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 facilitated 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 the 2'-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 unambiguous 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 unambiguous. 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

- \textit{x}: the input which can be of the different types depending on whether a \texttt{ModRiboMethSeq} or a \texttt{ModSetRiboMethSeq} object is to be constructed. For more information have a look at the documentation of the \texttt{Modifier} and \texttt{ModifierSet} classes.

- \textit{annotation}: annotation data, which must match the information contained in the BAM files. This is parameter is only required if \textit{x} if not a \texttt{Modifier} object.

- \textit{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 \textit{x} if not a \texttt{Modifier} object.

- \textit{seqinfo}: An optional \texttt{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

- \textit{weights}: The weights used for calculating the scores B and RMS (default: \texttt{weights = c(0.9,1,0,1,0,0.9)}).

- \textit{flankingRegion}: The size of the flanking region used for calculation of score A as an integer value (default: \texttt{flankingRegion = 6L}).

- \textit{minSignal}: The minimal signal at the position as integer value (default: \texttt{minSignal = 10L}). If the reaction is very specific a lower value and even 0L may need to be used.

- \textit{minScoreA}: minimum for score A to identify 2'-O methylated positions de novo (default: \texttt{minScoreA = 0.6}).

- \textit{minScoreB}: minimum for score B to identify 2'-O methylated positions de novo (default: \texttt{minScoreB = 3.0}).

- \textit{minScoreRMS}: minimum for score RMS to identify 2'-O methylated positions de novo (default: \texttt{minScoreRMS = 0.75}).

- \textit{minScoreMean}: minimum for ScoreMean to identify 2'-O methylated positions de novo (default: \texttt{minScoreMean = 0.75}).

- \textit{flankingRegionMean}: The size of the flanking region used for calculation of ScoreMean as an integer value (default: \texttt{flankingRegionMean = 2L}).

- \textit{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: \texttt{scoreOperator = ")&")}.

- \textit{maxLength}: The default read length. Reads with this length or longer are discarded, since they represent non-fragemented reads. This might need to be adjusted for individual samples dending on the experimental conditions. This is argument is passed on to \texttt{ProtectedEndSequenceData} (default: \texttt{maxLength = 50L}).

- other arguments which are passed on to \texttt{ProtectedEndSequenceData}.

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

Value

a \texttt{ModRiboMethSeq} or \texttt{ModSetRiboMethSeq} object
Author(s)
Felix G.M. Ernst [aut]

References

Examples

```r
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
mrmr <- ModRiboMethSeq(files[[1]], annotation = annotation, sequences = sequences)
# Creating a ModifierSet object of type ModSetRiboMethSeq
msrmr <- ModSetRiboMethSeq(files, annotation = annotation, sequences = sequences)
```

Description

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

Usage

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

## S4 method for signature 'ModRiboMethSeq'
aggregateData(x)
```
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 <- mrmzs[[1]]
settings(mrmzs)
aggregate(mrmzs)
modify(mrmzs)
getDataTrack(mrmzs, "1", mainScore(mrmzs))

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.
Description
This contains an example ModifierSet object of type ModSetRiboMethSeq

Usage
data(msrms)

Format
a ModSetRiboMethSeq instance
Index

* datasets
  RNAmodR.RiboMethSeq-datasets, 7
  aggregate, 6
  aggregate (ModRiboMethSeq-functions), 4
  aggregateData, ModRiboMethSeq-method
    (ModRiboMethSeq-functions), 4
  DataTrack, 6
  End5SequenceData, 2
  findMod, ModRiboMethSeq-method
    (ModRiboMethSeq-functions), 4
  getDataTrack, ModRiboMethSeq-method
    (ModRiboMethSeq-functions), 4
  Modifier, 3–6
  ModifierSet, 3–5
  modify, 6
  modify (ModRiboMethSeq-functions), 4
  ModRiboMethSeq, 2
  ModRiboMethSeq-class (ModRiboMethSeq), 2
  ModRiboMethSeq-functions, 4
  ModSetRiboMethSeq (ModRiboMethSeq), 2
  ModSetRiboMethSeq-class
    (ModRiboMethSeq), 2
  msrms (RNAmodR.RiboMethSeq-datasets), 7
  plotData, 6
  plotData (ModRiboMethSeq-functions), 4
  plotData, ModRiboMethSeq-method
    (ModRiboMethSeq-functions), 4
  plotData, ModSetRiboMethSeq-method
    (ModRiboMethSeq-functions), 4
  plotDataByCoord, 6
  plotDataByCoord
    (ModRiboMethSeq-functions), 4
  plotDataByCoord, ModRiboMethSeq, GRanges-method
    (ModRiboMethSeq-functions), 4
  ProtectedEndSequenceData, 2, 3
  RiboMethSeq (ModRiboMethSeq), 2
  RNAmodR.RiboMethSeq, 6
  RNAmodR.RiboMethSeq-datasets, 7
  Seqinfo, 3
  settings, 6
  settings (ModRiboMethSeq-functions), 4
  settings<-, ModRiboMethSeq-method
    (ModRiboMethSeq-functions), 4