

# Package ‘Rnits’

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**Type** Package

**Title** R Normalization and Inference of Time Series data

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**Depends** R (>= 3.6.0), Biobase, ggplot2, limma, methods

**Imports** affy, boot, impute, splines, graphics, qvalue, reshape2

**Suggests** BiocStyle, knitr, GEOquery, stringr

**Description** R/Bioconductor package for normalization, curve registration and inference in time course gene expression data.

**biocViews** GeneExpression, Microarray, TimeCourse, DifferentialExpression, Normalization

**Lazyload** yes

**LazyData** yes

**License** GPL-3

**VignetteBuilder** knitr

**NeedsCompilation** no

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## Contents

|                        |   |
|------------------------|---|
| build.Rnits . . . . .  | 2 |
| calculateGCV . . . . . | 3 |
| fit . . . . .          | 4 |
| getCID . . . . .       | 5 |
| getFitModel . . . . .  | 6 |
| getLR . . . . .        | 7 |

|                                |    |
|--------------------------------|----|
| getNormTwoChannel . . . . .    | 8  |
| getPval . . . . .              | 8  |
| getStat . . . . .              | 9  |
| plotResults . . . . .          | 10 |
| rnits . . . . .                | 11 |
| Rnits-class . . . . .          | 11 |
| summarizeProbes . . . . .      | 11 |
| summary,Rnits-method . . . . . | 12 |
| timeAlign . . . . .            | 13 |
| topData . . . . .              | 13 |
| yeastchemostat . . . . .       | 14 |
| \$ . . . . .                   | 15 |

|              |           |
|--------------|-----------|
| <b>Index</b> | <b>16</b> |
|--------------|-----------|

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|             |                                                                                                |
|-------------|------------------------------------------------------------------------------------------------|
| build.Rnits | <i>Input the RGList raw data, build a Rnits object and perform filtering and normalization</i> |
|-------------|------------------------------------------------------------------------------------------------|

---

## Description

This function takes high-dimensional expression data as a RGList, creates a [Rnits](#) object for subsequent filtering and normalization

## Usage

```
build.Rnits(obj, probedata = NULL, phenodata = NULL, filter = NULL,
  normalize = NULL, normmethod = NULL, plot = FALSE, center = FALSE,
  background = NULL, threshold = 0.8, logscale = FALSE)
```

## Arguments

|           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| obj       | Raw expression data in RGList, AffyBatch or simple data frame format                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| probedata | A data frame containing the probe names that should match the probe names in raw data (optional)                                                                                                                                                                                                                                                                                                                                                                                                                      |
| phenodata | A data frame with information about sample names. The rownames of the data frame must match column names of the expression values. If input data is data frame of log ratios, this is required.                                                                                                                                                                                                                                                                                                                       |
| filter    | An argument to perform background filtering of probes. If NULL, no filtering is done. If an integer (0-500), probes are flagged based on raw channel intensity. If a vector of two numbers is provided, the first will be used for red channel and the second for green channel. If 'background', probes whose intensities are lower than 2 standard deviations less than the mean of the background intensity for the channel are flagged.                                                                           |
| normalize | Character string specifying the normalization method for raw data. If Intensity, the reference channels for all arrays are used to construct an array-specific smoothing function which is then applied to normalize the sample channel. If Between, the normalization method normalizeBetweenArrays in the LIMMA package is used (use normmethod to further specify normalization methods. See packaged LIMMA for details.). If Within, the normalization method normalizeWithinArrays in the LIMMA package is used. |

|            |                                                                                               |
|------------|-----------------------------------------------------------------------------------------------|
| normmethod | Normalization method for input data. Default NULL. Can be one of 'quantile', 'vsn', 'Between' |
| background | Only for AffyBatch data. If TRUE, background filtering will be done on Affy data.             |
| center     | If TRUE, the log-ratio data will be mean centered to 0 in the column space.                   |
| plot       | If TRUE, boxplots of normalized channel intensities and log-ratios are drawn.                 |
| threshold  | Default 0.8. Fraction of samples with missing data for individual probes to be filtered out.  |
| logscale   | Default FALSE. Is the data in logscale? If FALSE, log2 transformation is done on the data.    |

### Details

See the Limma User's Guide for more details on `read.maimages`, `normalizeBetweenArrays`, `normalizeWithinArrays` and `RGList`. For importing microarray raw data, use the 'Targets file' to specify experimental design. The target file has columns SlideNumber, FileName, Cy3 (description of Cy3 channel ref/control/treatment), Cy5 (description of Cy5 channel ref/control/treatment) and Time. Time values should be identical for control and treatment.

