# Package 'ExiMiR'

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**Description** This package contains functions for reading raw data in ImaGene TXT format obtained from Exiqon miRCURY LNA arrays, annotating them with appropriate GAL files, and normalizing them using a spike-in probe-based method. Other platforms and data formats are also supported.

**License** GPL-2

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`ExiMiR-package` *R functions for the normalization of Exiqon miRNA array data*

---

## Description

This package contains functions for reading raw data in ImaGene TXT format obtained from Exiqon miRCURY LNA arrays, annotating them with appropriate GAL files, and normalizing them using a spike-in probe-based method. Other platforms and data formats are also supported by ExiMiR.

## Details

| | |
|---|---|
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## Author(s)

Sylvain Gubian, Alain Sewer, PMP SA
Maintainer: DL.RSupport@pmi.com

bg.correct.miR *ExiMiR low-level function for background correction*

## Description

This function performs background correction on an AffyBatch object.

The methods supported by bg.correct.miR are provided by the affy or limma packages, depending on whether the input AffyBatch object has been created with ReadAffy or ReadExi/createAB, respectively.

## Usage

```
bg.correct.miR(abatch,
        bgcorrect.method='auto',
        bgcorrect.param=list(),
        verbose=FALSE)
```

## Arguments

| | |
|---|---|
| abatch | An AffyBatch object. |
| bgcorrect.method | Character vector. It contains the name of the background correction method. Running NormiR.bgcorrect.methods(abatch) indicates which methods can be used, depending on the raw data contained in the abatch object. The auto option corresponds to the default choice of applying rma for single-channel arrays and normexp for dual-channel arrays. |
| bgcorrect.param | A R list containing the parameters required by the selected background correction method, as specified in the documentation of the original functions bg.correct of the affy package or backgroundCorrect of the limma package. As an illustration the parameters of the normexp method of the limma package are given below.<br>**normexp.method** Character vector. The variant of the normexp method, matching exactly the argument normexp.method of the backgroundCorrect function.<br>**offset** Numeric value to add to intensities. It matches exactly the argument offset of the backgroundCorrect function. |
| verbose | Logical. The default value is TRUE. The details of the function execution are displayed on the console. |

## Details

See accompanying vignette.

## Value

An AffyBatch object containing the background-corrected raw expression data.

## Author(s)

Sylvain.Gubian, Alain Sewer, PMP SA

## See Also

NormiR.bgcorrect.methods, NormiR.

## Examples

```
data(galenv)
data(GSE20122)
NormiR.bgcorrect.methods(GSE20122)
GSE20122.bgcorrected <- bg.correct.miR(GSE20122,
                                       bgcorrect.method='normexp',
                                       bgcorrect.param=list(offset=50))
```

---

createAB *ExiMiR function for creating an AffyBatch object from other object types (RGList, EListRaw, MAList or list)*

---

## Description

This function creates an AffyBatch object from a limma object (RGList, EListRaw, MAList) or from any appropriate list object.

## Usage

```
createAB(object,
         verbose=TRUE,
         ref.channel="R",
         genes.block=NULL,
         genes.row=NULL,
         genes.col=NULL,
         genes.id=NULL,
         genes.name=NULL,
         galname=NULL,
         env.overwrite=TRUE,
         ...)
```

## Arguments

| | |
|---|---|
| object | An appropriate EListRaw, RGList, MAList or list object. |
| verbose | Logical. The default value is TRUE. The details of the function execution are displayed on the console. |
| genes.block | Optional character vector in case the platform is neither ImaGene, Exiqon nor Agilent. The name of the column in the object$genes data frame that contains the block or field values. |

| | |
|---|---|
| genes.row | Optional character vector in case the platform is neither ImaGene, Exiqon nor Agilent. The name of the column in the object$genes data frame that contains the row values. |
| genes.col | Optional character vector in case the platform is neither ImaGene, Exiqon nor Agilent. The name of the column in the object$genes data frame that contains the column values. |
| genes.id | Optional character vector in case the platform is neither ImaGene, Exiqon nor Agilent. The name of the column in the object$genes data frame that contains the gene IDs. |
| genes.name | Optional character vector in case the platform is neither ImaGene, Exiqon nor Agilent. The name of the column in the object$genes data frame that contains gene names. |
| ref.channel | Character vector. The value of the reference channel for two-color arrays ('R' or 'G') |
| galname | Character vector. The default value is NULL. In this case the GAL annotation environment used by createAB for generating the resulting AffyBatch object is lost. Assigning galname a non-empty value allows to control this GAL environment, which is useful in two specific situations. First, it gives a handle to this GAL annotation environment for later use. Second, if an adequate GAL annotation environment already exists in the memory (e.g. after having been generated by createAB or by make.gal.env), galname allows to force createAB to use it for generating the resulting AffyBatch object. |
| env.overwrite | Logical. The default value is TRUE. If a GAL annotation environment with the same name already exists in the memory, it will be overwritten. This may be useful when the piece of code containing createAB is run several times. |
| ... | Any additional argument that can be given to the AffyBatch constructor, as specified in the documentation of the AffyBatch object provided in the affy package. |

## Details

See accompanying vignette.

