Package 'isomiRs'

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`isomiRs-package` *isomiRs*

Description

Characterization of miRNAs and isomiRs, clustering and differential expression.

Author(s)

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- Georgia Escaramis (CIBERESP - CIBER Epidemiologia y Salud Publica)

See Also

Useful links:

- Report bugs at https://github.com/lpantano/isomiRs/issues

---

counts *Accessors for the count matrix of a IsomirDataSeq object.*

Description

The counts slot holds the count data as a matrix of non-negative integer count values, one row for each isomiR, and one column for each sample. The normalized matrix can be obtained by using the parameter `norm=TRUE`.

Usage

```
counts.IsomirDataSeq(object, norm = FALSE)

## S4 method for signature 'IsomirDataSeq'
counts(object, norm = FALSE)

## S4 replacement method for signature 'IsomirDataSeq,matrix'
counts(object) <- value
```

Arguments

`object`	A `IsomirDataSeq` object.
`norm`	Boolean, return log2-normalized counts.
`value`	An integer matrix.

Value

base::matrix with raw or normalized count data.

Author(s)

Lorena Pantano

Examples

```
data(mirData)
head(counts(mirData))
```

dat286.long *Data frame containing mirna from Argyropoulos's paper*

Description

Argyropoulos, Christos, et al. "Modeling bias and variation in the stochastic processes of small RNA sequencing." Nucleic Acids Research (2017).

Usage

```
dat286.long
```

Format

mirna expression data in long format.

design *Accessors for the 'design' slot of a IsomirDataSeq object.*

Description

The design holds the R `formula` which expresses how the counts depend on the variables in `colData`. See IsomirDataSeq for details.

Usage

```
## S4 method for signature 'IsomirDataSeq'
design(object)

## S4 replacement method for signature 'IsomirDataSeq,formula'
design(object) <- value
```

Arguments

`object`	A IsomirDataSeq object.
`value`	A formula to pass to DESeq2.

Value

design for the experiment

Examples

```
data(mirData)
design(mirData) <- formula(~ 1)
```

ego *enrichResult class*

Description

enrichResult class

Usage

```
ego
```

Format

enrichResult class with the output of: ego <- enrichGO(row.names(assay(gene_ex_rse, "norm")), org.Mm.eg.db, "ENSEMBL", ont = "BP")

gene_ex_rse *Data frame containing gene expression data*

Description

Data frame containing gene expression data

Usage

```
gene_ex_rse
```

Format

gene expression data with 18 samples: example of a time series data

isoAnnotate *Annotate the rawData of the IsomirDataSeq object*

Description

Get the sequence and the name information for each isomiR, and the importance value (`isomir_reads/mirna_reads`) for each sample.

Usage

```
isoAnnotate(ids)
```

Arguments

ids	Object of class IsomirDataSeq.

Details

edit_mature_position represents the position at the mature sequence + nucleotide at reference + nucleotide at isomiR.

Value

data.frame with the sequence, isomir name, and importance for each sample and isomiR.

Examples

```
data(mirData)
head(isoAnnotate(mirData))
```

---

isoCounts *Create count matrix with different summarizing options*

Description

This function collapses isomiRs into different groups. It is a similar concept than how to work with gene isoforms. With this function, different changes can be put together into a single miRNA variant. For instance all sequences with variants at 3' end can be considered as different elements in the table or analysis having the following naming hsa-miR-124a-5p.iso.t3:AAA.

Usage

```
isoCounts(
  ids,
  ref = FALSE,
  iso5 = FALSE,
  iso3 = FALSE,
  add = FALSE,
  snv = FALSE,
  seed = FALSE,
  all = FALSE,
  minc = 1,
  mins = 1,
  merge_by = NULL
)
```

Arguments

ids	Object of class IsomirDataSeq.
ref	Differentiate reference miRNA from rest.
iso5	Differentiate trimming at 5 miRNA from rest.
iso3	Differentiate trimming at 3 miRNA from rest.
add	Differentiate additions miRNA from rest.
snv	Differentiate nt substitution miRNA from rest.
seed	Differentiate changes in 2-7 nts from rest.
all	Differentiate all isomiRs.
minc	Int minimum number of isomiR sequences to be included.
mins	Int minimum number of samples with number of sequences bigger than minc counts.
merge_by	Column in coldata to merge samples into a single column in counts. Useful to combine technical replicates.

