Package ‘isomiRs’

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BiocStyle
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GenomicRanges, SummarizedExperiment (>= 1.1.6)
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dplyr, RColorBrewer, gplots, methods, ggplot2, gtools,
gridExtra, grid, GGally
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NeedsCompilation no

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Accessors for the count matrix of an 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**

```r
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**: an IsomirDataSeq object
- **norm**: TRUE return log2-normalized counts
- **value**: an integer matrix

**Value**

- `matrix` with raw or normalized count data.

**Author(s)**

Lorena Pantano

**Examples**

```r
data(mirData)
head(counts(mirData))
```
**find_targets**

*Find miRNAs target using mRNA/miRNA expression*

**Description**

This function creates a matrix with rows (genes) and columns (mirnas) with values indicating if miRNA-gene pair is target according putative targets and negative correlation of the expression of both molecules.

**Usage**

```r
defind_targets(mirna_rse, gene_rse, target, summarize = "group", min_cor = -0.6)
```

**Arguments**

- **mirna_rse**: `SummarizedExperiment` with miRNA information. See details.
- **gene_rse**: `SummarizedExperiment` with gene information. See details.
- **target**: matrix with miRNAs (columns) and genes (rows) target prediction values (1 if it is a target, 0 if not).
- **summarize**: character column name in colData(rse) to use to group samples and compare between miRNA/gene expression.
- **min_cor**: numeric cutoff for correlation value that will be use to consider a miRNA-gene pair as valid.

**Examples**

```r
pairs <- as.matrix(data.frame(row.names=c("gene1", "gene2"),
mirna1=c(0,1), mirna2=c(1,0)))
mirna_matrix <- as.matrix(data.frame(row.names=c("mirna1", "mirna2"),
time0_1=c(1,1),time0_2=c(1,2,0.9),
time1_1=c(8,8),time1_2=c(8,2,7.9)))
gene_matrix <- as.matrix(data.frame(row.names=c("gene1", "gene2"),
time0_1=c(8,8),time0_2=c(8.2,7.9),
time1_1=c(1,1),time1_2=c(1,2,0.9)))
mirna_col <- data.frame(row.names=c("time0_1","time0_2","time1_1","time1_2"),
group=c("t0","t0","t1","t1"))
gene_col <- data.frame(row.names=c("time0_1","time0_2","time1_1","time1_2"),
group=c("t0","t0","t1","t1"))
mirna <- SummarizedExperiment(assays=SimpleList(norm=as.matrix(mirna_matrix)),
colData= mirna_col)
gene <- SummarizedExperiment(assays=SimpleList(norm=as.matrix(gene_matrix)),
colData= gene_col)
find_targets(mirna, gene, pairs)
```
**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**

```r
isoCounts(ids, ref = FALSE, iso5 = FALSE, iso3 = FALSE, add = FALSE, 
subs = FALSE, seed = FALSE, minc = 1, mins = 1)
```

**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
- `subs` differentiate nt substitution miRNA from rest
- `seed` differentiate changes in 2-7 nts from rest
- `minc` int minimum number of isomiR sequences to be included.
- `mins` int minimum number of samples with number of sequences bigger than `minc` counts.

**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](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**

```r
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))
```
Description

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

Usage

isDE(ids, formula, ...)

Arguments

ids object of class IsomirDataSeq
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-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 DESeqDataSet object.

Value

DESeqDataSet object. To get the differential expression isomiRs, use 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 object 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 and `isoPLSDA` for clustering. `isoPlot` helps with basic expression plot.

Metadata contains two lists: `rawList` is a list with the same length as the number of samples and stores the input files for each sample; `isoList` is a list with the same length as the number of samples and stores information for each isomiR type summarizing the different changes for the different isomiRs (trimming at 3', trimming a 5', addition and substitution). For instance, you can get the data stored in `isoList` for sample 1 and 5' changes with this code: `metadata(ids)['isoList'][[1]]$t5sum`.

Examples

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

head(counts(ids))
```

IsomirDataSeqFromFiles

`IsomirDataSeqFromFiles` loads miRNA annotation from seqbuster tool.

