

# Package ‘AgiMicroRna’

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**Title** Processing and Differential Expression Analysis of Agilent  
microRNA chips

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basicLimma

*Linear models Using limma*

---

## Description

Differential expression analysis using the linear model features implemented in the limma package. A linear model is fitted to each miRNA gene so that the fold change between different experimental conditions and their standard errors can be estimated. Empirical Bayes methods are applied to obtain moderated statistics

## Usage

```
basicLimma(eset, design, CM, verbose = FALSE)
```

## Arguments

eset	ExpressionSet containing the processed log-expression values
design	design matrix
CM	contrast matrix
verbose	logical, if TRUE prints out output

## Details

In our data example (see the target file in Table 1 in vignette), we have used a paired design (by subject) to assess the differential expression between two treatments B and C vs a control treatment A. That is, we want to obtain the microRNAs that are differentially expressed between conditions A vs B and A vs C. The linear model that we are going to fit to every miRNA is defined by equation:  $y = \text{Treatment} + \text{Subject} + \text{error term}$ . This model is going to estimate the treatment effect and then, the comparison between the different treatments are done in terms of contrasts between the estimates of the treatment effects. To fit the model, we need first to define a design matrix. The design matrix is an incidence matrix that relates each array/sample/file to its given experimental conditions, in our case, relates each file to one of the three treatments and with its particular subject. If treatment is a factor variable, we can define the design matrix using `model.matrix(~ -1 + treatment + subject)`. Then the linear model can be fitted using `fit=lmFit(eset,design)`. This will get the treatment estimates for each microRNA in the eset object:

```
treatmentA treatmentB treatmentC subject2 hsa-miR-152 7.5721 7.656 7.566 -0.1157 hsa-miR-15a* 0.9265 1.066 1.211 -0.2242 hsa-miR-337-5p 6.2448 7.298 7.084 -0.4489
```

We can define the contrasts of interest using a contrast matrix as in `CM=cbind(MSC\_AvsMSC\_B=c(1,-1,0), MSC\_AvsMSC\_C=c(1,0,-1))`

And then, we can estimate those contrasts using `fit2=contrasts.fit(fit,CM)`. Finally, we can obtain moderated statistics using `fit2=eBayes(fit2)`.

The function 'basicLimma' implemented in AgiMicroRna produces the last fit2 object, that has in `fit2$coeff` the M values, in `fit2$t` the moderated-t statistic of the contrasts, and in `fit2$p.value` the corresponding p value of each particular contrasts. Be aware that these p values must be corrected by multiple testing.

```
MSC\_AvsMSC\_B MSC\_AvsMSC\_C hsa-miR-152 0.67567761 0.977326746 hsa-miR-15a* 0.68019442 0.413657270 hsa-miR-337-5p 0.03737814 0.075248741
```

See `limmaUsersGuide()` for a complete description of the limma package.

## Value

An MArrayLM object of the package limma

## Author(s)

Pedro Lopez-Romero

## References

Smyth, G. K. (2005). Limma: linear models for microarray data. In: 'Bioinformatics and Computational Biology Solutions using R and Bioconductor'. R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds), Springer, New York, pages 397–420.

Smyth, G. K. (2004). Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Statistical Applications in Genetics and Molecular Biology*, Vol. 3, No. 1, Article 3. <http://www.bepress.com/sagmb/vol3/iss1/art3>

**See Also**

An 'RGList' example containing processed data is in ddPROC and an overview of how the processed data is produced is given in filterMicroRna. The ExpressionSet object can be generated using esetMicroRna

**Examples**

```
## Not run:
data(targets.micro)
data(ddPROC)
esetPROC=esetMicroRna(ddPROC,targets.micro,makePLOT=FALSE,verbose=FALSE)

levels.treatment=levels(factor(targets.micro$Treatment))
treatment=factor(as.character(targets.micro$Treatment),
  levels=levels.treatment)

levels.subject=levels(factor(targets.micro$Subject))
subject=factor(as.character(targets.micro$Subject),
  levels=levels.subject)

design=model.matrix(~ -1 + treatment + subject  )

CM=cbind(MSC_AvsMSC_B=c(1,-1,0,0),
  MSC_AvsMSC_C=c(1,0,-1,0))

fit2=basicLimma(esetPROC,design,CM,verbose=TRUE)

names(fit2)
head(fit2$coeff)
head(fit2$p.value)
plot(fit2\A$mean,fit2$coeff[,1],xlab="A",ylab="M")
abline(h=0)
abline(h=c(-1,1),col="red")
plot(fit2$coeff[,1],fit2$p.value[,1], xlab="M",ylab="p value")

## End(Not run)
```

---

 boxplotMicroRna

*Boxplot*


---

**Description**

It creates a Boxplot using the matrix columns as input

**Usage**

```
boxplotMicroRna(object, maintitle, colorfill, xlab, ylab)
```

**Arguments**

object	A matrix containing by columns the expression arrays in log2 scale
maintitle	character to indicate the title of the graph
colorfill	color to fill the boxplot
xlab	title for the x axis
ylab	title for the y axis

**Author(s)**

Pedro Lopez-Romero

**Examples**

```
data(dd.micro)
MMM=log2(dd.micro$meanS)
      boxplotMicroRna(MMM,
                      maintitle="log2 Mean Signal",
                      colorfill="green",
                      xlab="Samples",
                      ylab="expression")
```

---

 cvArray

*Coefficient of variation of replicated probes within array*


---

**Description**

Identifies replicated features at probe and at gene level and computes the coefficient of variation of the array

**Usage**

```
cvArray(ddDUP, foreground = c("MeanSignal", "ProcessedSignal"), targets, verbose=FALSE)
```

**Arguments**

ddDUP	uRNAList, containing the output from readMicroRnaAFE
foreground	Specifies the signal used, only "MeanSignal" or "ProcessedSignal" can be used
targets	data.frame with the target structure
verbose	logical, if TRUE prints out output

**Details**

In the Agilent microRNA platforms the features are replicated at a probe level and normally, a single microRNA is interrogated by either two or four sets of replicated probes. The replication of the probes allows computing the coefficient of variation (CV) for each array as a measure of the reproducibility of the array. The CV is computed for every set of replicated probes and the CV median is reported as the array CV. A lower array CV indicates a better array reproducibility.