Details

You can merge all isomiRs into miRNAs by calling the function only with the first parameter `isoCounts(ids)`. You can get a table with isomiRs altogether and the reference miRBase sequences by calling the function with `ref=TRUE`. You can get a table with 5' trimming isomiRS, miRBase reference and the rest by calling with `isoCounts(ids, ref=TRUE, iso5=TRUE)`. If you set up all parameters to TRUE, you will get a table for each different sequence mapping to a miRNA (i.e. all isomiRs).

Examples for the naming used for the isomiRs are at http://seqcluster.readthedocs.org/mirna_annotation.html#mirna-annotation.

Value

IsomirDataSeq object with new count table. The count matrix can be access with `counts(ids)`.

Examples

```
data(mirData)
ids <- isoCounts(mirData, ref=TRUE)
head(counts(ids))
# taking into account isomiRs and reference sequence.
ids <- isoCounts(mirData, ref=TRUE, minc=10, mins=6)
head(counts(ids))
```

isoDE *Differential expression analysis with DESeq2*

Description

This function does differential expression analysis with DESeq2::DESeq2-package using the specific formula. It will return a DESeq2::DESeqDataSet object.

Usage

```
isoDE(ids, formula = NULL, ...)
```

Arguments

ids	Object of class IsomirDataSeq.
formula	Formula used for DE analysis.
...	Options to pass to `isoCounts()` including ref, iso5, iso3, add, subs and seed parameters.

Details

First, this function collapses all isomiRs in different types. Read more at `isoCounts()` to know the different options available to collapse isomiRs.

After that, DESeq2::DESeq2-package is used to do differential expression analysis. It uses the count matrix and design experiment stored at (`counts(ids)` and `colData(ids)`) IsomirDataSeq object to construct a DESeq2::DESeqDataSet object.

Value

DESeq2::DESeqDataSet object. To get the differential expression isomiRs, use `DESeq2::results()` from DESeq2 package. This allows to ask for different contrast without calling again `isoDE()`. Read `results` manual to know how to access all the information.

Examples

```
data(mirData)
ids <- isoCounts(mirData, minc=10, mins=6)
dds <- isoDE(mirData, formula=~condition)
```

`IsomirDataSeq-class` *Class that contains all isomiRs annotation for all samples*

Description

The IsomirDataSeq is a subclass of `SummarizedExperiment`. used to store the raw data, intermediate calculations and results of an miRNA/isomiR analysis. This class stores all raw isomiRs data for each sample, processed information, summary for each isomiR type, raw counts, normalized counts, and table with experimental information for each sample.

Details

IsomirDataSeqFromFiles creates this object using seqbuster output files.

Methods for this objects are `counts()` to get count matrix and `isoSelect()` for miRNA/isomiR selection. Functions available for this object are `isoCounts()` for count matrix creation, `isoNorm()` for normalization, `isoDE()` for differential expression. `isoPlot()` helps with basic expression plot.

`metadata` contains one list:

- `rawData` is a data.frame with the information of each sequence found in the data and the counts for each sample.

The naming of isomiRs follows these rules:

- miRNA name
- type:ref if the sequence is the same as the miRNA reference. `iso` if the sequence has variations.
- `iso_5p tag`:indicates variations at 5 position. The naming contains two words: `direction - nucleotides`, where direction can be UPPER CASE NT (changes upstream of the 5 reference position) or LOWER CASE NT (changes downstream of the 5 reference position). `0` indicates no variation, meaning the 5 position is the same as the reference. After `direction`, it follows the nucleotide/s that are added (for upstream changes) or deleted (for downstream changes).
- `iso_3p tag`:indicates variations at 3 position. The naming contains two words: `direction - nucleotides`, where direction can be LOWER CASE NT (upstream of the 3 reference position) or UPPER CASE NT (downstream of the 3 reference position). `0` indicates no variation, meaning the 3 position is the same as the reference. After `direction`, it follows the nucleotide/s that are added (for downstream changes) or deleted (for upstream chanes).
- `iso_add tag`:indicates nucleotides additions at 3 position. The naming contains two words: `direction - nucleotides`, where direction is UPPER CASE NT (upstream of the 5 reference position). `0` indicates no variation, meaning the 3 position has no additions. After `direction`, it follows the nucleotide/s that are added.
- `iso_snv tag`: indicates nucleotides substitutions along the sequences. The naming contains three words: `position-nucleotide@isomiR-nucleotide@reference`.
- `iso_snv_seed tag`: same as `iso_snv` tag, but only if the change happens between nucleotide 2 and 8.

