Package 'RNAmodR.RiboMethSeq'

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Description RNAmodR.RiboMethSeq implements the detection of 2'-O methylations on RNA from experimental data generated with the RiboMethSeq protocol. The package builds on the core functionality of the RNAmodR package to detect specific patterns of the modifications in high throughput sequencing data.

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Author Felix G.M. Ernst [aut, cre] (ORCID:
<https://orcid.org/0000-0001-5064-0928>),
Denis L.J. Lafontaine [ctb, fnd]

Maintainer Felix G.M. Ernst <felix.gm.ernst@outlook.com>

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`RNAmodR.RiboMethSeq-package`
RNAmodR.RiboMethSeq: Detection of 2'-O methylations by RiboMethSeq

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Description

RNAmodR.RiboMethSeq implements the detection of 2'-O methylations on RNA from experimental data generated with the RiboMethSeq protocol. The package builds on the core functionality of the RNAmodR package to detect specific patterns of the modifications in high throughput sequencing data.

Author(s)

Maintainer: Felix G.M. Ernst <felix.gm.ernst@outlook.com> (ORCID)

Other contributors:

- Denis L.J. Lafontaine <denis.lafontaine@ulb.ac.be> [contributor, funder]

See Also

Useful links:

- https://github.com/FelixErnst/RNAmodR.RiboMethSeq
- Report bugs at https://github.com/FelixErnst/RNAmodR.RiboMethSeq/issues

ModRiboMethSeq *ModRiboMethSeq class to analyze RiboMethSeq data*

Description

Among the various post-transcriptional RNA modifications, 2'-O methylations are quite common in rRNA and tRNA. They confere resistance to alkaline degradation by preventing a nucleophilic attack on the 3'-phosphate especially in flexible RNA, which is fascilitated by high pH conditions. This property can be queried using a method called RiboMethSeq (Birkedahl et al. 2015, Marchand et al. 2017) for which RNA is treated in alkaline conditions and RNA fragments are used to prepare a sequencing library.

At position containing a 2'-O methylations, read ends are less frequent, which is used to detect and score the2'-O methylations.

`dataType` is `"ProtectedEndSequenceData"`:

The `ModRiboMethSeq` class uses the the `ProtectedEndSequenceData` class to store and aggregate data along the transcripts. The calculated scores follow the nomenclature of Birkedahl et al. (2015) with the names `scoreRMS` (default), `scoreA`, `scoreB` and `scoreMean`.

The ScoreMax as described by Marchand et al. (2017) are not implemented, yet, since an unambigeous description is not available from the literature.

The ScoreMean as described by Galvanin et al. (2018) is implemented. However, use with caution, since the description is not unambigeous. Currently it is calculated as as: 1 - (n / mean(areaL + areaR)). (n: counts at position, areaL: counts from x position upstream, areaR: counts from x position downstream)

Only samples named `treated` are used for this analysis. Normalization to untreated samples is currently not used.

The `ModRiboMethSeq5` class can be used as well. However, as `SequenceData` the `End5SequenceData` is employed using only the 5'-end positions of reads.

Usage

```
ModRiboMethSeq(x, annotation = NA, sequences = NA, seqinfo = NA, ...)

ModSetRiboMethSeq(x, annotation = NA, sequences = NA, seqinfo = NA, ...)
```

Arguments

`x`	the input which can be of the different types depending on whether a `ModRiboMethSeq` or a `ModSetRiboMethSeq` object is to be constructed. For more information have a look at the documentation of the `Modifier` and `ModifierSet` classes.
`annotation`	annotation data, which must match the information contained in the BAM files. This is parameter is only required if `x` if not a `Modifier` object.
`sequences`	sequences matching the target sequences the reads were mapped onto. This must match the information contained in the BAM files. This is parameter is only required if `x` if not a `Modifier` object.

`seqinfo` An optional `Seqinfo` argument or character vector, which can be coerced to one, to subset the sequences to be analyzed on a per chromosome basis.

`...` Optional arguments overwriting default values, which are

- weights: The weights used for calculating the scores B and RMS (default: `weights = c(0.9,1,0,1,0.9)`).
- flankingRegion: The size of the flanking region used for calculation of score A as an integer value (default: `flankingRegion = 6L`).
- minSignal: The minimal signal at the position as integer value (default: `minSignal = 10L`). If the reaction is very specific a lower value and even 0L may need to be used.
- minScoreA: minimum for score A to identify 2'-O methylated positions de novo (default: `minScoreA = 0.6`).
- minScoreB: minimum for score B to identify 2'-O methylated positions de novo (default: `minScoreB = 3.0`).
- minScoreRMS: minimum for score RMS to identify 2'-O methylated positions de novo (default: `minScoreRMS = 0.75`).
- minScoreMean: minimum for ScoreMean to identify 2'-O methylated positions de novo (default: `minScoreMean = 0.75`).
- flankingRegionMean: The size of the flanking region used for calculation of ScoreMean as an integer value (default: `flankingRegionMean = 2L`).
- scoreOperator: how the minimal score should be used as logical operator. "&" requires all minimal values to be exceeded, whereas "|" detects positions, if at least one minimal values is exceeded (default: `scoreOperator = "&"`).
- maxLength: The default read length. Reads with this length or longer are discarded, since they represent non-fragmented reads. This might need to be adjusted for individual samples depending on the experimental conditions. This is argument is passed on to `ProtectedEndSequenceData` (default: `maxLength = 50L`).
- other arguments which are passed on to `ProtectedEndSequenceData`.