**Value**

It prints out the results of the replication for the NON CONTROL FEATURES at a probe and gene level.

**Author(s)**

Pedro Lopez-Romero

**Examples**

```
## Not run:
data(dd.micro)
data(targets.micro)

cvArray(dd.micro, "MeanSignal", targets.micro, verbose=TRUE)

graphics.off()

## End(Not run)
```

---

dd.micro

*data example (uRNAList)*


---

**Description**

Data, extracted from scanned images using Agilent Feature Extraction Software, are stored in a uRNAList object.

**Usage**

```
data(dd.micro)
```

**Details**

A data example is provided. The data example includes 3 experimental conditions with two replicates.

For these data, chips were scanned using the Agilent G2567AA Microarray Scanner System (Agilent Technologies) Image analysis and data collection were carried out using the Agilent Feature Extraction 9.1.3.1. (AFE).

Data, collected with the Agilent Feature Extraction Software, are stored in a uRNAList object with the following components:

**uRNAList***\$*STGS matrix, 'gTotalGeneSignal'

**uRNAList***\$*TPS matrix, 'gTotalProbeSignal'

**uRNAList***\$*meanS matrix, 'gMeanSignal'

**uRNAList***\$*procS matrix, 'gProcessedSignal'

**uRNAList\$targets** data.frame, 'FileName'  
**uRNAList\$genes\$ProbeName** vector of characters, 'Agilent Probe Name'  
**uRNAList\$genes\$GeneName** vector of characters, 'microRNA Name'  
**uRNAList\$genes\$ControlType** vector of integers, '0'= Feature, '1'= Positive control, '-1'= Negative control  
**uRNAList\$other\$gIsGeneDetected** matrix, FLAG to classify signal if 'IsGeneDetected=1' or 'not=0'  
**uRNAList\$other\$gIsSaturated** matrix, FLAG to classify signal if 'IsSaturated = 1' or 'not=0'  
**uRNAList\$other\$gIsFeatPopnOL** matrix, FLAG to classify signal if 'IsFeatPopnOL = 0' or 'not=1'  
**uRNAList\$other\$gIsFeatNonUnifOL** matrix, FLAG to classify signal if 'gIsFeatNonUnifOL = 0' or 'not=1'  
**uRNAList\$other\$gBGMedianSignal** matrix, gBGMedianSignal  
**uRNAList\$other\$gBGUsed** matrix, gBGUsed

**Author(s)**

Pedro Lopez-Romero

**See Also**

readMicroRnaAFE.Rd

---

 ddPROC

---

*Processed miRNA data (uRNAList)*


---

**Description**

Filtered and Normalized miRNA data stored in a uRNAList object.

**Usage**

data(ddPROC)

**Details**

ddPROC is originated after the processing of the dd.micro raw data.

**Author(s)**

Pedro Lopez-Romero

**See Also**

An overview of how ddPROC is obtained is given in filterMicroRna

---

dim.uRNAList                    *Retrieve the Dimensions of an uRNAList Object*

---

### Description

Retrieve the number of rows (genes) and columns (arrays) for an uRNAList object.

### Usage

```
## S3 method for class 'uRNAList'  
dim(x)  
## S3 method for class 'uRNAList'  
length(x)
```

### Arguments

x                    an object of class uRNAList

### Details

This function and this file, has been borrowed from the files created by Gordon Smyth for the limma package.

### Value

Numeric vector of length 2. The first element is the number of rows (genes) and the second is the number of columns (arrays).

### Author(s)

Pedro Lopez-Romero

---

dimnames.uRNAList                    *Retrieve the Dimension Names of an uRNAList Object*

---

### Description

Retrieve the dimension names of a microarray data object.

### Usage

```
## S3 method for class 'uRNAList'  
dimnames(x)  
## S3 replacement method for class 'uRNAList'  
dimnames(x) <- value
```



**Arguments**

x                    an object of class uRNAList  
 value                a possible value for dimnames(x)

**Details**

The dimension names of a microarray object are the same as those of the most important matrix component of that object. A consequence is that rownames and colnames will work as expected. This function and this file, has been borrowed from the files created by Gordon Smyth for the limma package.

**Value**

Either NULL or a list of length 2.

**Author(s)**

Pedro Lopez-Romero

---

esetMicroRna                    *ExpressionSet object from a uRNAList*

---

**Description**

It creates an 'ExpressionSet' object from a 'uRNAList' with unique probe names. Typically, the 'uRNAList object' contains the Total Gene Processed data.

**Usage**

esetMicroRna(uRNAList, targets, makePLOT=FALSE, verbose=FALSE)

**Arguments**

uRNAList            An uRNAList containing normally the processed data  
 targets             data.frame with the targets structure  
 makePLOT           logical, if TRUE it makes a 'heatmap' with the 100 greater variance genes, a 'hierarchical cluster' with all the genes and a pca plot  
 verbose             logical, if TRUE prints out output

**Details**

It creates an ExpressionSet object from a uRNAList. Usually this function is applied to an uRNAList object containing the Total Gene Processed data.