In general nucleotides in UPPER case mean insertions respect to the reference sequence, and nucleotides in LOWER case mean deletions respect to the reference sequence.

Examples

```
path <- system.file("extra", package="isomiRs")
fn_list <- list.files(path, pattern="mirna", full.names = TRUE)
de <- data.frame(row.names=c("f1" , "f2"),
                 condition = c("newborn", "newborn"))
ids <- IsomirDataSeqFromFiles(fn_list, coldata=de)

head(counts(ids))
```

`IsomirDataSeqFromFiles` *Loads miRNA annotation from seqbuster tool or pre-processed data.*

Description

This function parses output of seqbuster tool to allow isomiRs/miRNAs analysis of samples in different groups such as characterization, differential expression and clustering. It creates an IsomirDataSeq object.

Usage

```
IsomirDataSeqFromFiles(
  files,
  coldata,
  rate = 0.2,
  canonicalAdd = TRUE,
  uniqueMism = TRUE,
  uniqueHits = FALSE,
  design = ~1L,
  minHits = 1L,
  header = TRUE,
  skip = 0,
  quiet = TRUE,
  ...
)
```

Arguments

`files`	files with the output of seqbuster tool
`coldata`	data frame containing groups for each sample
`rate`	minimum counts fraction to consider a mismatch a real mutation
`canonicalAdd`	`boolean` only keep A/T non-template addition. All non-template nucleotides at the 3' end will be removed if they contain C/G nts.
`uniqueMism`	`boolean` only keep mutations that have a unique hit to one miRNA molecule. For instance, if the sequence map to two different miRNAs, then it would be removed.

uniqueHits	boolean whether filtering ambigous sequences or not.
design	a formula to pass to DESeq2::DESeqDataSet
minHits	Minimum number of reads in the sample to consider it in the final matrix.
header	boolean to indicate files contain headers
skip	skip first line when reading files
quiet	boolean indicating to print messages while reading files. Default FALSE.
...	arguments provided to SummarizedExperiment and IsomirDataSeqFromRawData. including rowData.

Details

This function parses the output of http://seqcluster.readthedocs.org/mirna_annotation.html for each sample to create a count matrix for isomiRs, miRNAs or isomiRs grouped in types (i.e all sequences with variations at 5' but ignoring any other type). It creates IsomirDataSeq object (see link to example usage of this class) to allow visualization, queries, differential expression analysis and clustering. To create the IsomirDataSeq, it parses the isomiRs files, and generates an initial matrix having all isomiRs detected among samples. As well, it creates a summary for each isomiR type (trimming, addition and substitution) to visualize general isomiRs distribution.

Value

IsomirDataSeq class object.

Examples

```
path <- system.file("extra", package="isomiRs")
fn_list <- list.files(path, pattern="mirna", full.names = TRUE)
de <- data.frame(row.names=c("f1" , "f2"),
                 condition = c("newborn", "newborn"))
ids <- IsomirDataSeqFromFiles(fn_list, coldata=de)

head(counts(ids))
IsomirDataSeqFromRawData(metadata(ids)[["rawData"]], de)
```

IsomirDataSeqFromMirtop

Import mirtop *output into* IsomirDataSeq

Description

The tabular output of mirtop is compatible with IsomirDataSeq. This function allows to import the data and filter low confidence isomiRs for downstream analysis.

Usage

```
IsomirDataSeqFromMirtop(mirtop, coldata, ...)
```

Arguments

`mirtop`	data.frame with the output of `mirtop export`
`coldata`	data.frame with the metadata of the samples
`...`	It supports the same parameters as in IsomirDataSeqFromRawData.

Details

The output is generated with `mirtop export --format isomir`.

Value

IsomirDataSeq class object.

Examples

```
library(readr)
path <- system.file("extra", "mirtop", package="isomiRs")
fn <- list.files(path, full.names = TRUE)
de <- data.frame(row.names=c("sample1" , "sample2"),
                 condition = c("cc", "cc"))
# mirtop export --format isomir ....
IsomirDataSeqFromMirtop(read_tsv(fn), de)
```

IsomirDataSeqFromRawData

Loads miRNA annotation from seqbuster tool or pre-processed data.

Description

Process raw data like tables to speed up filtering steps.