### Value

An object of S4 class `Rnits` (which is derived from class `exprSet`), containing the probe data, design data, expression data, phenotypical data (i.e. Time).

### See Also

`ExpressionSet`

### Examples

```
# load pre-compiled expressionSet object for Ronen and Botstein yeast chemostat data
data(yeastchemostat)
rnitsobj = build.Rnits(yeastchemostat, logscale = TRUE, normmethod = 'Between')
```

---

|              |                                                                                |
|--------------|--------------------------------------------------------------------------------|
| calculateGCV | <i>Calculate the optimal B-spline model using generalized cross-validation</i> |
|--------------|--------------------------------------------------------------------------------|

---

### Description

Calculate the optimal B-spline model using generalized cross-validation

### Usage

```
calculateGCV(object, topcomp = 5)

## S4 method for signature 'Rnits'
calculateGCV(object, topcomp = 5)
```

**Arguments**

object            [Rnits](#) object

topcomp          The number of top eigenvectors to be used for computation

**Details**

The optimal B-spline model is chosen as the largest model that minimizes the cross validation error of the top N eigenvectors of each time series data.

**Value**

A list object with fields 'degree', 'df' for each time series data set.

**Examples**

```
# load pre-compiled expressionSet object for Ronen and Botstein yeast chemostat data
data(yeastchemostat)
rnitsobj = build.Rnits(yeastchemostat, logscale = TRUE, normmethod = 'Between')
opt_model <- calculateGCV(rnitsobj)
## Not run:
rnitsobj <- fit(rnitsobj, gene.level = TRUE, model = opt.model)

## End(Not run)
```

---

|     |                                      |
|-----|--------------------------------------|
| fit | <i>Fit model on time series data</i> |
|-----|--------------------------------------|

---

**Description**

Fit a model comparing time series data set [Rnits](#) objects

**Usage**

```
fit(object, cluster = TRUE, B = 100, verbatim = FALSE, nclus = NULL,
     modelhistplot = FALSE, seed = 123, gene.level = TRUE,
     clusterallsamples = FALSE, model = NULL)

## S4 method for signature 'Rnits'
fit(object, cluster = TRUE, B = 100, verbatim = FALSE,
     nclus = NULL, modelhistplot = FALSE, seed = 123, gene.level = TRUE,
     clusterallsamples = FALSE, model = NULL)
```

**Arguments**

object            [Rnits](#) object

cluster          if TRUE, perform clustering to identify groups of genes/probes with similar expression profiles.

B                Default 100. Number of bootstrap iterations for p-value calculation

verbatim        If FALSE, print out details of fitting models.

nclus            Default NULL. Number of clusters to use for k-means clustering.

|                   |                                                                                                                                           |
|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------|
| modelhistplot     | If TRUE, p-value histograms of multiple models are plotted.                                                                               |
| seed              | Random seed for bootstrap iterations                                                                                                      |
| gene.level        | If TRUE, collapse probes to gene level information.                                                                                       |
| clusterallsamples | If TRUE, Use all time series for clustering. By default, only the sample labeled 'control' is used or the lexically first sample is used. |
| model             | A data frame with fields 'degree' and 'df' indicating a specific B-spline model to be used. If provided, model selection is not run.      |

## Details

The function compares multiple time-series expression data sets by i) (optional) summarizing probes into gene-level information ii) (optional) identifying a set of co-expressed genes by clustering iii) For each cluster (or for all genes /probes), fit a series of B-splines with varying curvature and degrees of freedom. Under the null hypothesis  $H_0$ , a single model is fit for all data sets, while under  $H_1$ , each data set is fit separately. P-values from the hypothesis test are then plotted and the least complex spline parameters that result in uniformly distributed null p-values are automatically chosen.