**Value**

An ExpressionSet object

**Author(s)**

Pedro Lopez-Romero

**See Also**

An 'uRNAList' example containing processed data is in ddPROC and an overview of how the processed data is produced is given in filterMicroRna

---

filterMicroRna	<i>Filtering Genes</i>
----------------	------------------------

---

**Description**

Filter genes out according to their Quality Flag

**Usage**

```
filterMicroRna(ddNORM,
  dd,
  control,
  IsGeneDetected,
  wellaboveNEG,
  limIsGeneDetected,
  limNEG,
  makePLOT,
  targets,
  verbose,
  writeout)
```

**Arguments**

ddNORM	uRNAList with the Total Gene Signal in log2 scale to be FILTERED out according to a Quality FLAG
dd	uRNAList, containing the output from readMicroRnaAFE
control	logical, if TRUE it removes controls
IsGeneDetected	logical, if TRUE it filters genes according to gIsGeneDetected Flag. Flag = 1, then gene is detected
wellaboveNEG	logical, if TRUE it filter genes whose expression is not above a limit value defined by the expression of negative controls. Limit= Mean(negative) + 1.5 x sd(negative)
limIsGeneDetected	for a given feature xi accros samples, is the minimum in at least one experimental condition with a IsGeneDetected-FLAG = 1 (Is Detected)
limNEG	for a given feature xi accros samples, is the minimum in at least one experimental condition with intensity > Limit established for negative controls (Mean + 1.5 x SD)

makePLOT	logical, if TRUE makes QC plots with the remaining signals
targets	data.frame with the targets structure
verbose	logical, if TRUE prints out output
writeout	logical, if TRUE writes out output files

### Details

Agilent Feature Extraction software provides a flag for each spot that identifies different quantification errors of the signal. Quantification flags were used to filter out signals that did not reach a minimum established criterion of quality.

### Value

The function returns a uRNAList containing the FILTERED data. In order to allow the tracking of those microRNAs that may have been filtered out from the original raw data, the following files are given:

NOCtrl\_exprs.txt: Log2 Normalized Total Gene Signals for the Non Control Genes  
 NOCtrl\_FlagIsGeneDetected.txt: IsGeneDetected Flag for the Non Control Genes, 1 = detected  
 IsNOTGeneDetected.txt: Genes that not are not detected according to IsGeneDetected Flag

### Author(s)

Pedro Lopez-Romero

### References

Agilent Feature Extraction Reference Guide <http://www.Agilent.com>

### Examples

```
data(dd.micro, verbose=FALSE)
data(targets.micro, verbose=FALSE)
ddTGS=tgsMicroRna(dd.micro, half=TRUE, makePLOT=FALSE, verbose=FALSE)
ddNORM=tgsNormalization(ddTGS, 'quantile',
                        makePLOTpre=FALSE, makePLOTpost=TRUE, targets.micro, verbose=FALSE)
ddPROC=filterMicroRna(ddNORM,
                      dd.micro,
                      control=TRUE,
                      IsGeneDetected=TRUE,
                      wellaboveNEG=FALSE,
                      limIsGeneDetected=50,
                      limNEG=25,
                      makePLOT=FALSE,
                      targets.micro,
                      verbose=FALSE,
                      writeout=FALSE)
```

---

getDecideTests	<i>Differential expression analysis an multiplicity of the tests</i>
----------------	--

---

**Description**

It Uses the decideTests function of the 'limma' package to classify the list of genes as up, down or not significant after correcting by the multiplicity of the tests.

**Usage**

```
getDecideTests(fit2, DEmethod, MTestmethod, PVcut, verbose=FALSE)
```

**Arguments**

fit2	MArrayLM object
DEmethod	method for decideTests, only 'separate' or 'nestedF' are implemented. see decideTests in limma package.
MTestmethod	method for multiple test, choices are 'none', 'BH', 'BY', ... see p.adjust
PVcut	p value threshold to declare significant features
verbose	logical, if TRUE prints out output

**Value**

A 'TestResults' object of the 'limma' package It prints out the number of UP and DOWN genes for every contrasts according to the p value limit specified

**Author(s)**

Pedro Lopez-Romero

**References**

Smyth, G. K. (2005). Limma: linear models for microarray data. In: 'Bioinformatics and Computational Biology Solutions using R and Bioconductor'. R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds), Springer, New York, pages 397–420.

**See Also**

An overview of miRNA differential expression analysis is given in basicLimma

**Examples**

```
## Not run:
DE=getDecideTests(fit2,
  DEmethod="separate",
  MTestmethod="BH",
  PVcut=0.10,
  verbose=TRUE)

## End(Not run)
```

---

HeatMapMicroRna	<i>HeatMap</i>
-----------------	----------------

---

**Description**

Creates a HeatMap graph using the 'heatmap.2' function

**Usage**

```
HeatMapMicroRna(object, size, maintitle)
```

**Arguments**

object	A expression Matrix
size	number of highest variance genes to be considered in the plot
maintitle	title of the plot

**Author(s)**

Pedro Lopez-Romero

**See Also**

heatmap.2

**Examples**

```
data(ddPROC)
HeatMapMicroRna(ddPROC$TGS,
  size=100,
  maintitle="100 High Var genes")
```

---

hierclusMicroRna      *Hierarchical clustering*

---

**Description**

Hierarchical cluster of samples using the 'hclust' function

**Usage**

```
hierclusMicroRna(object, GRep, methdis, methclu, sel, size)
```

**Arguments**

object	An expression Matrix
GRep	Numerical vector that relates each sample with its experimental condition
methdis	the distance measure to be used. Options are 'euclidean' and 'pearson'. see 'dist' function
methclu	the agglomeration method to be used by the 'hclust' function
sel	logical, if TRUE selects the 'size' highest variance genes for the plot
size	selects the 'size' highest variance genes for the plot if 'sel=TRUE'

**Author(s)**

Pedro Lopez-Romero

**See Also**

hclust,dist

**Examples**

```
data(targets.micro)
data(ddPROC)

hierclusMicroRna(ddPROC$TGS,GRep,
methdis="euclidean",
methclu="complete",
sel=FALSE,100)
```

---

`mvaBASIC`*MVA plot*

---

**Description**

For each array, the M value is computed for every spot as the difference between the spot intensity in the array and the averaged intensity for that feature over the whole set of arrays. It does not make a distinction between the different kind of features in the array as the `mvaMicroRna()` does.

