10() prefix when log_x = TRUE
ylab	y-axis label (string or expression); same rules as xlab
log_x	logical; apply log10 to x before plotting
log_y	logical; apply log10 to y before plotting
color_var	optional character; column name for discrete color grouping; when supplied geom_point is used instead of geom_pointdensity
color_values	optional named character vector passed to scale_color_manual when color_var is set
color_lab	legend title for the color scale; defaults to "Density" or color_var

Value

an ggplot2 object

an ggplot2 object

an ggplot2 object

an ggplot2 object

a ggplot object

an ggplot2 object

Slots

rates a tbl_df containing the estimated rates

name a character value for the name of the experiment

stericHindrance a logical value indicating whether steric hindrance was modeled

Examples

```
# Create an ExperimentTranscriptionRates object
load(system.file("extdata", "granges_for_read_counting_DLD1_chr21.RData",
package = "STADyUM"))
expRates <- estimateTranscriptionRates(system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_plus_chr21_subset.bw", package = "STADyUM"),
bigwigMinus = system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_minus_chr21_subset.bw", package = "STADyUM"),
    pauseRegions = bw_pause_filtered,
    geneBodyRegions = bw_gb_filtered,
    name = "Control"
)
plotPauseSiteContourMap(expRates, file="pause_sites_contour_map.png")
```

`TranscriptionRatesLRT-class`

Constructor for TranscriptionRatesLRT object

Description

Constructs results of likelihood ratio test comparing transcription data estimated from two different sets of experimental read counts.

Likelihood ratio test comparing aspects of transcriptional dynamics of the same TU under different conditions using Likelihood Ratio Test Statistics. Uses read counts and rate estimates estimated from estimateTranscriptionRates. The method also requires scaling factors to determine changes in χ estimates. They can be the numbers of total mapped reads or spike-in reads from the samples. Likelihood ratio test computes the log 2 fold change in χ estimates between conditions, the log 2 fold change in beta estimates between conditions, the t-statistics for the likelihood ratio tests, and the adjusted p-values based on the "BH" method.

Accessor for the first ExperimentTranscriptionRates object from a TranscriptionRatesLRT object.

Accessor for the second ExperimentTranscriptionRates object from a TranscriptionRatesLRT object.

Accessor for the spike-in scaling factor from a TranscriptionRatesLRT object.

Accessor for the chi table from a TranscriptionRatesLRT object.

Accessor for the beta table from a TranscriptionRatesLRT object.

Accessor for the merged per-gene LRT summary table from a TranscriptionRatesLRT object. This table is built automatically by `likelihoodRatioTest` via `mergeBetaChiLRTStats` and is the table consumed by the package's LRT plotting methods (e.g. `plotBetaQuantileHeatmap`, `plotBetaScatter`, `plotDeltaBetaSigma`, `plotFksdDensity`).

Builds a single per-gene summary tibble from a TranscriptionRatesLRT object's beta LRT results, joined with per-sample rate groupings, and enriched with derived chi LRT statistics (mean and log2 fold change between samples, scaled by `scale_factor`), as well as derived pause-site shape statistics (`pause_change`, `deltaSD`, `deltaMean`) used by the package's LRT plotting methods. This is the table populated automatically in the `lrtTbl` slot by `likelihoodRatioTest`.

Plot a heatmap comparing quantile-based beta groupings (Q1-Q5) between two conditions, with tiles showing the number of genes that fall into each combination of groups. Ported from `plot_beta_quantile_heatmap` in `1_2_LRT_viz.R`. Operates on the `lrtTbl` slot of a `TranscriptionRatesLRT` object, as populated by `likelihoodRatioTest`.

Plot a scatter of log10(beta1) vs log10(beta2), colored by betaCategory, comparing pause-escape rate estimates between two conditions. Ported from `plot_beta_scatter` in `1_2_LRT_viz.R`. Operates on the `lrtTbl` slot of a `TranscriptionRatesLRT` object, as populated by `likelihoodRatioTest`.

Plot a 2D density scatter of the log2 fold change in beta (`lfc`) against the normalized change in pause site standard deviation (`deltaSD`) between two conditions, annotated with the Spearman correlation. Ported from `plot_delta_beta_sigma` in `1_2_LRT_viz.R`. Operates on the `lrtTbl` slot of a `TranscriptionRatesLRT` object, as populated by `likelihoodRatioTest`.