## Value

An AffyBatch object containing the raw expression data.

## Author(s)

Sylvain Gubian, Alain Sewer, PMP SA

## See Also

ReadExi, make.gal.env.

galenv *R annotation environment for GEO series GSE20122*

## Description

The galenv environment is a hash table for the annotation of the Exiqon miRCURY LNA arrays used in the GEO series GSE20122 (Exiqon miRCURY LNA array v.11).

## Details

See accompanying vignette.

## See Also

make.gal.env.

GSE19183 *Affybatch object for the raw data from GEO series GSE19183*

## Description

The Affybatch object GSE19183 contains the raw expression data contained in the CEL files of the GEO series GSE19183, obtained from the Affymetrix miRNA-1_0 platform. The annotation is included in the Affybatch object.

## Details

See accompanying vignette.

GSE20122 *Affybatch object for the raw data from GEO series GSE20122*

## Description

The Affybatch object GSE20122 contains the raw expression data contained in the ImaGene TXT files of the GEO series GSE20122, obtained from the Exiqon miRCURY LNA platform v.11.

## Details

See accompanying vignette.

make.gal.env *GAL Environment Maker*

## Description

Reads an Exiqon GAL file and creates an annotation environment used as a hash table for the probeset mapping location.

## Usage

```
make.gal.env(galname=NULL,
             filename=NULL,
             gal.path=getwd(),
             verbose=FALSE)
```

## Arguments

| | |
|---|---|
| galname | Character vector. Name to be used for the annotation environment. |
| filename | Character vector. Name of the GAL file. |
| gal.path | Character vector. Path to the GAL file. |
| verbose | Logical. The default value is TRUE. The details of the function execution are displayed on the console. |

## Details

This function is designed similarly to make.cdf.env from the makecdfenv package. If no filename is provided as argument, the function tries to read the first GAL file in the input path. The returned environment is a hash table. For every probeset name we have a matrix with 2 columns. The first column contains the PM locations and the second column the MM locations. For PM only chips the MM column will have NAs.

## Value

None.

## Author(s)

Sylvain Gubian, Alain Sewer, PMP SA

## Examples

```
# The folder 'Exiqon' contains a GAL file
## Not run: make.gal.env(galname='galenv', gal.path='Exiqon')
```

norm.miR *ExiMiR low-level function for miRNA raw data normalization.*

## Description

This function performs low-level normalization on an AffyBatch object and returns the result in a new AffyBatch object.

By default, it applies the spike-in probe-based normalization method. In case the spike-in probe-based method cannot be applied, a median normalization is executed instead. Several options allow however to force the execution of the spike-in probe-based normalization and to fine-tune the resulting correction functions.

## Usage

```
norm.miR(abatch,
         normalize.method="spikein",
         normalize.param=list(),
         verbose=TRUE,
  ...)
```

## Arguments

abatch An AffyBatch object.

normalize.method

Character vector. It contains the name of normalization method. By default, the spikein method is used. Running NormiR.normalize.methods(abatch) indicates which other methods can be chosen, depending on the raw data contained in the abatch object.

normalize.param

A R list of the arguments that are used to control the spikein normalization. Running NormiR.spikein.args() provides a complete list of all the tunable parameters supported by norm.miR and explained below.

**figures.output** Character vector. By default, display is used. Figures are shown to the screen. Using file generates the figures in PDF format in the working directory.

**min.corr** Numeric. Default value is 0.5. Minimal allowed value for the average of the off-diagonal elements of the Pearson correlation matrix of the spike-in probeset intensities across the arrays.

**loess.span** Numeric. Default value is -1, which corresponds to a loess smoothing neighbourhood spanning a fraction 3/(number of spike-in probesets) of the total number of points. Other positive values are allowed, see the span argument of the R loess function

**extrap.points** Numeric. Default value is 2. The number of spike-in probesets used in the high-intensity extrapolation of the normalization correction function.

