Package 'dStruct'

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```
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Title Identifying differentially reactive regions from RNA structurome
     profiling data
Version 1.17.0
Depends R (>= 4.1)
Description dStruct identifies differentially reactive regions from RNA structurome profil-
     ing data. dStruct is compatible with a broad range of structurome profiling technolo-
     gies, e.g., SHAPE-MaP, DMS-MaPseq, Structure-Seq, SHAPE-Seq, etc. See Choud-
     hary et al., Genome Biology, 2019 for the underlying method.
Imports zoo, ggplot2, purrr, reshape2, parallel, IRanges, S4Vectors,
     rlang, grDevices, stats, utils
License GPL (>= 2)
biocViews StatisticalMethod, StructuralPrediction, Sequencing,
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```

2 calcDis

```
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```

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calcDis

Calculates d score.

Description

d score of a nucleotide is a measure of dissimilarity of its normalized reactivity scores. Consider a transcript and its reactivity profiles from a group of samples. Then, the d score of a nucleotide is $(2/\pi)$ times the arc-tangent of the ratio of the sample standard deviation of its reactivities to their mean.

Usage

calcDis(x)

Arguments

Х

A numeric vector or matrix.

Value

If input is a numeric vector, a number is returned. For a matrix, a numeric vector is returned.

Author(s)

Krishna Choudhary

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References

Choudhary, K., Lai, Y. H., Tran, E. J., & Aviran, S. (2019). dStruct: identifying differentially reactive regions from RNA structurome profiling data. *Genome biology*, 20(1), 1-26.

Choudhary K, Shih NP, Deng F, Ledda M, Li B, Aviran S. Metrics for rapid quality control in RNA structure probing experiments. *Bioinformatics*. 2016; 32(23):3575–3583.

Examples

```
#Lower standard deviation of reactivites results in lower d-score. calcDis(rnorm(10, 1, 0.2)) calcDis(rnorm(10, 1, 0.6))
```

dCombs

Assesses within-group or between-group variation.

Description

Given the reactivity profiles for a transcript from multiple samples, and a list of sample identifiers, this function computes the dissimilarity of reactivity scores between the specified samples. These are returned as a sequence of nucleotide-wise *d* scores.

Usage

```
dCombs(rdf, combs)
```

Arguments

rdf Data.frame of reactivities for each sample.

combs Data.frame with each column containing groupings of samples.

Value

Nucleotide-wise d scores.

Author(s)

Krishna Choudhary

References

Choudhary, K., Lai, Y. H., Tran, E. J., & Aviran, S. (2019). dStruct: identifying differentially reactive regions from RNA structurome profiling data. *Genome biology*, 20(1), 1-26.

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Examples

```
#Example of a data frame with reactivities.
reacs <- data.frame(matrix(runif(30, 0, 10), 10, 3))
#The columns of data frame with must indicate sample grouping and id.
colnames(reacs) <- c("A1", "A2", "B1")
#Get nucleotide-wise dissimilarity scores for a set of samples.
dCombs(rdf = reacs, combs = data.frame(c("A1", "B1")))</pre>
```

dStruct

Performs de novo discovery of differentially reactive regions.

Description

This function takes reactivity profiles for samples of two groups as input and identifies differentially reactive regions in three steps (see Choudhary et al., $Genome\ Biology$, 2019 for details). First, it regroups the samples into homogeneous and heteregenous sub-groups, which are used to compute the within-group and between-group nucleotide-wise d scores. Second, smoothed between- and within-group d score profiles are compared to construct candidate differential regions. Finally, unsmoothed between- and within-group d scores are compared using the Wilcoxon signed-rank test. The resulting p-values quantify the significance of difference in reactivity patterns between the two input groups.

Usage