**Usage**

```
mvaBASIC(object, colorfill, maintitle)
```

**Arguments**

<code>object</code>	An expression matrix in log2 scale
<code>colorfill</code>	color of the plot
<code>maintitle</code>	title of the plot

**Author(s)**

Pedro Lopez-Romero

**Examples**

```
data(dd.micro)
op=par(mfrow=c(1,1),ask=TRUE)
mvaBASIC(log2(dd.micro$meanS),
colorfill="red",
        maintitle=" log2 Mean Signal")
par(op)
```

---

`mvaMicroRna`*MA plot*

---

**Description**

For each array, the M value is computed for every spot as the difference between the spot intensity in the array and the median intensity for that feature over the whole set of arrays. Every kind of feature is identified with different color (microRNA genes, positive controls, etc ...) The input must be an `uRNAList` object created by the user, in such a way that the `uRNAList$meanS` field contains the expression matrix that we want to use in log2 scale (see example below) The `gProcessedSignal` computed by the Agilent Feature Extaction software normally contains negative values, so a small constant has to be added to the signals before log transformation.

**Usage**

```
mvaMicroRna(uRNAList, maintitle, verbose=FALSE)
```

**Arguments**

uRNAList	A uRNAList object. It uses the expression matrix stored in the uRNAList\$meanS slot. Input expression matrix should be in log2 scale
maintitle	character to indicate the title of the graph
verbose	logical, if TRUE it prints details

**Author(s)**

Pedro Lopez-Romero

**Examples**

```
data(dd.micro)
op=par(mfrow=c(1,1),ask=TRUE)

MMM=dd.micro$procS      ## gProcessedSignal

min=min(MMM)           ## transforming gProcessedSignal to positive values
for(i in 1:dim(MMM)[2]){ ## before log2 transformation
  MMM[,i]=MMM[,i]+(abs(min)+ 5)
}
  ddaux=dd.micro
  ddaux$meanS=log2(MMM)
  mvaMicroRna(ddaux,maintitle="ProcessedSignal",verbose=FALSE)
  rm(ddaux)
  par(op)
```

---

PCApplotMicroRna	<i>PCA plot</i>
------------------	-----------------

---

**Description**

It is a wrapper for the 'plotPCA' of the 'affycoretools' package

**Usage**

```
PCApplotMicroRna(eset, targets)
```

**Arguments**

eset	An Expression Set object
targets	data.frame with the target structure



**Author(s)**

Pedro Lopez-Romero

**Examples**

```
data(targets.micro)
data(ddPROC)
esetPROC=esetMicroRna(ddPROC, targets.micro, makePLOT=FALSE, verbose=FALSE)

PCApplotMicroRna(esetPROC, targets.micro)
```

---

*plotDensityMicroRna*     *Density Plots of Intensity Signals*

---

**Description**

Creates a density plot with the arrays intensities

**Usage**

```
plotDensityMicroRna(object, maintitle)
```

**Arguments**

object	An expression matrix, in log2 scale
maintitle	title of the plot

**Author(s)**

Pedro Lopez-Romero

**Examples**

```
data(dd.micro)
plotDensity(log2(dd.micro$meanS), maintitle="log2 Mean Signal")
```

---

pvalHistogram

*Histogram of the p values*


---

### Description

Creates an histogram of the pvalues. For multiple contrasts, creates an histogram for every t.test pvalue (separate) or a single histogram for the F.test pvalue (nestedF). A uniform histogram will indicate no differential expression in the data set, whereas a right skewed histogram, will indicate some significant differential expression

### Usage

```
pvalHistogram(fit2, DE, PVcut, DEmethod, MTestmethod, CM, verbose=FALSE)
```

### Arguments

fit2	MArrayLM object
DE	TestResults object
PVcut	limit p value to declare significant features
DEmethod	method for decideTests, only 'separate' or 'nestedF' are implemented
MTestmethod	method for multiple test
CM	contrast matrix
verbose	logical, if TRUE prints out output

### Author(s)

Pedro Lopez-Romero

### See Also

An overview of miRNA differential expression analysis is given in `basicLimma`. An example of how to get the 'TestResults' object is in `getDecideTests`

### Examples

```
## Not run:
pvalHistogram(fit2, DE, PVcut=0.10,
              DEmethod="separate", MTestmethod="BH", CM)

## End(Not run)
```

---

 qcPlots

*Plots for Quality Assessment*


---

### Description

It creates BoxPlots, Density Plots, MA plots, RLE plots and hierachical clustering plots with the sample data set.

### Usage

```
qcPlots(dd,
  offset,
  MeanSignal=TRUE,
  ProcessedSignal=FALSE,
  TotalProbeSignal=FALSE,
  TotalGeneSignal=FALSE,
  BGMedianSignal=FALSE,
  BGUsed=FALSE,
  targets)
```

### Arguments

dd	A uRNAList object containing the ouput from readMicroRnaAFE
offset	numeric value to add to the intensities before log transforming
MeanSignal	logical, if TRUE "gMeanSignal" is used
ProcessedSignal	logical, if TRUE "gProcessedSignal" is used
TotalProbeSignal	logical, if TRUE "gTotalProbeSignal" is used
TotalGeneSignal	logical, if TRUE "gTotalGeneSignal" is used
BGMedianSignal	logical, if TRUE "gBGMedianSignal" is used
BGUsed	logical, if TRUE "gBGUsed" is used
targets	data.frame with the target structure

### Details

The signals loaded from the AFE data files can be used for the quality assesment using the graphical utilities included in the qcPlots function. For the gMeanSignal, the BoxPlots, Density Plots, MA plots, RLE plots and hierachical clustering plots are done. For the gProcessedSignal the same plots are done, except the hierachical clustering. For the gTotalProbeSignal and the gTotalGeneSignal only the BoxPlots and Density Plots are done, and finally, for the Background signals only the Boxplots are done.