Plot a 2D density of pause site standard deviation (`fkSD`) between two conditions, with quadrant labels showing how many genes have stable or changing pause-site sharpness between conditions. Ported from `plot_fksd_density` in `1_2_LRT_viz.R`. Operates on the `lrtTbl` slot of a `TranscriptionRatesLRT` object, as populated by `likelihoodRatioTest`, restricted to genes with a non-missing `sdGroup` call in both samples. Sharp/broad cutoffs default to `getSdGroupCutoff` computed on that restricted table when not supplied explicitly.

Plot a contour map with mean pause site position on the x-axis and pause site standard deviation on the y-axis. The plot is a comparison between two conditions.

Plot a violin plot comparing the beta values between two conditions.

Plot a violin plot comparing the chi values between two conditions.

Plot a MA plot for log fold change treated/control vs log mean beta*zeta.

Usage

```
likelihoodRatioTest(
  transcriptionRates1,
  transcriptionRates2,
  scaleFactor = 1,
  spikeInFile = NULL
)

transcriptionRates1(object)

## S4 method for signature 'TranscriptionRatesLRT'
transcriptionRates1(object)

transcriptionRates2(object)

## S4 method for signature 'TranscriptionRatesLRT'
transcriptionRates2(object)

spikeInFile(object)

## S4 method for signature 'TranscriptionRatesLRT'
spikeInFile(object)

chiTbl(object)

## S4 method for signature 'TranscriptionRatesLRT'
chiTbl(object)

betaTbl(object)

## S4 method for signature 'TranscriptionRatesLRT'
```

```
betaTbl(object)

lrtTbl(object)

## S4 method for signature 'TranscriptionRatesLRT'
lrtTbl(object)

mergeBetaChiLRTStats(
  object,
  scale_factor,
  lfc_threshold = 0.5,
  prefix1 = "control",
  prefix2 = "treatment"
)

## S4 method for signature 'TranscriptionRatesLRT'
mergeBetaChiLRTStats(
  object,
  scale_factor,
  lfc_threshold = 0.5,
  prefix1 = "control",
  prefix2 = "treatment"
)

plotBetaQuantileHeatmap(
  object,
  file = NULL,
  width = 8,
  height = 6,
  dpi = 300,
  fill_label = "Gene count",
  show_text = TRUE,
  text_size = 5,
  show_legend = FALSE
)

## S4 method for signature 'TranscriptionRatesLRT'
plotBetaQuantileHeatmap(
  object,
  file = NULL,
  width = 8,
  height = 6,
  dpi = 300,
  fill_label = "Gene count",
  show_text = TRUE,
  text_size = 5,
  show_legend = FALSE
)

plotBetaScatter(
  object,
  file = NULL,
```

```
  width = 8,
  height = 6,
  dpi = 300,
  label_x = -6,
  label_y = -1,
  x_lim = c(-6, -1),
  y_lim = c(-6, -1)
)

## S4 method for signature 'TranscriptionRatesLRT'
plotBetaScatter(
  object,
  file = NULL,
  width = 8,
  height = 6,
  dpi = 300,
  label_x = -6,
  label_y = -1,
  x_lim = c(-6, -1),
  y_lim = c(-6, -1)
)

plotDeltaBetaSigma(
  object,
  file = NULL,
  width = 8,
  height = 6,
  dpi = 300,
  filter_lfc = FALSE
)

## S4 method for signature 'TranscriptionRatesLRT'
plotDeltaBetaSigma(
  object,
  file = NULL,
  width = 8,
  height = 6,
  dpi = 300,
  filter_lfc = FALSE
)

plotFksdDensity(
  object,
  file = NULL,
  width = 8,
  height = 6,
  dpi = 300,
  cutoff_cd4 = NULL,
  cutoff_cd14 = NULL
)

## S4 method for signature 'TranscriptionRatesLRT'
```

```
plotFksdDensity(
  object,
  file = NULL,
  width = 8,
  height = 6,
  dpi = 300,
  cutoff_cd4 = NULL,
  cutoff_cd14 = NULL
)

plotPauseSiteContourMapTwoConditions(
  object,
  file = NULL,
  width = 8,
  height = 6,
  dpi = 300
)

## S4 method for signature 'TranscriptionRatesLRT'
plotPauseSiteContourMapTwoConditions(
  object,
  file = NULL,
  width = 8,
  height = 6,
  dpi = 300
)

BetaViolinPlot(object, file = NULL, width = 8, height = 6, dpi = 300)

## S4 method for signature 'TranscriptionRatesLRT'
BetaViolinPlot(object, file = NULL, width = 8, height = 6, dpi = 300)

ChiViolinPlot(object, file = NULL, width = 8, height = 6, dpi = 300)

## S4 method for signature 'TranscriptionRatesLRT'
ChiViolinPlot(object, file = NULL, width = 8, height = 6, dpi = 300)

plotLfcMa(object, file = NULL, width = 8, height = 6, dpi = 300)

## S4 method for signature 'TranscriptionRatesLRT'
plotLfcMa(object, file = NULL, width = 8, height = 6, dpi = 300)
```