**extrap.method** Character vector. Default value is `mean`. The method used for the high-intensity extrapolation of the normalization correction function.

**force.zero** Logical. Default value is `FALSE`. If `TRUE`, it forces the normalization correction functions to have zero values at the lower end of the probe intensity range.

**cover.ext** Numeric. Default value is 1/2. Minimal allowed relative coverage of the spike-in probesets intensities. It is computed as the ratio between the intensity range covered by the spike-in probes and the one covered by all probes on the array.

**cover.int** Numeric. Default value is 1/3. Maximal allowed relative intensity interval between two consecutive spike-in probesets. It is computed as the largest intensity difference between two consecutive spike-in probesets divided by the overall probe intensity range.

**verbose** Logical. Default is `TRUE`; some details are provided on the console.

**max.log2span** Numeric. Default value is 1. Gives the maximal (log2) intensity interval allowed for the probes belonging to one spike-in probeset.

**probeset.list** Vector of probes names that will be used as the "spike-in probes". By default, `norm.miR` uses the probes annotated as "spike-in" by Exiqon or Affymetrix.

`verbose` Logical. The default value is `TRUE`. The details of the function execution are displayed on the console.

`...` Any additional argument. Used for backward compatibility.

## Details

See accompanying vignette.

## Value

An `AffyBatch` object containing the normalized (but not summarized) expression data.

## Author(s)

Sylvain.Gubian, Alain.Sewer, PMP SA

## See Also

`NormiR.normalize.methods`, `NormiR.spikein.args`, `NormiR`.

## Examples

```
data(galenv)
data(GSE20122)
GSE20122.normalized <- norm.miR(GSE20122,
                                normalize.param=list(figures.show=FALSE))
# Apply the affy method hist on the generated AffyBatch object GSE20122.normalized
layout(matrix(c(1,2), 1, 2, byrow = TRUE))
hist(GSE20122)
hist(GSE20122.normalized)
```

```
layout(1)
```

---

NormiR *ExiMiR high-level function for miRNA raw data normalization.*

---

## Description

This function applies a standard raw data normalization pipeline (i.e. background correction, normalization, PM correction if needed, and summarization) on the input AffyBatch object and returns the result in an ExpressionSet object.

The methods supported by NormiR for the background correction are provided by the affy or limma packages, depending on whether the input AffyBatch object has been created with ReadAffy or ReadExi/createAB, respectively.

By default, it applies the spike-in probe-based method for the second step of normalization. In case the spike-in probe-based method cannot be applied, a median normalization is executed instead. Several options allow however to force the execution of the spike-in probe-based normalization and to fine-tune the resulting correction functions.

The next step of PM correction is enabled only when numerical values are available for the MM probes of the input AffyBatch object. In this case the methods proposed by NormiR are provided by the affy package.

The methods supported by NormiR for the last step of summarization are also provided by the affy package. They do not depend on how the input AffyBatch object has been created.

## Usage

```
NormiR(abatch,
       # background correction
       bg.correct=TRUE,
       bgcorrect.method='auto',
       bgcorrect.param=list(),
       # normalize
       normalize=TRUE,
       normalize.method='spikein',
       normalize.param=list(),
       # pm correction (enabled only when MM-values are available)
       pmcorrect.method='pmonly',
       pmcorrect.param=list(),
       # expression values
       summary.method='medianpolish',
       summary.param=list(),
       summary.subset=NULL,
       # misc.
       verbose=FALSE,
       ...)
```

## Arguments

| | |
|---|---|
| abatch | An AffyBatch object. |
| bg.correct | Logical. Default is TRUE: the background correction step will be performed. |
| bgcorrect.method | Character vector. It contains the name of the background correction method. Running NormiR.bgcorrect.methods(abatch) indicates which methods can be used, depending on the raw data contained in the abatch object. The auto option corresponds to the default choice of applying rma for single-channel arrays and normexp for dual-channel arrays. |
| bgcorrect.param | A R list containing the parameters required by the selected background correction method, as specified in the documentation of the original functions bg.correct of the affy package or backgroundCorrect of the limma package. As an illustration the parameters of the normexp method of the limma package are given below. |

**normexp.method** Character vector. The variant of the normexp method, matching exactly the argument normexp.method of the backgroundCorrect function.