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, design, header = FALSE, skip = 1,
quiet = TRUE, ...)
```

Arguments

- `files`: files with the output of seqbuster tool
- `design`: data frame containing groups for each sample
- `header`: boolean to indicate files contain headers
- `skip`: boolean indicating to skip first line when reading files
- `quiet`: boolean indicating to print messages while reading files. Default `FALSE`.
- `...`: arguments provided to `SummarizedExperiment` 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

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

isoNetwork

Clustering miRNAs-genes pairs in similar pattern expression

Description

Clustering miRNAs-genes pairs in similar pattern expression

Usage

```r
isoNetwork(mirna_rse, gene_rse, target, summarize = "group", org,
genename = "ENSEMBL", min_cor = -0.6)
```

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>mirna_rse</td>
<td>SummarizedExperiment with miRNA information. See details.</td>
</tr>
<tr>
<td>gene_rse</td>
<td>SummarizedExperiment with gene information. See details.</td>
</tr>
<tr>
<td>target</td>
<td>matrix with miRNAs (columns) and genes (rows) target prediction (1 if it is a target, 0 if not).</td>
</tr>
<tr>
<td>summarize</td>
<td>character column name in colData(rse) to use to group samples and compare between miRNA/gene expression.</td>
</tr>
<tr>
<td>org</td>
<td>AnnotationDb. (org.Mm.eg.db)</td>
</tr>
<tr>
<td>genename</td>
<td>character keytype of the gene names in gene_rse object.</td>
</tr>
<tr>
<td>min_cor</td>
<td>numeric cutoff to consider a miRNA to regulate a target</td>
</tr>
</tbody>
</table>
Details

This function will correlate miRNA and gene expression data using a specific metadata variable to group samples and detect pattern of expression that will be annotated with GO terms. `mirna_rse` and `gene_rse` can be created using the following code:

```r
mi_rse = SummarizedExperiment(assays=SimpleList(norm=mirna_matrix), colData, metadata=list(sign=mirna_keep))
```

where, `mirna_matrix` is the normalized counts expression, `colData` is the metadata information and `mirna_keep` the list of miRNAs to be used by this function.

Examples

```r
library(org.Mm.eg.db)
library(clusterProfiler)
data(isoExample)
ego <- enrichGO(row.names(assay(gene_ex_rse, "norm")), org.Mm.eg.db, ont = "BP", keytype="ENSEMBL")
data = isoNetwork(mirna_ex_rse, gene_ex_rse, ma_ex, org=ego@result)
isoPlotNet(data)
```

---

### isoNorm

**Normalize count matrix**

**Description**

This function normalizes raw count matrix using `rlog` function from `DESeq2-package`.

**Usage**

```r
isoNorm(ids, formula = ~condition)
```

**Arguments**

- `ids` object of class `IsomirDataSeq`
- `formula` formula that will be used for normalization

**Value**

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

**Examples**

```r
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 plots different isomiRs proportion for each sample. It can show trimming events at both side, additions and nucleotides changes.

Usage

isoPlot(ids, type = "iso5", column = "condition")

Arguments

ids

object of class IsomirDataSeq

type

string (iso5, iso3, add, subs) to indicate what isomiRs to use for the plot. See details for explanation.

column

string indicating the column in colData to color samples.

Details

There are four different values for type parameter. To plot trimming at 5’ or 3’ end, use type="iso5" or type="iso3". In this case, it will plot 3 positions at both side of the reference position described at miRBase site. Each position refers to the number 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 number of total counts. The position at y is the number of different sequences.

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.

Value

 ggplot object showing different isomiRs changes at different positions.

Examples

data(mirData)
isoPlot(mirData)
isoPlotNet  

Functional miRNA / gene expression profile plot

Description
Functional miRNA / gene expression profile plot

Usage
isoPlotNet(obj)

Arguments
obj  
output from isoNetwork

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 = 1, column = "condition")

Arguments
ids  
oobject 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 number of different sequences supporting the change.

Value

ggplot object showing nucleotide changes at a given position.

Examples

data(mirData)
isoPlotPosition(mirData)
isoPLSDA

Partial Least Squares Discriminant Analysis for IsomirDataSeq

Description

Use PLS-DA method with the normalized count data to detect the most important features (miRNAs/isomiRs) that explain better the group of samples given by the experimental design. It is a supervised clustering method with permutations to calculate the significance of the analysis.

Usage

isoPLSDA(ids, group, validation = NULL, learn = NULL, test = NULL, tol = 0.001, nperm = 400, refinement = FALSE, vip = 1.2)

Arguments

- **ids**: object of class `IsomirDataSeq`
- **group**: column name in `colData(ids)` to use as variable to explain.
- **validation**: type of validation, either NULL or "learntest". Default NULL
- **learn**: optional vector of indexes for a learn-set. Only used when validation="learntest". Default NULL
- **test**: optional vector of indices for a test-set. Only used when validation="learntest". Default NULL
- **tol**: tolerance value based on maximum change of cumulative R-squared coefficient for each additional PLS component. Default tol=0.001
- **nperm**: number of permutations to compute the PLD-DA p-value based on R2 magnitude. Default nperm=400
- **refinement**: logical indicating whether a refined model, based on filtering out variables with low VIP values
- **vip**: Variance Importance in Projection threshold value when a refinement process is considered. Default vip=1.2

Details

Partial Least Squares Discriminant Analysis (PLS-DA) is a technique specifically appropriate for analysis of high dimensionality data sets and multicollinearity (Perez-Enciso, 2013). PLS-DA is a supervised method (i.e. makes use of class labels) with the aim to provide a dimension reduction strategy in a situation where we want to relate a binary response variable (in our case young or old status) to a set of predictor variables. Dimensionality reduction procedure is based on orthogonal transformations of the original variables (miRNAs/isomiRs) into a set of linearly uncorrelated latent variables (usually termed as components) such that maximizes the separation between the different classes in the first few components (Xia, 2011). We used sum of squares captured by the model (R2) as a goodness of fit measure.