Arguments

transcriptionRates1	an TranscriptionRates object
transcriptionRates2	an TranscriptionRates object
scaleFactor	a numeric value to scale the beta estimates. Defaults to 1
spikeInFile	path to a csv file containing scale factors based on total or spike-in reads or NULL if not provided. Defaults to NULL.
object	an TranscriptionRatesLRT object

scale_factor	a numeric scale factor used to normalize chi between the two samples (e.g. a spike-in based library size ratio)
lfc_threshold	the threshold for the log2 fold change used to categorize beta and chi changes
prefix1	the prefix for the first sample's joined rate columns
prefix2	the prefix for the second sample's joined rate columns
file	the path to a file to save the plot to
width	the width of the plot in inches
height	the height of the plot in inches
dpi	the resolution of the plot in dpi
fill_label	the label for the fill legend
show_text	whether to display the gene count in each tile
text_size	the size of the gene count labels
show_legend	whether to display the fill legend
label_x	the x position of the correlation label
label_y	the y position of the correlation label
x_lim	the x-axis limits
y_lim	the y-axis limits
filter_lfc	if TRUE, restricts to genes with abs(lfc) < 5 before computing the correlation and plotting
cutoff_cd4	the fkSD cutoff between "Sharp" and "Broad" for the first sample. If NULL (the default), computed via getSdGroupCutoff
cutoff_cd14	the fkSD cutoff between "Sharp" and "Broad" for the second sample. If NULL (the default), computed via getSdGroupCutoff

Value

a TranscriptionRatesLRT object

tbl_df

tbl_df

tbl_df

tbl_df

a ggplot2 object

a ggplot2 object

a ggplot2 object

a ggplot2 object

an ggplot2 object

an ggplot2 object

an ggplot2 object

an ggplot2 object

Examples

```
load(system.file("extdata", "granges_for_read_counting_DLD1_chr21.RData",
package = "STADyUM"))
transcriptionRates1 <- estimateTranscriptionRates(system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_plus_chr21_subset.bw", package = "STADyUM"),
bigwigMinus = system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_minus_chr21_subset.bw", package = "STADyUM"),
    pauseRegions = bw_pause_filtered,
    geneBodyRegions = bw_gb_filtered,
    name = "Control"
)
transcriptionRates2 <- estimateTranscriptionRates(system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_plus_chr21_subset.bw", package = "STADyUM"),
bigwigMinus = system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_minus_chr21_subset.bw", package = "STADyUM"),
    pauseRegions = bw_pause_filtered,
    geneBodyRegions = bw_gb_filtered,
    name = "Treated"
)
spikeInFile <- system.file("extdata", "spikein_scaling_factor.csv",
package = "STADyUM")
lrts <- likelihoodRatioTest(transcriptionRates1, transcriptionRates2,
scaleFactor=1, spikeInFile)
# Print the likelihood ratio test object
print(lrts)
# Create an ExperimentTranscriptionRates object
load(system.file("extdata", "granges_for_read_counting_DLD1_chr21.RData",
package = "STADyUM"))
transcriptionRates1 <- estimateTranscriptionRates(system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_plus_chr21_subset.bw", package = "STADyUM"),
bigwigMinus = system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_minus_chr21_subset.bw", package = "STADyUM"),
    pauseRegions = bw_pause_filtered,
    geneBodyRegions = bw_gb_filtered,
    name = "Control"
)
transcriptionRates2 <- estimateTranscriptionRates(system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_plus_chr21_subset.bw", package = "STADyUM"),
bigwigMinus = system.file("extdata",
"PROseq-DLD1-aoi-NELFC_Auxin_Ctrl-SE_minus_chr21_subset.bw", package = "STADyUM"),
    pauseRegions = bw_pause_filtered,
    geneBodyRegions = bw_gb_filtered,
    name = "Treated"
)
spikeInFile <- system.file("extdata", "spikein_scaling_factor.csv",
package = "STADyUM")
lrts <- likelihoodRatioTest(transcriptionRates1, transcriptionRates2,
scaleFactor=1, spikeInFile)
plotPauseSiteContourMapTwoConditions(lrts,
file="pause_sites_contour_map.png")
```

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