**offset** Numeric value to add to intensities. It matches exactly the argument offset of the backgroundCorrect function.

| | |
|---|---|
| normalize | Logical. Default is TRUE: the normalization step will be performed. |
| normalize.method | Character vector. It contains the name of normalization method. By default, the spikein method is used. Running NormiR.normalize.methods(abatch) indicates which other methods can be chosen, depending on the raw data contained in the abatch object. |
| normalize.param | A R list of the arguments that are used to control the spikein normalization. Running NormiR.spikein.args() provides a complete list of all the tunable parameters supported by NormiR and explained below. |

**figures.output** Character vector. By default, display is used. Figures are shown to the screen. Using file generates the figures in PDF format in the working directory.

**min.corr** Numeric. Default value is 0.5. Minimal allowed value for the average of the off-diagonal elements of the Pearson correlation matrix of the spike-in probeset intensities across the arrays.

**loess.span** Numeric. Default value is -1, which corresponds to a loess smoothing neighbourhood spanning a fraction 3/(number of spike-in probesets) of the total number of points. Other positive values are allowed, see the span argument of the R loess function

**extrap.points** Numeric. Default value is 2. The number of spike-in probesets used in the high-intensity extrapolation of the normalization correction function.

**extrap.method** Character vector. Default value is mean. The method used for the high-intensity extrapolation of the normalization correction function.

**force.zero** Logical. Default value is FALSE. If TRUE, it forces the normalization correction functions to have zero values at the lower end of the probe intensity range.

**cover.ext** Numeric. Default value is 1/2. Minimal allowed relative coverage of the spike-in probesets intensities. It is computed as the ratio between the intensity range covered by the spike-in probes and the one covered by all probes on the array.

**cover.int** Numeric. Default value is 1/3. Maximal allowed relative intensity interval between two consecutive spike-in probesets. It is computed as the largest intensity difference between two consecutive spike-in probesets divided by the overall probe intensity range.

**verbose** Logical. Default is TRUE; some details are provided on the console.

**max.log2span** Numeric. Default value is 1. Gives the maximal (log2) intensity interval allowed for the probes belonging to one spike-in probeset.

**probeset.list** Vector of probes names that will be used as the "spike-in probes". By default, NormiR uses the probes annotated as "spike-in" by Exiqon or Affymetrix.

`pmcorrect.method`
: Character vector. It contains the name of the PM correction method, which is enabled only when numerical values are available for the MM probes of the input AffyBatch object. Running `NormiR.pmcorrect.methods(abatch)` indicates which other methods can be chosen instead of the default one `pmonly`.

`pmcorrect.param`
: A R list of optional parameters for the selected `pmcorrect.method`, as specified in the documentation of the original function `pmcorrect` function of the `affy` package.

`summary.method`
: Character vector. It contains the name of the summarization method. Running `NormiR.summary.methods()` indicates which other methods can be chosen instead of the default one `medianpolish`.

`summary.param`
: A R list of optional parameters for the selected `summary.method`, as specified in the documentation of the original AffyBatch method `computeExprSet` contained in the `affy` package.

`summary.subset`
: A R list of probe set identifiers. When set to its default NULL value, the summarized expression values are computed for all probe sets available on the array.

`verbose`
: Logical. The default value is TRUE. The details of the function execution are displayed on the console.

`...`
: Any additional argument. Used for backward compatibility.

## Details

See accompanying vignette.

## Value

An ExpressionSet object containing the normalized expression data.

### Author(s)

Sylvain Gubian, Alain Sewer, PMP SA

### See Also

bg.correct.miR, NormiR.bgcorrect.methods, norm.miR, NormiR.normalize.methods, NormiR.spikein.args, NormiR.pmcorrect.methods, summarize.miR, NormiR.summary.methods.

### Examples

```
data(galenv)
data(GSE20122)
eset.spike <- NormiR(GSE20122,
                     bg.correct=FALSE,
                     normalize.method='spikein',
                     summary.method='medianpolish')
```

---

## NormiR.methods *ExiMiR functions for enumerating the names of low-level normalization methods or arguments*

---

### Description

These functions enumerate the names of methods or arguments of the low-level functions for miRNA raw data normalization (i.e. background correction, (spike-in probe-based) normalization, PM corrrection, summarization). They take into account how the input AffyBatch object was created as well as the underlying array type.

### Usage

```
NormiR.bgcorrect.methods(object)
NormiR.normalize.methods(object)
NormiR.pmcorrect.methods(object)
NormiR.summary.methods()
NormiR.spikein.args()
```

### Arguments

| | |
|---|---|
| object | An AffyBatch object. |

### Details

See accompanying vignette.

### Value

List of strings containing the names of the methods or arguments available for the input AffyBatch object.

### Author(s)

Sylvain.Gubian, Alain.Sewer, PMP SA

### See Also

NormiR, bg.correct.miR, norm.miR, summarize.miR.