**Author(s)**

Pedro Lopez-Romero

**References**

Boldstad B.M., Collin F., Brettschneider J., Simpson, K., Cope L., Irizarry R. A., Speed T. P. Quality Assesement of Affymetrix GeneChip Data. In Bioinformatics and Computational Biology Solutions Using R and Bioconductor. (eds.) Gentleman R., Carey V. J., Huber W., Irizarry R. A., Dudoit S. (2005). Springer.

**See Also**

boxplotMicroRna, plotDensityMicroRna, RleMicroRna, mvaMicroRna and hierclusMicroRna

**Examples**

```
## Not run:
data(dd.micro)
qcPlots(dd.micro, offset=5,
        MeanSignal=TRUE,
        ProcessedSignal=TRUE,
        TotalProbeSignal=TRUE,
        TotalGeneSignal=TRUE,
        BGMedianSignal=TRUE,
        BGUsed=TRUE,
        targets.micro)
graphics.off()

## End(Not run)
```

---

readMicroRnaAFE

*Read Agilent Feature Extraction txt data files*

---

**Description**

Read the data files generated by the Agilent Feature Extraction image analysis software

**Usage**

```
readMicroRnaAFE(targets, verbose=FALSE)
```

**Arguments**

targets	A data frame that specifies experimental conditions under which each sample has been obtained.
verbose	logical, if TRUE prints out output

## Details

The function reads the \*.txt files generated by the AFE Software using the 'read.maimages' function of 'limma' package.

Data, collected with the Agilent Feature Extraction Software, are stored in a uRNAList object with the following components:

```
- dd.micro\TGS 'gTotalGeneSignal' - dd.micro\TPS 'gTotalProbeSignal' - dd.micro\$meanS 'gMeanSignal'
- dd.micro\$procS 'gProcessedSignal' - dd.micro\$targets 'targets' - dd.micro\$genes\$ProbeName
'Probe Name' - dd.micro\$genes\$GeneName 'microRNA Name' - dd.micro\$genes\$ControlType
'FLAG to specify the sort of feature' - dd.micro\$other\$gIsGeneDetected 'FLAG IsGeneDetected'
- dd.micro\$other\$gIsSaturated 'FLAG IsSaturated' - dd.micro\$other\$gIsFeatNonUnifOL 'FLAG
IsFeatNonUnifOL' - dd.micro\$other\$gIsFeatPopnOL 'FLAG IsFeatPopnOL' - dd.micro\$other\$gBGMedianSignal
'gBGMedianSignal' - dd.micro\$other\$gBGUsed 'gBGUsed'
```

## Value

A uRNAList containing the following elements:

```
uRNAList\TGS    matrix, 'gTotalGeneSignal'
uRNAList\TPS    matrix, 'gTotalProbeSignal'
uRNAList\$meanS
                matrix, 'gMeanSignal'
uRNAList\$procS
                matrix, 'gProcessedSignal'
uRNAList\$targets
                data.frame, 'FileName'
uRNAList\$genes\$ProbeName
                character, 'AGilent Probe Name'
uRNAList\$genes\$GeneName
                character, 'microRNA Name'
uRNAList\$genes\$ControlType
                integer, '0'= Feature, '1'= Positive control, '-1'= Negative control
uRNAList\$other\$gIsGeneDetected
                matrix, FLAG to classify signal if 'IsGeneDetected=1' or 'not=0'
uRNAList\$other\$gIsSaturated
                matrix, FLAG to classify signal if 'IsSaturated = 1' or 'not=0'
uRNAList\$other\$gIsFeatPopnOL
                matrix, FLAG to classify signal if 'IsFeatPopnOL = 0' or 'not=1'
uRNAList\$other\$gIsFeatNonUnifOL
                matrix, FLAG to classify signal if 'gIsFeatNonUnifOL = 0' or 'not=1'
uRNAList\$other\$gBGMedianSignal
                matrix, gBGMedianSignal
uRNAList\$other\$gBGUsed
                matrix, gBGUsed
```

## Author(s)

Pedro Lopez-Romero

## References

Agilent Feature Extraction Reference Guide <http://www.Agilent.com>

Smyth, G. K. (2005). Limma: linear models for microarray data. In: 'Bioinformatics and Computational Biology Solutions using R and Bioconductor'. R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds), Springer, New York, pages 397–420.

## See Also

A data example can be found in `dd.micro` See also `readTargets` to see how to build the target file and the example given in `targets.micro`

## Examples

```
## Not run:
data(targets.micro)
dd.micro = readMicroRnaAFE(targets.micro)

## End(Not run)
```

---

`readTargets`

*read the target file*

---

## Description

The target file is a txt file created by the user where every input file (array, sample) is attached to a experimental condition

## Usage

```
readTargets(infile, verbose=FALSE)
```

## Arguments

<code>infile</code>	name of the target file, for instance 'targets.micro.txt'
<code>verbose</code>	logical, if TRUE prints out output

## Details

In the 'target' file (see Table 1 in vignette) we specify the experimental conditions under which the data have been generated. The target file **MUST** contain the following mandatory columns:  
 -FileName : Name of the array data file  
 -Treatment : Treatment effect  
 -Gerep : Treatment effect in numeric code, from '1' to 'n', being 'n' the number of the levels of the treatment effect

Other explanatory variables specifying the experimental conditions might be also included.

## Value

A 'data.frame' containing by the columns specified in the input file `targets.txt`. This 'targets.txt' file must be created by the user.

**Author(s)**

Pedro Lopez-Romero

**References**

Smyth, G. K. (2005). Limma: linear models for microarray data. In: 'Bioinformatics and Computational Biology Solutions using R and Bioconductor'. R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds), Springer, New York, pages 397–420.