## Value

An object of S4 class `Rnits` with fitted results data containing cluster information, ratio statistics and p-values.

## Examples

```
# load pre-compiled expressionSet object for Ronen and Botstein yeast chemostat data
data(yeastchemostat)
rnitsobj = build.Rnits(yeastchemostat, logscale = TRUE, normmethod = 'Between')
## Not run:
# Fit model using gene-level summarization
rnitsobj <- fit(rnitsobj, gene.level = TRUE, clusterallsamples = FALSE)

## End(Not run)
```

---

getCID

---

Cluster IDs of probes/genes from fitted `Rnits`


---

## Description

Retrieve cluster IDs of probes/genes from fitted `Rnits` object after `fit` has been run.

## Usage

```
getCID(object)

## S4 method for signature 'Rnits'
getCID(object)
```

## Arguments

object                    `Rnits`

**Details**

If cluster = False during fitting, a vector of 1s will be returned.

**Value**

A vector of cluster IDs corresponding to gene/probe names

**Examples**

```
# load pre-compiled expressionSet object for Ronen and Botstein yeast chemostat data
data(yeastchemostat)
rnitsobj = build.Rnits(yeastchemostat, logscale = TRUE, normmethod = 'Between')
## Not run:
# Fit model using gene-level summarization
rnitsobj <- fit(rnitsobj, gene.level = TRUE, clusterallsamples = FALSE)

# Get cluster IDs from fitted model
cid <- getCID(rnitsobj)

## End(Not run)
```

---

getFitModel

---

*Extract fit data from [Rnits](#) object*


---

**Description**

Retrieve model fit data from [Rnits](#) object after fit has been run.

**Usage**

```
getFitModel(object)

## S4 method for signature 'Rnits'
getFitModel(object)
```

**Arguments**

object            [Rnits](#)

**Details**

Contains Ratio statistic, p-value and cluster ID data

**Value**

A data frame containing the model fit results for all genes

## Examples

```
# load pre-compiled expressionSet object for Ronen and Botstein yeast chemostat data
data(yeastchemostat)
rnitsobj = build.Rnits(yeastchemostat, logscale = TRUE, normmethod = 'Between')
## Not run:
# Fit model using gene-level summarization
rnitsobj <- fit(rnitsobj, gene.level = TRUE, clusterallsamples = FALSE)

# P-values, ratio statistics and cluster ID's can be retrieved for all genes together
fitdata <- getFitModel(rnitsobj)

## End(Not run)
```

---

getLR

*Get log-ratios*

---

## Description

Extract normalized log-ratios from [Rnits](#) object

## Usage

```
getLR(object, impute = FALSE)

## S4 method for signature 'Rnits'
getLR(object, impute = FALSE)
```

## Arguments

|        |                                                         |
|--------|---------------------------------------------------------|
| object | <a href="#">Rnits</a> object                            |
| impute | If TRUE, perform K-NN imputation to fill missing values |

## Value

A matrix of normalized log-ratios.

## Examples

```
# load pre-compiled expressionSet object for Ronen and Botstein yeast chemostat data
data(yeastchemostat)
rnitsobj = build.Rnits(yeastchemostat, logscale = TRUE, normmethod = 'Between')
## Not run:
# Fit model using gene-level summarization
rnitsobj <- fit(rnitsobj, gene.level = TRUE, clusterallsamples = FALSE)

# Get logratios
lr <- getLR(rnitsobj)

## End(Not run)
```

---

|                   |                                                           |
|-------------------|-----------------------------------------------------------|
| getNormTwoChannel | <i>Get Normalized channel data for two channel arrays</i> |
|-------------------|-----------------------------------------------------------|

---

**Description**

For two color data, extract normalized channel data from [Rnits](#) object

**Usage**

```
getNormTwoChannel(object)

## S4 method for signature 'Rnits'
getNormTwoChannel(object)
```

**Arguments**

object            [Rnits](#) object

**Value**

A list containing R and G fields for normalized Red and Green channel data respectively.