Usage

```
IsomirDataSeqFromRawData(
  rawdata,
  coldata,
  design = ~1L,
  pct = 0.1,
  n_snv = 1,
  whitelist = NULL,
  ...
)
```

Arguments

rawdata	data.frame stored in metadata slot of IsomirDataSeq object.
coldata	data frame containing groups for each sample
design	a formula to pass to DESeq2::DESeqDataSet
pct	numeric used to remove isomiRs with an importance lower than this value. Importance is calculated by dividing the isomiR count by the total counts of the miRNA to which it maps.
n_snv	numeric used to remove isomiRs with more than this number of single nucleotide variants (indels are counted here).
whitelist	character vector with sequences to keep even if the filtering step would have removed them. They have to match the seq column in the table.
...	arguments provided to SummarizedExperiment. including rowData.

Value

IsomirDataSeq class object.

Examples

```
path <- system.file("extra", package="isomiRs")
fn_list <- list.files(path, pattern="mirna", full.names = TRUE)
de <- data.frame(row.names=c("f1" , "f2"),
                 condition = c("newborn", "newborn"))
ids <- IsomirDataSeqFromFiles(fn_list, coldata=de)

head(counts(ids))
IsomirDataSeqFromRawData(metadata(ids)[["rawData"]], de)
```

---

isoNorm *Normalize count matrix*

Description

This function normalizes raw count matrix using DESeq2::rlog() function from DESeq2::DESeq2-package.

Usage

```
isoNorm(ids, formula = NULL, maxSamples = 50)
```

Arguments

ids	Object of class IsomirDataSeq.
formula	Formula that will be used for normalization.
maxSamples	Maximum number of samples to use with DESeq2::rlog(), if not limma::voom() is used.

Value

IsomirDataSeq object with the normalized count matrix in a slot. The normalized matrix can be access with `counts(ids, norm=TRUE)`.

Examples

```
data(mirData)
ids <- isoCounts(mirData, minc=10, mins=6)
ids <- isoNorm(mirData, formula=~condition)
head(counts(ids, norm=TRUE))
```

`isoPlot` *Plot the amount of isomiRs in different samples*

Description

This function plot different isomiRs proportion for each sample. It can show trimming events at both side, additions and nucleotides changes.

Usage

```
isoPlot(ids, type = "iso5", column = NULL, use = NULL, nts = FALSE)
```

Arguments

`ids`	Object of class IsomirDataSeq.
`type`	String (iso5, iso3, add, snv, all) to indicate what isomiRs to use for the plot. See details for explanation.
`column`	String indicating the column in `colData` to color samples.
`use`	Character vector to only use these isomiRs for the plot. The id used is the rownames that comes from using isoCounts with all the arguments on TRUE.
`nts`	Boolean to indicate whether plot positions of nucleotides changes when showing single nucleotides variants.

Details

There are four different values for `type` parameter. To plot trimming at 5' or 3' end, use `type="iso5"` or `type="iso3"`. Get a summary of all using `type="all"`. In this case, it will plot 3 positions at both side of the reference position described at miRBase site. Each position refers to the % of sequences that start/end before or after the miRBase reference. The color indicates the sample group. The size of the point is proportional to the abundance considering the total as all the sequences in the sample. The position at `y` is the % of different sequences considering the total as all sequences with changes for the specific isomiR showed.

Same logic applies to `type="add"` and `type="subs"`. However, when `type="add"`, the plot will refer to addition events from the 3' end of the reference position. Note that this additions don't match to the precursor sequence, they are non-template additions. In this case, only 3 positions

after the 3' end will appear in the plot. When `type="subs"`, it will appear one position for each nucleotide in the reference miRNA. Points will indicate isomiRs with nucleotide changes at the given position. When `type="all"` a colar coordinate map will show the abundance of each isomiR type in a single plot. Note the position is relatively to the sequence not the miRNA.

Value

`ggplot2::ggplot()` Object showing different isomiRs changes at different positions.

Examples

```
data(mirData)
isoPlot(mirData)
```

`isoPlotPosition` *Plot nucleotides changes at a given position*

Description

This function plot different isomiRs proportion for each sample at a given position focused on the nucleotide change that happens there.

Usage

```
isoPlotPosition(ids, position = 1L, column = NULL)
```

Arguments

`ids`	Object of class IsomirDataSeq.
`position`	Integer indicating the position to show.
`column`	String indicating the column in colData to color samples.