```
dStruct(
  rdf,
  reps_A,
  reps_B,
  batches = FALSE,
 min_length = 11,
  check_signal_strength = TRUE,
  check_nucs = TRUE,
  check_quality = TRUE,
  quality = "auto",
  evidence = 0,
  signal_strength = 0.1,
 within_combs = NULL,
  between_combs = NULL,
  ind_regions = TRUE,
  gap = 1,
  get_FDR = TRUE,
  proximity_assisted = FALSE,
 proximity = 10,
  proximity_defined_length = 30
)
```

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Arguments

rdf Dataframe of reactivities for each sample.

reps_A Number of replicates of group A.
reps_B Number of replicates of group B.

batches Logical suggesting if replicates of group A and B were performed in batches and

are labelled accordingly. If TRUE, a heterogeneous/homogeneous subset may

not have multiple samples from the same batch.

min_length Minimum length of constructed regions.

check_signal_strength

Logical, if TRUE, construction of regions must be based on nucleotides that

have a minimum absolute value of reactivity.

check_nucs Logical, if TRUE, constructed regions must have a minimum number of nu-

cleotides participating in Wilcoxon signed rank test.

check_quality Logical, if TRUE, check constructed regions for quality.

quality Worst allowed quality for a region to be tested.

evidence Minimum evidence of increase in variation from within-group comparisons to

between-group comparisons for a region to be tested.

signal_strength

Threshold for minimum signal strength.

within_combs Data.frame with each column containing groupings of replicates of groups A or

B, which will be used to assess within-group variation.

between_combs Dataframe with each column containing groupings of replicates of groups A and

B, which will be used to assess between-group variation.

ind_regions Logical, if TRUE, test each region found in the transcript separately.

gap Integer. Join regions if they are separated by these many nucleotides.

get_FDR Logical, if FALSE, FDR is not reported.

proximity_assisted

Logical, if TRUE, proximally located regions are tested together.

proximity Maximum distance between constructed regions for them to be considered prox-

imal.

proximity_defined_length

If performing a "proximity-assisted" test, minimum end-to-end length of a re-

gion to be tested.

Value

Constructs regions, reports p-value and median difference of between-group and within-group d-scores for each region, and FDR for them.

Author(s)

Krishna Choudhary

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References

Choudhary, K., Lai, Y. H., Tran, E. J., & Aviran, S. (2019). dStruct: identifying differentially reactive regions from RNA structurome profiling data. *Genome biology*, 20(1), 1-26.

Examples

```
#Load data from Lai et al., 2019
data(lai2019)

#Run dStruct in de novo discovery mode for a transcript with id YAL042W.
dStruct(rdf = lai2019[["YAL042W"]], reps_A = 3, reps_B = 2,
    batches = TRUE, min_length = 21,
    between_combs = data.frame(c("A3", "B1", "B2")),
    within_combs = data.frame(c("A1", "A2", "A3")),
    ind_regions = TRUE)
```

dStructGuided

Performs guided discovery of differentially reactive regions.

Description

This function takes as input reactivity profiles for a transcript region from samples of two groups. First, it regroups the samples into homogeneous and heteregenous sub-groups, which are used to compute the within-group and between-group nucleotide-wise d scores. If the region meets the quality criteria, the between- and within-group d scores are compared using the Wilcoxon signed-rank test. The resulting p-values quantify the significance of difference in reactivity patterns between the two input groups.

Usage

```
dStructGuided(
  rdf,
  reps_A,
  reps_B,
  batches = FALSE,
  within_combs = NULL,
  between_combs = NULL,
  check_quality = TRUE,
  quality = "auto",
  evidence = 0
)
```

Arguments

rdf Dataframe of reactivities for each sample. Each column must be labelled as A1, A2, ..., B1, B2, ...

reps_A Number of replicates of group A.

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reps_B	Number of replicates of group B.
batches	Logical suggesting if replicates of group A and B were performed in batches and are labelled accordingly. If TRUE, a heterogeneous/homogeneous subset may not have multiple samples from the same batch.
within_combs	Data.frame with each column containing groupings of replicates of groups A or B, which will be used to assess within-group variation.
between_combs	Dataframe with each column containing groupings of replicates of groups A and B, which will be used to assess between-group variation.
check_quality	Logical, if TRUE, check regions for quality.
quality	Worst allowed quality for a region to be tested.
evidence	Minimum evidence of increase in variation from within-group comparisons to between-group comparisons for a region to be tested.

Value

p-value for the tested region (estimated using one-sided Wilcoxon signed rank test) and the median of nucleotide-wise difference of between-group and within-group d-scores.

Author(s)

Krishna Choudhary

References

Choudhary, K., Lai, Y. H., Tran, E. J., & Aviran, S. (2019). dStruct: identifying differentially reactive regions from RNA structurome profiling data. *Genome biology*, 20(1), 1-26.

Examples