**See Also**

An example of a target file can be found in `targets.micro`

---

RleMicroRna	<i>Relative Log Expression</i>
-------------	--------------------------------

---

**Description**

RLE: Relative Log Expression

**Usage**

```
RleMicroRna(object, maintitle, colorfill)
```

**Arguments**

<code>object</code>	An expression matrix
<code>maintitle</code>	title of the plot
<code>colorfill</code>	color of the plot

**Details**

Each Boxplot corresponds to a sample and displays the Relative Log Expression computed for every spot in the array as the difference between the spot intensity and the median intensity for the same feature accros all the arrays. Since majority of the spots are expected not to be differentially expressed, the plot should show boxplots centered around zero and all of them having the approximately the same dispersion. An array showing greater dispersion than the other, or being not centered at zero could have quality problems.

**Author(s)**

Pedro Lopez-Romero

## References

Boldstad B.M., Collin F., Brettschneider J., Simpson, K., Cope L., Irizarry R. A., Speed T. P. Quality Assesement of Affymetrix GeneChip Data. In Bioinformatics and Computational Biology Solutions Using R and Bioconductor. (eds.) Gentleman R., Carey V. J., Huber W., Irizarry R. A., Dudoit S. (2005). Springer.

## Examples

```
data(dd.micro)
      RleMicroRna(log2(dd.micro$meanS),
      maintitle="log2 Mean Signal RLE",
      colorfill="orange")
```

---

rmaMicroRna

*Getting the Total Gene Signal by RMA algorithm*

---

## Description

The function creates an `uRNAList` containing the `TotalGeneSignal` computed by the RMA algorithm. This signal can be used for the statistical analysis.

## Usage

```
rmaMicroRna(dd, normalize, background)
```

## Arguments

<code>dd</code>	<code>uRNAList</code> , containing the output from <code>readMicroRnaAFE</code>
<code>normalize</code>	logical, if TRUE the signal is normalized between arrays using the 'quantile' method
<code>background</code>	logical, if TRUE the signal is background corrected by fitting a normal + exponential convolution model to a vector of observed intensities

## Details

The function creates an `uRNAList` output that contains in the `uRNAList$TGS`, `uRNAList$TPS`, `uRNAList$meanS` & `uRNAList$procS` slots the Total Gene Signal (TGS) computed by the RMA algorithm. The function uses the robust multiarray average (RMA) method from the 'affy' package. RMA obtains an estimate of the expression measure for each gene using all the replicated probes for that gene. First, RMA obtains a background corrected intensity by fitting a normal + exponential convolution model to a vector of observed intensities. The normal part represents the background and the exponential part represents the signal intensities. Then the arrays are normalized using 'quantile' normalization. Finally, for each probe set that interrogates the same microRNA, RMA fits a linear model to the background-corrected, normalized and log2 transformed probe intensities. This model produces an estimate of the gene signal taking into account the probe effect. The model parameters estimates are obtained by median polish. The estimates of the gene expression signals are referred as RMA estimates. Normally, each microRNA is interrogated by 16



probes either using 2 different probes, each of them replicated 8 times, or using 4 different probes replicated 4 times. First, function 'rmaMicroRna' obtains a background corrected signal using the 'rma.background.correct' function of the package 'preprocessCore', then the signal is normalized between arrays using the 'limma' function 'normalizeBetweenArrays' with the 'quantile' method. Then, the median of the replicated probes is obtained, leading to either 2 or 4 different measures for each gene. These measures correspond to different probes for the same genes that are summarized into a single RMA linear model described above.

### Value

uRNAList containing the Total Gene Signal computed by the RMA algorithm in log 2 scale.

### Author(s)

Pedro Lopez-Romero

### References

- Irizarry, R., Hobbs, B., Collin, F., Beazer-Barclay, Y., Antonellis, K., Scherf, U., Speed, T. (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics*. 4, 249-264
- Gautier, L., Cope, L., Bolstad, B. M., and Irizarry, R. A. (2004). *affy*—analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics* 20, 3, 307-315.
- Bolstad B. M. (). *preprocessCore*: A collection of pre-processing functions. R package version 1.4.0
- Smyth, G. K. (2005). *Limma*: linear models for microarray data. In: 'Bioinformatics and Computational Biology Solutions using R and Bioconductor'. R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds), Springer, New York, pages 397 - 420

### Examples

```
data(dd.micro)
ddTGS.rma=rmaMicroRna(dd.micro, normalize=TRUE, background=TRUE)
dim(ddTGS.rma)
RleMicroRna(ddTGS.rma$TGS, "RLE TGS.rma", "blue")
```

---

significantMicroRna      *Summarize Differential Expression Results*

---

### Description

The function summarizes the results from the differential expression analysis using the different objects that are obtained after 'limma' has been used, such as the 'MArrayLM' object with the statistics and the 'TestResults' object highlighting the significant features.

**Usage**

```
significantMicroRna(eset, ddset, targets, fit2,
CM, DE, DEmethod, MTestmethod, PVcut, Mcut, verbose=FALSE)
```

**Arguments**

eset	ExpressionSet containing the Total Gene processed data
ddset	An uRNAList object containing the Total Gene processed data
targets	data.frame with the target structure
fit2	MArrayLM object from eBayes 'limma' function
CM	Contrast matrix
DE	TestResults object
DEmethod	method used in decideTests, only 'separate' or 'nestedF' are implemented
MTestmethod	method for multiple test
PVcut	p value threshold to declare significant features
Mcut	M value threshold to select within significant features
verbose	logical, if TRUE prints out output

**Details**

A list containing the genes with their statistics is generated. The significant genes above the PVcut p values are also given in a html file that links the selected miRNAs to the miRBase <http://microrna.sanger.ac.uk/>. A MA plots indicating the differentially expressed genes are also displayed.

When multiple contrasts are done, the method for the selection of the significant genes can be either 'separated' or 'nestedF'. See decideTests in package limma *limma* for a detailed description on these two methods. When 'separated' is used a list with all the genes that have been analyzed in limma is given. The list includes the following columns:

PROBE - Probe name (one of the probes interrogating the gene) GENE - miRNA name PROBE chr\_coord - Agilent chromosomal location M - Fold change A - Mean of the intensity for that miRNA t - moderated t-statistic pval - p value of the t-statistic adj.pval - p value adjusted by 'MTestmethod' fdr.pval - p value adjusted by fdr

Some times, the user can be set 'MTestmethod = none', in this case, it might be interesting to still see the fdr value, despite of the fact that the user has decided not apply any multiple testing correction.