Details

It shows the nucleotides changes at the given position for each sample in each group. The color indicates the sample group. The size of the point is proportional to the number of total counts of isomiRs with changes. The position at `y` is the % of different isomiRs supporting the change. Note the position is relatively to the sequence not the miRNA.

Value

`ggplot2::ggplot()` Object showing nucleotide changes at a given position.

Examples

```
data(mirData)
isoPlotPosition(mirData)
```

`isoSelect` *Method to select specific miRNAs from an IsomirDataSeq object.*

Description

This method allows to select a miRNA and all its isomiRs from the count matrix.

Usage

```
isoSelect.IsomirDataSeq(object, mirna, minc = 10)

## S4 method for signature 'IsomirDataSeq'
isoSelect(object, mirna, minc = 10)
```

Arguments

`object`	A IsomirDataSeq object.
`mirna`	String referring to the miRNA to show.
`minc`	Minimum number of isomiR reads needed to be included in the table.

Value

S4Vectors::DataFrame with count information. The `row.names` show the isomiR names, and each of the columns shows the counts for this isomiR in that sample. Mainly, it will return the count matrix only for isomiRs belonging to the miRNA family given by the `mirna` parameter. IsomiRs need to have counts bigger than `minc` parameter at least in one sample to be included in the output. Annotation of isomiRs follows these rules:

- miRNA name
- mismatches
- additions
- 5 trimming events
- 3 trimming events

Author(s)

Lorena Pantano

Examples

```
data(mirData)
# To select isomiRs from let-7a-5p miRNA
# and with 10000 reads or more.
isoSelect(mirData, mirna="hsa-let-7a-5p", minc=10000)
```

isoTop *Heatmap of the top expressed isomiRs*

Description

This function creates a heatmap with the top N isomiRs/miRNAs. It uses the matrix under `counts(ids)` to get the top expressed isomiRs/miRNAs using the average expression value and plot a heatmap with the raw counts for each sample.

Usage

```
isoTop(ids, top = 20, condition = NULL)
```

Arguments

`ids`	Object of class IsomirDataSeq.
`top`	Number of isomiRs/miRNAs used.
`condition`	Give condition to color PCA samples

Value

PCA of the top expressed miRNAs

Examples

```
data(mirData)
isoTop(mirData)
```

ma_ex *Data frame containing gene-mirna relationship*

Description

Data frame containing gene-mirna relationship

Usage

```
ma_ex
```

Format

A data frame with rows sames as gene_ex_rse and columns same as mirna_ex_rse.

mirData *Example of IsomirDataSeq with human brain miRNA counts data*

Description

This data set is the object return by IsomirDataSeqFromFiles. It contains miRNA count data from 14 samples: 7 control individuals (pc) and 7 patients with Parkinson's disease in early stage (Pantano et al, 2016). Use colData to see the experiment design.

Usage

```
data("mirData")
```

Format

a IsomirDataSeq class.

Author(s)

Lorena Pantano, 2018-04-27

Source

Data is available from GEO dataset under accession number GSE97285

Every sample was analyzed with seqbuster tool, see http://seqcluster.readthedocs.org/mirna_annotation.html for more details. You can get same files running the small RNA-seq pipeline from https://github.com/bcbio/bcbio-nextgen.

bcbio_nextgen was used for the full analysis.

See raw-data.R to know how to recreate the object. This script is inside "extra" folder of the package.

References

Pantano L, Friedlander MR, Escaramis G, Lizano E et al. Specific small-RNA signatures in the amygdala at premotor and motor stages of Parkinson's disease revealed by deep sequencing analysis. Bioinformatics 2016 Mar 1;32(5):673-81. PMID: 26530722

mirna_ex_rse *Data frame containing mirna expression data*

Description

Data frame containing mirna expression data

Usage

```
mirna_ex_rse
```

Format

mirna expression data with 18 samples: example of a time series data

mirTritation *Data frame containing mirna from Argyropoulos's paper*

Description

Argyropoulos, Christos, et al. "Modeling bias and variation in the stochastic processes of small RNA sequencing." Nucleic Acids Research (2017).

Usage

```
mirTritation
```

Format

mirna expression data in long format. Train and test data to use with isoCorrect

updateIsomirDataSeq *Update IsomirDataSeq object from version < 1.7*

Description

In version 1.9 IsomirDataSeq object changed their internal structure to save space and speed up loading and downstream functions.

Usage

```
updateIsomirDataSeq(object)
```

Arguments

`object`	IsomirDataSeq.

Details

This function will update to the current structure.

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