```
#Load Wan et al., 2014 data
data(wan2014)

#Run dStruct in the guided mode on first region in wan2014.
dStructGuided(wan2014[[1]], reps_A = 2, reps_B = 1)
```

dStructome	Performs de novo or guided discovery of differentially reactive regions
	for transcriptome-wide data.

Description

This function provides a convenient way to call the dStruct or dStructGuided functions for multiple transcripts simultaneously. By default, the transcripts are processed in using multiple parallel processes if available.

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Usage

```
dStructome(
  rl,
  reps_A,
  reps_B,
 batches = FALSE,
 min_length = 11,
  check_signal_strength = TRUE,
  check_nucs = TRUE,
  check_quality = TRUE,
  quality = "auto",
  evidence = 0,
  signal_strength = 0.1,
 within_combs = NULL,
  between_combs = NULL,
  ind_regions = TRUE,
  gap = 1,
  processes = "auto",
 method = "denovo",
  proximity_assisted = FALSE,
 proximity = 10,
  proximity_defined_length = 30
```

Arguments

rl List of dataframes of reactivities for each sample.

reps_A Number of replicates of group A.
reps_B Number of replicates of group B.

batches Logical suggesting if replicates of group A and B were performed in batches and

are labelled accordingly. If TRUE, a heterogeneous/homogeneous subset may

not have multiple samples from the same batch.

min_length Minimum length of constructed regions.

check_signal_strength

Logical, if TRUE, construction of regions must be based on nucleotides that

have a minimum absolute value of reactivity.

check_nucs Logical, if TRUE, constructed regions must have a minimum number of nu-

cleotides participating in Wilcoxon signed rank test.

check_quality Logical, if TRUE, check constructed regions for quality.

quality Worst allowed quality for a region to be tested.

evidence Minimum evidence of increase in variation from within-group comparisons to

between-group comparisons for a region to be tested.

signal_strength

Threshold for minimum signal strength.

within_combs Data.frame with each column containing groupings of replicates of groups A or

B, which will be used to assess within-group variation.

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between_combs Dataframe with each column containing groupings of replicates of groups A and

B, which will be used to assess between-group variation.

ind_regions Logical, if TRUE, test each region found in the transcript separately.

gap Integer. Join regions if they are separated by these many nucleotides.

processes Number of parallel processes to use.

method Character specifying either guided or de novo discovery approach.

proximity_assisted

Logical, if TRUE, proximally located regions are tested together.

proximity Maximum distance between constructed regions for them to be considered prox-

imal.

proximity_defined_length

If performing a "proximity-assisted" test, minimum end-to-end length of a re-

gion to be tested.

Value

Constructs regions, reports p-value and median difference of between-group and within-group d-scores for each region, and FDR for them.

Author(s)

Krishna Choudhary

References

Choudhary, K., Lai, Y. H., Tran, E. J., & Aviran, S. (2019). dStruct: identifying differentially reactive regions from RNA structurome profiling data. *Genome biology*, 20(1), 1-26.

10 getCombs

getCombs	Identifies subgroupings of replicates for assessing within-group and between-group variation.
	gent mannen

Description

Regroup all the samples of A and B groups into homogoneous and heterogeneous sub-groups. Each homogenous sub-group contains replicates of either group A only or group B only. Each heterogeneous sub-group has a mix of samples from both the groups A and B.

Usage