If the 'nestedF' is used, then two lists are provided for each contrasts. A first containing the selected significant genes, and a second list containing the rest of the genes that have been analyzed. The columns given in this case is:

PROBE - Probe name (one of the probes interrogating the gene) GENE - miRNA name PROBE chr\_coord - Agilent chromosomal location M - Fold change A - Mean of the intensity for that miRNA t - moderated t-statistic t pval - p value of the t-statistic F - F statistic (null hypothesis:  $C_i = C_j$ , for all contrasts  $i, j$ ) adj.F.pval - F p value adjusted by 'MTestmethod' fdr.F.pval - F p value adjusted by fdr

The html files, both for the 'separated' and 'nestedF' method, includes only the selected as significant genes.

**Author(s)**

Pedro Lopez-Romero

**References**

Smyth, G. K. (2005). Limma: linear models for microarray data. In: 'Bioinformatics and Computational Biology Solutions using R and Bioconductor'. R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds), Springer, New York, pages 397–420.

miRBase: the home of microRNA data <http://microrna.sanger.ac.uk/>

**See Also**

A 'uRNAList' example containing processed data is in `ddPROC` and an overview of how the processed data is produced is given in `filterMicroRna`. The `ExpressionSet` object can be generated using `esetMicroRna`. An overview of miRNA differential expression analysis is given in `basicLimma`. An example of how to get the 'TestResults' object is in `getDecideTests`.

**Examples**

```
data(targets.micro)
data(ddPROC)
esetPROC=esetMicroRna(ddPROC,targets.micro,makePLOT=FALSE)

levels.treatment=levels(factor(targets.micro$Treatment))
treatment=factor(as.character(targets.micro$Treatment),
  levels=levels.treatment)

levels.subject=levels(factor(targets.micro$Subject))
subject=factor(as.character(targets.micro$Subject),
  levels=levels.subject)

design=model.matrix(~ -1 + treatment + subject )

CM=cbind(MSC_AvsMSC_B=c(1,-1,0,0),
  MSC_AvsMSC_C=c(1,0,-1,0))

fit2=basicLimma(esetPROC,design,CM,verbose=TRUE)

DE=getDecideTests(fit2,
  DEmethod="separate",
  MTestmethod="BH",
  PVcut=0.10)

significantMicroRna(esetPROC,
  ddPROC,
  targets.micro,
  fit2,
  CM,
  DE,
  DEmethod="separate",
  MTestmethod="BH",
```

```
PVcut=0.10,  
Mcut=0,  
verbose=TRUE)
```

---

summary.uRNAList      *Summaries of Microarray Data Objects*

---

### **Description**

Briefly summarize microarray data objects.

### **Usage**

```
## S3 method for class 'uRNAList'  
summary(object, ...)
```

### **Arguments**

object	an object of class uRNAList
...	other arguments are not used

### **Details**

The data objects are summarized as if they were lists, i.e., brief information about the length and type of the components is given. This function and this file, has been borrowed from the files created by Gordon Smyth for the limma package.

### **Value**

A table.

### **Author(s)**

Pedro Lopez-Romero

---

targets.micro	<i>Example of target file</i>
---------------	-------------------------------

---

**Description**

Example of target file

**Usage**

```
data(targets.micro)
```

**Format**

A data frame with 4 observations on the following 5 variables.

FileName names of the Files Ast.txt Bst.txt Aunst.txt Bunst.txt

Treatment Assigns level for Treatment Effect to each File (mandatory)

GErep a numeric vector tha numerates the FACTOR of the Treatment Effect (mandatory)

Subject Assigns level for Subject Effect to each File

**Details**

It is a tab-delimited text format file. The target file is created by the user with the intention of carrying out a differential expression analysis in future steps using 'limma'. Here is where the factors that are going to be included in the linear model that is fitted to each gen are specified. The targets file assigns each data file to a particular experimental conditions. First column 'FileName' is mandatory and includes the image data files names. Second column 'Treatment' is also mandatory and includes the treatment effect. Third column 'GErep' is also mandatory, and includes the treatment effect in a numeric code, from 1 to n, being n the number of Treatment effect levels.

**Author(s)**

Pedro Lopez-Romero

**References**

Gordon K. Smyth, M. Ritchie, N. Thorne, J. Wettenhall (2007). limma: Linear Models for Microarray Data User's Guide.

**See Also**

readTargets

tgsMicroRna

*Getting the Total Gene Signal***Description**

The function creates an `uRNAList` containing the `TotalGeneSignal` computed by the Agilent Feature Extraction software. This signal can be used for the statistical analysis after a possible normalization step.

**Usage**

```
tgsMicroRna(dd, offset, half, makePLOT=FALSE, verbose=FALSE)
```

**Arguments**

<code>dd</code>	<code>uRNAList</code> , containing the output from <code>readMicroRnaAFE</code>
<code>offset</code>	integer. To use this option set <code>half = FALSE</code>
<code>half</code>	logical, if TRUE half option is used
<code>makePLOT</code>	logical, if TRUE QC plots with the Total Gene Signal are displayed
<code>verbose</code>	logical, if TRUE prints out some summary results

**Details**

The function creates a `uRNAList` object that contains in the `uRNAList$TGS`, `uRNAList$TPS`, `uRNAList$meanS` & `uRNAList$procS` the Total Gene Signal (TGS) as computed by the Agilent Feature Extraction algorithms. This TGS is not in  $\log_2$  scale. All the replicated genes have the same estimated TGS, and the function simply picks one gene from each set of replicated genes. To maintain the format of the `uRNAList`, every selected gene retains a probe name attached to them. This probe name is not meaningful any more, since the signal corresponds to the total gene signal and not to the probe signal. The TGS processed by AFE contains some negative values. To get signals with positive values we can either add a positive small constant to all the signals (`offset`) or we can select the 'half' option, which set to 0.5 all the values that are smaller than 0.5. To use the `offset` option we have to set `half=FALSE`, otherwise the half method is used by default. The `offset` option, adds to each signal the quantity  $(\text{abs}(\min(\text{ddTGS}\$TGS)) + \text{offset})$ , where `ddTGS$TGS` is the matrix that contains the `TotalGeneSignal`.