---

ReadExi *ExiMiR function for reading Exiqon raw data into an AffyBatch object.*

---

### Description

This function reads Exiqon raw data in ImaGene file format and creates an AffyBatch object.

### Usage

```
ReadExi(txtfile.path=getwd(),
        galname=NULL,
        description=NULL,
 notes='',
 rm.background=FALSE,
 verbose=TRUE)
```

### Arguments

| | |
|---|---|
| txtfile.path | Character vector. It contains the path to the folder containing the samplesinfo.txt file and the Exiqon raw data files in ImaGene txt format. |
| galname | Character vector. The default value is NULL. In this case the GAL annotation environment used by ReadExi for generating the resulting AffyBatch object is lost. Assigning galname a non-empty value allows to control this GAL environment, which is useful in two specific situations. First, it gives a handle to this GAL annotation environment for later use. Second, if an adequate GAL annotation environment already exists in the memory (e.g. after having been generated by ReadExi or by make.gal.env), galname allows to force ReadExi to use it for generating the resulting AffyBatch object. |
| description | Object of class MIAME, as specified in the documentation of the AffyBatch object provided in the affy package. |
| notes | Character vector, as specified in the documentation of the AffyBatch object provided in the affy package. |
| rm.background | Logical. This option is kept for compatibility reasons but it is not used anymore. See the NormiR options for background correction. |
| verbose | Logical. The default value is TRUE. The details of the function execution are displayed on the console. |

### Details

The Exiqon miRNA raw expression data are normally in ImageGene `txt` file format and accompanied by a `samplesinfo.txt` description file. It enumerates the names of the sample files for each channel. Therefore the `txtfile.path` argument of `ReadExi` must be a folder that contains the ImageGene and the `samplesinfo.txt` files. If this is not the case, `ReadExi` stops.

The `galname` argument of `ReadExi` must be the name of a GAL annotation environment created with the `make.gal.env` or the `ReadExi` functions. If `galname` is provided a `NULL` value, which is the default situation, a minimal GAL annotation environment is created based on the annotation contained in the ImageGene `txt` files.

### Value

An `AffyBatch` object containing the raw expression data.

### Warning

The `image` method of the `AffyBatch` object might not work properly when the `galname` argument of `ReadExi` has not been assigned.

### Author(s)

Sylvain Gubian, Alain Sewer, PMP SA

### See Also

`make.gal.env`, `createAB`.

### Examples

```
# The folder 'Exiqon' contains the file 'samplesinfo.txt' and  the corresponding raw data files in ImaGene format
## Not run: ebatch <- ReadExi(txtfile.path='Exiqon')
# If the GAL environment has already created by the function make.gal.env
## Not run: ebatch <- ReadExi(galenv='galenv', txtfile.path='Exiqon')
```

---

## summarize.miR *ExiMiR low-level function for summarization.*

---

### Description

This function performs summarization on an `AffyBatch` object using a GAL or CDF annotation environment and generates an `ExpressionSet` object containing the results.

## Usage

```
summarize.miR(abatch,
       pmcorrect.method='pmonly',
       pmcorrect.param=list(),
       summary.method='medianpolish',
       summary.param=list(),
       summary.subset=NULL)
```

## Arguments

| | |
|---|---|
| abatch | An AffyBatch object. |
| pmcorrect.method | Character vector. It contains the name of the PM correction method, which is enabled only when numerical values are available for the MM probes of the input AffyBatch object. Running NormiR.pmcorrect.methods(abatch) indicates which other methods can be chosen instead of the default one pmonly. |
| pmcorrect.param | A R list of optional parameters for the selected pmcorrect.method, as specified in the documentation of the original function pmcorrect function of the affy package. |
| summary.method | Character vector. It contains the name of the summarization method. Running NormiR.summary.methods() indicates which other methods can be chosen instead of the default one medianpolish. |
| summary.param | A R list of optional parameters for the selected summary.method, as specified in the documentation of the original AffyBatch method computeExprSet contained in the affy package. |
| summary.subset | A R list of probe set identifiers. When left to its default NULL value, the summarized expression values are computed for all probe sets available on the array. |

## Value

An ExpressionSet containing the summarized expression data.

## Author(s)

Sylvain.Gubian, Alain Sewer, PMP SA

## See Also

NormiR.pmcorrect.methods, NormiR.summary.methods, NormiR.

## Examples

```
data(galenv)
data(GSE20122)
eset <- summarize.miR(GSE20122,
                      summary.method="medianpolish")
```

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