```
getCombs(
  reps_A,
  reps_B,
  batches = FALSE,
  between_combs = NULL,
  within_combs = NULL
)
```

Arguments

reps_A	Number of replicates of group A.
reps_B	Number of replicates of group B.
batches	Logical suggesting if replicates of group A and B were performed in batches and are labelled accordingly. If TRUE, a heterogeneous/homogeneous subset may not have multiple samples from the same batch.
between_combs	Dataframe with each column containing groupings of replicates of groups A and B, which will be used to assess between-group variation.
within_combs	Data.frame with each column containing groupings of replicates of groups A or B, which will be used to assess within-group variation.

Value

List of two dataframes, containing groupings for within-group and between-group variation.

Author(s)

Krishna Choudhary

References

Choudhary, K., Lai, Y. H., Tran, E. J., & Aviran, S. (2019). dStruct: identifying differentially reactive regions from RNA structurome profiling data. *Genome biology*, 20(1), 1-26.

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Examples

#Get heterogeneous and homogeneous set combinations of samples when there are 2 samples of group A and 1 of group B. getCombs(2, 1)

getContigRegions

Identifies contiguous regions from a list of nucleotide indices.

Description

Given a sequence of nucleotide indices, this function returns integer ranges covered by the indices. There is an option to merge ranges if they are separated by less than a user-specified distance.

Usage

```
getContigRegions(x, gap = 0)
```

Arguments

x A vector of integers.

gap Include gaps in the ranges if they are shorter than or equal to this length.

Value

IRanges object storing start and end sites of continguous regions.

Author(s)

Krishna Choudhary

```
#Convert an integer vector of nucleotide positions to an IRanges object containing the coordinates of contiguous reg
nucleotide_positions <- c(1, 3, 2, 8, 4:7, 11:20)
getContigRegions(nucleotide_positions)
```

```
#Merge regions if their end points are within 3 nt of each other.
getContigRegions(nucleotide_positions, gap = 3)
```

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getRegions

Constructs potential differentially reactive regions.

Description

This function takes between- and within-group d scores for a transcript as input and identifies regions where the former is generally larger. Regions that pass minimum quality and minimum signal criteria are returned.

Usage

```
getRegions(
   d_within,
   d_spec,
   rdf,
   min_length = 11,
   check_signal_strength = TRUE,
   check_nucs = TRUE,
   check_quality = TRUE,
   quality = 0.5,
   evidence = 0,
   signal_strength = 0.1
)
```

Arguments

d_within
 Nucleotide-wise d score for within-group variation.
 d_spec
 Nucleotide-wise d score for between-group variation.
 rdf
 Dataframe of reactivities for each sample.
 min_length
 Minimum length of constructed regions.
 check_signal_strength

Logical, if TRUE, construction of regions must be based on nucleotides that

have a minimum absolute value of reactivity.

check_nucs Logical, if TRUE, constructed regions must have a minimum number of nu-

cleotides participating in Wilcoxon signed rank test.

check_quality Logical, if TRUE, check constructed regions for quality.

quality Worst allowed quality for a region to be tested.

evidence Minimum evidence of increase in variation from within-group comparisons to

between-group comparisons for a region to be tested.

signal_strength

Threshold for minimum signal strength.

Value

Integer vector of nucleotides that constitute potential differentially reactive regions.

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Author(s)

Krishna Choudhary

References

Choudhary, K., Lai, Y. H., Tran, E. J., & Aviran, S. (2019). dStruct: identifying differentially reactive regions from RNA structurome profiling data. *Genome biology*, 20(1), 1-26.

lai2019

Saccharomyces cerevisiae Structure-seq data

Description

Data from a Structure-seq assay of five samples of *S. cerevisiae*, three of which were wild-type samples and two mutant samples. The data was pre-processed to obtain DMS reactivities as described by Lai et al. (2019).

Usage

```
data("lai2019")
```

Format

An object of class "list".

Source

Raw data from Lai et al., 2019 in processed form.

References

```
Lai et al. (2019) Genetics, Vol. 212, 153–174 (Genetics)
```

```
data("lai2019")
```

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normalizer

Returns normalizer for reactivity vector.

Description

Assesses normalization factor for raw reactivities using the 2-8 % method. Given a reactivity profile, first, remove 2% of the nucleotides with the highest reactivities. Then, the normalization factor is the mean of reactivities of the 8% of the nucleotides with the next highest reactivities.

Usage

```
normalizer(raw.estimates)
```

Arguments

raw.estimates A vector of raw reactivities.

Value

The normalization factor.

Author(s)

Krishna Choudhary

References

Low JT, Weeks KM. SHAPE-directed RNA secondary structure prediction. *Methods*. 2010; 52(2):150–8.

Sloma MF, Mathews DH, Chen SJ, Burke-Aguero DH. Chapter four – improving RNA secondary structure prediction with structure mapping data. In: *Methods in Enzymology*, vol. 553. Cambridge: Academic Press: 2015. p. 91–114.

Choudhary K, Deng F, Aviran S. Comparative and integrative analysis of RNA structural profiling data: current practices and emerging questions. *Quant Biol.* 2017; 5(1):3–24.