---

|         |                     |
|---------|---------------------|
| getPval | <i>Get p-values</i> |
|---------|---------------------|

---

**Description**

Extract p-values from fitted [Rnits](#) object

**Usage**

```
getPval(object)

## S4 method for signature 'Rnits'
getPval(object)
```

**Arguments**

object            [Rnits](#) object

**Value**

An vector of p-values

### Examples

```
# load pre-compiled expressionSet object for Ronen and Botstein yeast chemostat data
data(yeastchemostat)
rnitsobj = build.Rnits(yeastchemostat, logscale = TRUE, normmethod = 'Between')
## Not run:
# Fit model using gene-level summarization
rnitsobj <- fit(rnitsobj, gene.level = TRUE, clusterallsamples = FALSE)

#Get pvalues from fitted model
pval <- getPval(rnitsobj)

## End(Not run)
```

---

getStat

*Retrieve ratio statistics*

---

### Description

Extract ratio statistics from fitted [Rnits](#) object

### Usage

```
getStat(object)

## S4 method for signature 'Rnits'
getStat(object)
```

### Arguments

object            [Rnits](#) object

### Value

An vector of ratio statistics

### Examples

```
# load pre-compiled expressionSet object for Ronen and Botstein yeast chemostat data
data(yeastchemostat)
rnitsobj = build.Rnits(yeastchemostat, logscale = TRUE, normmethod = 'Between')
## Not run:
# Fit model using gene-level summarization
rnitsobj <- fit(rnitsobj, gene.level = TRUE, clusterallsamples = FALSE)

# Get ratio statistics from fitted model
stat <- getStat(rnitsobj)

## End(Not run)
```

---

plotResults

*Plot profiles of top genes/probes*


---

## Description

After fit has been applied on [Rnits](#) object, plot the profiles of N top ranking genes/probes.

## Usage

```
plotResults(object, id = NULL, fdr = NULL, top = 48, pdf = FALSE,
  sort.by = "p-value", filename = "TopPlots.pdf", scale_y = NULL)

## S4 method for signature 'Rnits'
plotResults(object, id = NULL, fdr = NULL, top = 48,
  pdf = FALSE, sort.by = "p-value", filename = "TopPlots.pdf",
  scale_y = NULL)
```

## Arguments

|          |                                                                                  |
|----------|----------------------------------------------------------------------------------|
| object   | <a href="#">Rnits</a> object.                                                    |
| id       | Names of specific genes or probes to be plotted. Overrides fdr and top argument. |
| fdr      | FDR cut-off plotting top probes or genes. Overrides top argument.                |
| top      | Number of top genes or probes whose profile is to be plotted. Default 48.        |
| pdf      | Save plot as pdf? Default FALSE.                                                 |
| sort.by  | Criteria for sorting top genes or probes. Default 'p-value'.                     |
| filename | Name of pdf file. Default topplots.pdf.                                          |
| scale_y  | If 'free', use free scales for plots. Default NULL.                              |
| ...      | Optional arguments to plot                                                       |

## Examples

```
# load pre-compiled expressionSet object for Ronen and Botstein yeast chemostat data
data(yeastchemostat)
rnitsobj = build.Rnits(yeastchemostat, logscale = TRUE, normmethod = 'Between')
## Not run:
# Fit model using gene-level summarization
rnitsobj <- fit(rnitsobj, gene.level = TRUE, clusterallsamples = FALSE)

# Plot top results
plotResults(rnitsobj, top = 16)

## End(Not run)
```

---

|       |               |
|-------|---------------|
| rnits | <i>rnits.</i> |
|-------|---------------|

---

**Description**

rnits.

---

|             |                    |
|-------------|--------------------|
| Rnits-class | <i>rnits class</i> |
|-------------|--------------------|

---

**Description**

Class rnits for time series

**Details**

Some details

---

|                 |                                                      |
|-----------------|------------------------------------------------------|
| summarizeProbes | <i>Summarize probe level data to gene level data</i> |
|-----------------|------------------------------------------------------|

---

**Description**

The code utilizes the probe-gene mapping from features file to summarize probe-level log ratios to gene level ratios.