To disable minimal values for modification calling, set them to `0`. It is not advised to set them all to `0`.

Value

a `ModRiboMethSeq` or `ModSetRiboMethSeq` object

Author(s)

Felix G.M. Ernst [aut]

References

- Birkedal U, Christensen-Dalsgaard M, Krogh N, Sabarinathan R, Gorodkin J, Nielsen H (2015): "Profiling of ribose methylations in RNA by high-throughput sequencing." Angewandte Chemie (International ed. in English) 54 (2), P. 451–455. DOI: 10.1002/anie.201408362.

- Marchand V, Ayadi L, El Hajj A, Blanloeil-Oillo F, Helm M, Motorin Y (2017): "High-Throughput Mapping of 2'-O-Me Residues in RNA Using Next-Generation Sequencing (Illumina RiboMethSeq Protocol)." Methods in molecular biology (Clifton, N.J.) 1562, P. 171–187. DOI: 10.1007/978-1-4939-6807-7_12.

- Galvanin A, Ayadi L, Helm M, Motorin Y, Marchand V (2017): "Mapping and Quantification of tRNA 2'-O-Methylation by RiboMethSeq". Wajapeyee N., Gupta R. (eds) Epitranscriptomics. Methods in Molecular Biology (Humana Press, New York, NY) 1870, P. 273-295. DOI: 10.1007/978-1-4939-8808-2_21

Examples

```
library(RNAmodR.Data)
library(rtracklayer)
annotation <- GFF3File(RNAmodR.Data.example.RMS.gff3())
sequences <- RNAmodR.Data.example.RMS.fasta()
files <- list("Sample1" = c(treated = RNAmodR.Data.example.RMS.1()),
              "Sample2" = c(treated = RNAmodR.Data.example.RMS.1()))
# Creating a Modifier object of type ModRiboMethSeq
mrms <- ModRiboMethSeq(files[[1]], annotation = annotation, sequences = sequences)
# Creating a ModifierSet object of type ModSetRiboMethSeq
msrms <- ModSetRiboMethSeq(files, annotation = annotation, sequences = sequences)
```

ModRiboMethSeq-functions

Functions for ModRiboMethSeq

Description

All of the functions of `Modifier` and the `ModifierSet` classes are inherited by the `ModRiboMethSeq` and `ModSetRiboMethSeq` classes.

Usage

```
## S4 replacement method for signature 'ModRiboMethSeq'
settings(x) <- value

## S4 method for signature 'ModRiboMethSeq'
aggregateData(x)

## S4 method for signature 'ModRiboMethSeq'
findMod(x)

## S4 method for signature 'ModRiboMethSeq'
getDataTrack(x, name, type, ...)

## S4 method for signature 'ModRiboMethSeq,GRanges'
plotDataByCoord(
  x,
```

```
  coord,
  type = c("ends", "scoreA", "scoreB", "scoreRMS", "scoreMean"),
  window.size = 15L,
  ...
)

## S4 method for signature 'ModRiboMethSeq'
plotData(
  x,
  name,
  from = 1L,
  to = 30L,
  type = c("ends", "scoreA", "scoreB", "scoreRMS", "scoreMean"),
  ...
)

## S4 method for signature 'ModSetRiboMethSeq,GRanges'
plotDataByCoord(
  x,
  coord,
  type = c("scoreRMS", "ends", "scoreA", "scoreB", "scoreMean"),
  window.size = 15L,
  ...
)

## S4 method for signature 'ModSetRiboMethSeq'
plotData(
  x,
  name,
  from = 1L,
  to = 30L,
  type = c("scoreRMS", "ends", "scoreA", "scoreB", "scoreMean"),
  ...
)
```

Arguments

x	a Modifier or a ModifierSet object. For more details see also the man pages for the functions mentioned below.
value	See settings
coord, name, from, to, type, window.size, ...	See plotData

Details

ModRiboMethSeq specific arguments for plotData:

- colour - a named character vector of length = 4 for the colours of the individual histograms. The names are expected to be c("ends","scoreA","scoreB","scoreRMS","scoreMean").

Value

- settings: See settings.
- aggregate: See aggregate.
- modify: See modify.
- getDataTrack: a list of DataTrack object.
- plotData: See plotDataByCoord.
- plotDataByCoord: See plotDataByCoord.

Examples

```
data(msrms,package="RNAmodR.RiboMethSeq")
mrms <- msrms[[1]]
settings(mrms)
aggregate(mrms)
modify(mrms)
getDataTrack(mrms, "1", mainScore(mrms))
```

RNAmodR.RiboMethSeq *RNAmodR.RiboMethSeq*

Description

'RNAmodR.RiboMethSeq' implements the detection of 2'-O methylations from RiboMethSeq data using the workflow and class the package 'RNAmodR' provides.

Author(s)

Felix G M Ernst [aut], Denis L J Lafontaine [fnd]

See Also

Further details are described in the man pages of the Modifier object and the vignettes.

RNAmodR.RiboMethSeq-datasets *Example data in the RNAmodR.RiboMethSeq package*

Description

This contains an example ModifierSet object of type ModSetRiboMethSeq

Usage

```
data(msrms)
```

Format

a ModSetRiboMethSeq instance

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