```
normalizer(c(NA, rnorm(20, 0.5, 0.3), NA, -999))
```

plotDStructurome 15

Plots differentially reactive regions.	plotDStructurome
--	------------------

Description

Given the table of results from dStruct or dStructGuided and the corresponding lists with reactivity scores for all transcripts, this function saves a PDF file with detailed visualizations of reactivities for all differential regions.

Usage

```
plotDStructurome(
  rl,
  diff_regions,
  outfile,
  fdr = 0.05,
  ylim = c(-0.05, 3),
  del_d_cutoff = 0.01
)
```

Arguments

rl	List of dataframes of reactivities for each sample.
diff_regions	Output from dStruct or dStructGuided containing coordinates of regions with significance of differentially reactivity.
outfile	The name for pdf file which will be saved.
fdr	FDR threshold for plotted regions.
ylim	Y-axis limits for plots.
del_d_cutoff	Minimum effect size for plotted regions specified in terms of median difference of the between-group and within-group d-scores.

Value

Saves a PDF for all differentially reactive regions. Returns NULL.

Author(s)

Krishna Choudhary

References

Choudhary, K., Lai, Y. H., Tran, E. J., & Aviran, S. (2019). dStruct: identifying differentially reactive regions from RNA structurome profiling data. *Genome biology*, 20(1), 1-26.

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Examples

```
#Load data from Lai et al., 2019
data(lai2019)

#Run dStruct in de novo discovery mode for all the transcripts in this data in one step.
res <- dStructome(lai2019, 3, 2, batches= TRUE, min_length = 21,
    between_combs = data.frame(c("A3", "B1", "B2")),
    within_combs = data.frame(c("A1", "A2", "A3")),
    ind_regions = TRUE, processes = 1)

#Plot the significant results and save to a PDF file.
plotDStructurome(rl = lai2019,
    diff_regions = res,
    outfile = "significantly_differential_regions",
    fdr = 0.05,
    ylim = c(-0.05, 3))</pre>
```

twoEightNormalize

Normalizes reactivity vector.

Description

Given a reactivity profile, first, remove 2% of the nucleotides with the highest reactivities. Then, the normalization factor is the mean of reactivities of the 8% of the nucleotides with the next highest reactivities. The raw reactivities are divided by the normalization factor to get normalized reactivities. This is called as 2-8 % normalization and has been a common way to normalize data from RNA structurome profiling technologies such as SHAPE-Seq, Structure-Seq, etc. (see Low and Weeks, 2010, Sloma et al., 2015, and Choudhary et al., 2017).

Usage

```
twoEightNormalize(raw.estimates)
```

Arguments

raw.estimates A vector of raw reactivities.

Value

A vector of normalized reactivities.

Author(s)

Krishna Choudhary

wan2014

References

Low JT, Weeks KM. SHAPE-directed RNA secondary structure prediction. Methods. 2010; 52(2):150-8.

Sloma MF, Mathews DH, Chen SJ, Burke-Aguero DH. Chapter four – improving RNA secondary structure prediction with structure mapping data. In: *Methods in Enzymology*, vol. 553. Cambridge: Academic Press: 2015. p. 91–114.

Choudhary K, Deng F, Aviran S. Comparative and integrative analysis of RNA structural profiling data: current practices and emerging questions. *Quant Biol.* 2017; 5(1):3–24.

Examples

```
twoEightNormalize(c(NA, rnorm(20, 0.5, 0.3), NA, -999))
```

wan2014

Homo sapiens PARS data

Description

Data from a PARS assay of a family trio of mother, father, and child. The data was pre-processed to obtain PARS scores as described in Choudhary et al. (2019).

Usage

```
data(wan2014)
```

Format

An object of class "list".

Source

Counts data from Wan et al., 2014 in processed form.

References

```
Wan et al., Nature, 505, 706–709 (2014) (Nature)
```

```
data(wan2014)
```

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