**Usage**

```
summarizeProbes(object)
```

```
## S4 method for signature 'Rnits'  
summarizeProbes(object)
```

**Arguments**

object      [Rnits](#) object

**Details**

Tukey's biweight is used to compute gene level summary

**Value**

An object of class [Rnits](#) with gene level log ratios, which can be retrieved by `getLR(object)`

**Examples**

```
# load pre-compiled expressionSet object for Ronen and Botstein yeast chemostat data
data(yeastchemostat)
rnitsobj = build.Rnits(yeastchemostat, logscale = TRUE, normmethod = 'Between')

# Summarize gene-level data
rnitsobj <- summarizeProbes(rnitsobj)
```

---

summary,Rnits-method    *Summary of fit*

---

**Description**

Summarize top genes or probes from Rnits fit method

**Usage**

```
## S4 method for signature 'Rnits'
summary(object, top = 48, fdr = NULL, plot = FALSE,
        sort.by = "p-value")
```

**Arguments**

|         |                                                                                                 |
|---------|-------------------------------------------------------------------------------------------------|
| object  | <a href="#">Rnits</a> object on which fit has been applied                                      |
| top     | Display results for top N genes/probes. Default 50                                              |
| fdr     | Display results for genes/probes less than FDR (%) cutoff (if provided). Overrides top argument |
| plot    | If TRUE, plot histogram of p-values                                                             |
| sort.by | Sort top results by either p-value or FDR                                                       |

**Value**

A table of top genes/profiles listing the ratio statistics, p-values, q-values and cluster information.

**Examples**

```
# load pre-compiled expressionSet object for Ronen and Botstein yeast chemostat data
data(yeastchemostat)
rnitsobj = build.Rnits(yeastchemostat, logscale = TRUE, normmethod = 'Between')
## Not run:
# Fit model using gene-level summarization
rnitsobj <- fit(rnitsobj, gene.level = TRUE, clusterallsamples = FALSE)

# Get summary of top genes
summary(rnitsobj, FDR = 5)

## End(Not run)
```

---

|           |                                                 |
|-----------|-------------------------------------------------|
| timeAlign | <i>Curve registration of time series curves</i> |
|-----------|-------------------------------------------------|

---

**Description**

Align multiple time series to the average series

**Usage**

```
timeAlign(object, iterMax = 5, seed = 123, null.frac = 0.75,
  anchor = NULL, rerun = FALSE, plot = FALSE)

## S4 method for signature 'Rnits'
timeAlign(object, iterMax = 5, seed = 123,
  null.frac = 0.75, anchor = NULL, rerun = FALSE, plot = FALSE)
```

**Arguments**

|           |                                                                                                |
|-----------|------------------------------------------------------------------------------------------------|
| object    | rnits object                                                                                   |
| iterMax   | Maximum iterations to be performed                                                             |
| seed      | Random seed                                                                                    |
| null.frac | Fraction of genes that are considered as null                                                  |
| anchor    | Sample to be considered as base for aligning time series. If not provided, the average is used |
| rerun     | If TRUE, re-align previously aligned data                                                      |
| plot      | If TRUE, plot results                                                                          |

**Examples**

```
# load pre-compiled expressionSet object for Ronen and Botstein yeast chemostat data
data(yeastchemostat)
rnitsobj = build.Rnits(yeastchemostat, logscale = TRUE, normmethod = 'Between')

# Do curve-registration on data
rnitsobj <- timeAlign(rnitsobj)
```

---

|         |                                 |
|---------|---------------------------------|
| topData | <i>Data of top genes/probes</i> |
|---------|---------------------------------|

---

**Description**

Extract expression data for top genes/probes

**Usage**

```
topData(object, id = NULL, fdr = NULL, top = 16, sort.by = "p-value")

## S4 method for signature 'Rnits'
topData(object, id = NULL, fdr = NULL, top = 16,
  sort.by = "p-value")
```