We implemented this method using the `DiscriMiner-package` into `isoPLSDA` function. The output p-value of this function will tell about the statistical significant of the group separation using miRNA/isomiR expression data.

Read more about the parameters related to the PLS-DA directly from `plsDA` function.
**Value**

A list with the following elements: \( R_2 \text{Matrix} \) (R-squared coefficients of the PLS model), \( \text{components} \) (of the PLS, similar to PCs in a PCA), \( \text{vip} \) (most important isomiRs/miRNAs), \( \text{group} \) (classification of the samples), \( \text{p.value} \) and \( \text{R2PermutationVector} \) obtained by the permutations.

If the option \textit{refinement} is set to \texttt{TRUE}, then the following elements will appear: \( R_2 \text{RefinedMatrix} \) and \( \text{componentsRefinedModel} \) (R-squared coefficients of the PLS model only using the most important miRNAs/isomiRs). As well, \( \text{p.valRefined} \) and \( \text{R2RefinedPermutationVector} \) with p-value and R2 of the permutations where samples were randomized. And finally, \( \text{p.valRefinedFixed} \) and \( \text{R2RefinedFixedPermutationVector} \) with p-value and R2 of the permutations where miRNAs/isomiRs were randomized.

**References**


**Examples**

data(mirData)

# Only miRNAs with > 10 reads in all samples.
ids <- isoCounts(mirData, minc=10, mins=6)
ids <- isoNorm(ids)
pls.ids = isoPLSDA(ids, "condition", nperm = 2)
cat(paste0("pval:",pls.ids$p.val))
cat(paste0("components:",pls.ids$components))

**isoPLSDAplot**

\textit{Plot components from isoPLSDA analysis (pairs plot)}

**Description**

Plot the most significant components that come from \textit{isoPLSDA} analysis together with the density of the samples scores along those components.

**Usage**

isoPLSDAplot(pls)

**Arguments**

\begin{itemize}
\item \texttt{pls} \hspace{1cm} output from \texttt{isoPLSDA} function.
\end{itemize}

**Details**

The function \textit{isoPLSDAplot} helps to visualize the results from \textit{isoPLSDA}. It will plot the samples using the significant components (t1, t2, t3 ...) from the PLS-DA analysis and the samples score distribution along the components. It uses \texttt{ggpairs} for the plot.
Value

`ggpairs` plot showing the scores for each sample using isomiRs/miRNAs expression to explain variation.

`data.frame` object with a first column referring to the sample group, and the following columns referring to the score that each sample has for each component from the PLS-DA analysis.

Examples

```r
data(mirData)
# Only miRNAs with > 10 reads in all samples.
ids <- isoCounts(mirData, minc=10, mins=6)
ids <- isoNorm(ids)
pls.ids <- isoPLSDA(ids, "condition", nperm = 2)
isoPLSDAplot(pls.ids)
```

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

```r
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`: int minimum number of isomiR reads needed to be included in the table.

Value

DataFrame-class 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 to be included in the output.

Author(s)

Lorena Pantano

Examples

```r
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)`

Arguments

- `ids`: object of class `IsomirDataSeq`
- `top`: number of isomiRs/miRNAs used

Examples

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

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 6 samples: 3 newborns and 3 elderly human individuals (Somel et al, 2010). Use `colData` to see the experiment design.

Usage

`data("mirData")`

Format

- a `IsomirDataSeq` class.

Author(s)

Lorena Pantano, 2015-05-19
Source

Data is available from GEO dataset under accession number GSE18069. Samples used are: GSM450597, GSM450598, GSM450600, GSM450604, GSM450610 and GSM450609.

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/chapmanb/bcbio-nextgen.

Adapter removal with the following parameters: adrec fastq_file TCGTATGCCGTCTT 8 1 0.34

miRNAs annotation with the following parameters: miraligner adrec_output mirbase_files hsa 1 3 3 out_prefix

The data was created with isomiRs-package package:

library(isomiRs)
fns <- c("GSM450597.mirna", "GSM450598.mirna", "GSM450600.mirna","GSM450604.mirna", "GSM450610.mirna")
design <- data.frame(condition=c('nb', 'nb', 'nb','o', 'o', 'o'))
mirData <- IsomirDataSeqFromFiles(fns, design)

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