**Value**

`uRNAList` containing the `TotalGeneSignal` computed by the Agilent Feature Extraction software. Optionally, it can generate a boxplot, a density plot and a MA plot with the Total Gene Signal.

**Author(s)**

Pedro Lopez-Romero

## References

Agilent Feature Extraction Reference Guide <http://www.Agilent.com>

## Examples

```
data(dd.micro)
data(targets.micro)
ddTGS=tgsMicroRna(dd.micro, half=TRUE, makePLOT=FALSE, verbose=FALSE)
```

---

tgsNormalization	<i>Normalization Between Arrays</i>
------------------	-------------------------------------

---

## Description

Normalization between arrays of the Total Gene Signal. The function is a wrapper of the 'limma' 'normalizeBetweenArrays' with ('none', 'quantile', 'scale') methods

## Usage

```
tgsNormalization(ddTGS, NORMmethod = "quantile", makePLOTpre = FALSE, makePLOTpost = FALSE, targets, verbose)
```

## Arguments

ddTGS	uRNAList, containing the output from tgsMicroRna
NORMmethod	character specifying the normalization method, 'none', 'quantile', 'scale'. The default is quantile
makePLOTpre	logical, if TRUE QC plots with the Raw Total Gene Signal are displayed
makePLOTpost	logical, if TRUE QC plots with the Normalized Total Gene Signal are displayed
targets	data.frame with the target structure
verbose	logical, if TRUE prints out output

## Value

A uRNAList object containing the Normalized Total Gene Signal in log 2 scale

## Author(s)

Pedro Lopez-Romero

## References

Smyth, G. K. (2005). Limma: linear models for microarray data. In: 'Bioinformatics and Computational Biology Solutions Using R and Bioconductor'. R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds), Springer, New York, pages 397 - 420

Smyth, G. K., and Speed, T. P. (2003). Normalization of cDNA microarray data. *Methods* 31, 265-273.

**Examples**

```
## Not run:
data(dd.micro)
data(targets.micro)
ddTGS=tgsMicroRna(dd.micro, half=TRUE, makePLOT=FALSE, verbose=FALSE)

ddNORM=tgsNormalization(ddTGS, 'quantile',
                        makePLOTpre=FALSE, makePLOTpost=TRUE, targets.micro, verbose=TRUE)
graphics.off()

## End(Not run)
```

---

uRNAList-class

*uRNAList* - class

---

**Description**

A list-based class (similar to the RGList class in limma package) for the storing of Agilent chips microRNA data uRNAList objects are created by read.agiMicroRna

**uRNAList Components**

uRNAList objects are created by new("uRNAList", Newagi) where Newagi is a list. with the following components:

**uRNAList\$TGS** matrix, 'gTotalGeneSignal'

**uRNAList\$TPS** matrix, 'gTotalProbeSignal'

**uRNAList\$meanS** matrix, 'gMeanSignal'

**uRNAList\$procS** matrix, 'gProcessedSignal'

**uRNAList\$targets** data.frame, 'FileName'

**uRNAList\$genes\$ProbeName** vector of characters, 'AGilent Probe Name'

**uRNAList\$genes\$GeneName** vector of characters, 'microRNA Name'

**uRNAList\$genes\$ControlType** vector of integers, '0'= Feature, '1'= Positive control, '-1'= Negative control

**uRNAList\$other\$gIsGeneDetected** matrix, FLAG to classify signal if 'IsGeneDetected=1' or 'not=0'

**uRNAList\$other\$gIsSaturated** matrix, FLAG to classify signal if 'IsSaturated = 1' or 'not=0'

**uRNAList\$other\$gIsFeatPopnOL** matrix, FLAG to classify signal if 'IsFeatPopnOL = 0' or 'not=1'

**uRNAList\$other\$gIsFeatNonUnifOL** matrix, FLAG to classify signal if 'gIsFeatNonUnifOL = 0' or 'not=1'

**uRNAList\$other\$gBGMedianSignal** matrix, gBGMedianSignal

**uRNAList\$other\$gBGUsed** matrix, gBGUsed



**Author(s)**

Pedro Lopez-Romero

**Examples**

```
## Not run:
  data(dd.micro)

## End(Not run)
```

---

writeEset	<i>Writes the expression data matrix of an ExpressionSet object in a txt file</i>
-----------	---

---

**Description**

Writes the expression data matrix of an ExpressionSet object in a file.

**Usage**

```
writeEset(eset, ddPROC, targets, verbose=FALSE)
```

**Arguments**

eset	An Expression object, normally containing the processed data
ddPROC	An RGList object, normally containing the processed data
targets	data.frame with the targets structure
verbose	logical, if TRUE prints out output

**Details**

Writes the expression data matrix of an ExpressionSet object in a file.

**Author(s)**

Pedro Lopez-Romero

**See Also**

An 'RGList' example containing processed data is in ddPROC and an overview of how the processed data is produced is given in filterMicroRna. The ExpressionSet object can be generated using esetMicroRna

**Examples**

```
## Not run:  
data(ddPROC)  
data(targets.micro)  
esetPROC=esetMicroRna(ddPROC,targets.micro,makePLOT=TRUE,verbose=FALSE)  
writeEset(esetPROC,ddPROC,targets.micro,verbose=TRUE)  
  
## End(Not run)
```

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