**Arguments**

|         |                                                                                             |
|---------|---------------------------------------------------------------------------------------------|
| object  | <b>Rnits</b> object on which fit has been applied                                           |
| id      | Names of probes or genes                                                                    |
| top     | Display results for top N genes/probes. Default 50                                          |
| fdr     | Display results for genes/probes less than FDR cutoff (if provided). Overrides top argument |
| sort.by | Sort top results by either p-value or FDR                                                   |

**Value**

A table of expression values of top genes/profiles

**Examples**

```
# load pre-compiled expressionSet object for Ronen and Botstein yeast chemostat data
data(yeastchemostat)
rnitsobj = build.Rnits(yeastchemostat, logscale = TRUE, normmethod = 'Between')
## Not run:
# Fit model using gene-level summarization
rnitsobj <- fit(rnitsobj, gene.level = TRUE, clusterallsamples = FALSE)

#Get data for top genes
td <- topData(rnitsobj, FDR = 5)

## End(Not run)
```

---

|                |                                                                                                                              |
|----------------|------------------------------------------------------------------------------------------------------------------------------|
| yeastchemostat | <i>Yeast chemostat data from Ronen and Botstein (Proc Natl Acad Sci U S A. 2006 Jan 10;103(2):389-94. Epub 2005 Dec 28.)</i> |
|----------------|------------------------------------------------------------------------------------------------------------------------------|

---

**Description**

(From author's GEO submission) Transcriptional response of steady-state yeast cultures to transient perturbations in carbon source

**Usage**

```
yeastchemostat
```

**Format**

An ExpressionSet object with containing 'Sample' and 'Time' columns and replicates removed.

**Source**

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE4158>

---

|    |                              |
|----|------------------------------|
| \$ | <i>replace slot of Rnits</i> |
|----|------------------------------|

---

**Description**

replace slot of Rnits

# Index

\$, [15](#)  
\$<-, Rnits-method (\$), [15](#)

build.Rnits, [2](#)

calculateGCV, [3](#)  
calculateGCV, character, ANY-method  
    (calculateGCV), [3](#)  
calculateGCV, Rnits-method  
    (calculateGCV), [3](#)

exprSet, [3](#)

fit, [4](#)  
fit, character, ANY-method (fit), [4](#)  
fit, Rnits-method (fit), [4](#)

getCID, [5](#)  
getCID, character, ANY-method (getCID), [5](#)  
getCID, Rnits-method (getCID), [5](#)  
getFitModel, [6](#)  
getFitModel, character, ANY-method  
    (getFitModel), [6](#)  
getFitModel, Rnits-method (getFitModel),  
    [6](#)

getLR, [7](#)  
getLR, character, ANY-method (getLR), [7](#)  
getLR, Rnits-method (getLR), [7](#)  
getNormTwoChannel, [8](#)  
getNormTwoChannel, character, ANY-method  
    (getNormTwoChannel), [8](#)  
getNormTwoChannel, Rnits-method  
    (getNormTwoChannel), [8](#)

getPval, [8](#)  
getPval, character, ANY-method (getPval),  
    [8](#)  
getPval, Rnits-method (getPval), [8](#)

getStat, [9](#)  
getStat, character, ANY-method (getStat),  
    [9](#)  
getStat, Rnits-method (getStat), [9](#)

plotResults, [10](#)  
plotResults, character, ANY-method  
    (plotResults), [10](#)

plotResults, Rnits-method (plotResults),  
    [10](#)

Rnits, [2–12](#), [14](#)  
rnits, [11](#)  
Rnits-class, [11](#)  
rnits-package (rnits), [11](#)  
Rnits.getLR (getLR), [7](#)

summarizeProbes, [11](#)  
summarizeProbes, character, ANY-method  
    (summarizeProbes), [11](#)  
summarizeProbes, Rnits-method  
    (summarizeProbes), [11](#)  
summary, Rnits-method, [12](#)

timeAlign, [13](#)  
timeAlign, character, ANY-method  
    (timeAlign), [13](#)  
timeAlign, Rnits-method (timeAlign), [13](#)

topData, [13](#)  
topData, character, ANY-method (topData),  
    [13](#)  
topData, Rnits-method (topData), [13](#)

yeastchemostat, [14](#)