

# Package ‘LVSmRNA’

October 9, 2013

**Type** Package

**Title** LVS normalization for Agilent miRNA data

**Version** 1.10.0

**Date** 2012-11-15

**Depends** R (>= 2.10), Biobase, quantreg, splines, MASS, limma, affy, methods, SparseM, vsn

**Imports** BiocGenerics, stats4

**Enhances** parallel, snow, Rmpi

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**biocViews** Microarray, AgilentChip, OneChannel, Preprocessing

**Description** Normalization of Agilent miRNA arrays.

**License** GPL-2

**LazyLoad** yes

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boxplot-methods	<i>Methods for Function boxplot</i>
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### Description

Methods for function boxplot for objects of class EList and RGList

### Methods

signature(x = "EList") boxplot for EList object  
signature(x = "RGList") boxplot for RGList object

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estVC	<i>Robust Linear Model to Estimate Residual Variance and Array Effect</i>
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### Description

Given intensities from microRNA data, fits a robust linear model at probe level and return the residual standard deviations and the array effects.

### Usage

```
estVC(object,method=c("joint","rlm"),cov.formula=c("weighted","asymptotic"),clName,verbose=FALSE)
## S3 method for class 'RGList'
estVC(object,method=c("joint","rlm"),cov.formula=c("weighted","asymptotic"),clName,verbose=FALSE)
## S3 method for class 'EList'
estVC(object,method=c("joint","rlm"),cov.formula=c("weighted","asymptotic"),clName,verbose=FALSE)
```

### Arguments

object	an object of class EList or RGList.
method	character string specifying the estimating algorithm to be used. Choices are "joint" and "rlm".
cov.formula	character string specifying the covariance formula to be used. Choices are "weighted" and "asymptotic".
clName	Cluster object produced by makeCluster function. Used only if snow is loaded.
verbose	Print some debug messages.

## Details

estVC is the first step in LVS normalization. It fits a robust linear model at the probe-level data in order to estimate the variability of probe intensities due to array-to-array variability. Depending on whether probes show considerable differences in within-probe variance, user can choose the more complex joint model to accommodate the potential heteroscedasticity or standard robust linear model if within-probe variance can be ignored.

The array effects are then captured by the chi-square statistic. The covariance matrix can be estimated based either on the sandwich form of weighted covariance matrix or an asymptotic form.

## Value

An object of class RA containing three components as follows:

ArrayEffects	a matrix containing the array effect with samples as columns and miRNAs as rows.
ArrayChi2	vector giving chi-square statistics of the miRNAs as a measure of array-to-array variability.
logStdDev	vector giving standard deviations of the genes on log scale.

## Author(s)

Stefano Calza <stefano.calza@biostatistics.it>, Suo Chen and Yudi Pawitan.

## References

Calza et al., 'Normalization of oligonucleotide arrays based on the least variant set of genes', (2008, BMCBioinformatics); Pawitan, Y. 'In All Likelihood: Statistical Modeling and Inference Using Likelihood', (2001, Oxford University Press); Huber, P. J., 'Robust estimation of a location parameter', (1964, Annals of Mathematical Statistics).

## See Also

[read.mir](#), [lvs](#)

## Examples

```
## Not run:

# Starting from an EList object called MIR
data("MIR-spike-in")
AA <- estVC(MIR,method="joint")

# Parallel execution using multicore

library(multicore)

# use this to set the desired number of
#cores. Otherwise multicore would use all the available
options(cores=8)
```

```

AA <- estVC(MIR,method="joint")

detach('package:multicore')

# Parellel execution using snow

library(snow)

cl <- makeCluster(8,type="SOCK")

# Or also...see ?makeCluster
# cl <- makeCluster(8,type="MPI")

AA <- estVC(MIR,method="joint",clName=cl)

## End(Not run)

```

---

exprs-methods

*Methods for Function exprs*

---

### Description

Methods for function exprs for objects of class EList and RGList

### Methods

signature(x = "EList") exprs for EList object  
signature(x = "RGList") exprs for RGList object

---

exprs<--methods

*~~ Methods for Function exprs<- in Package 'Biobase' ~~*

---

### Description

*~~ Methods for function exprs<- in Package 'Biobase' ~~*

### Methods

signature(object = "AffyBatch", value = "ANY")  
signature(object = "EList", value = "ANY")  
signature(object = "ExpressionSet", value = "matrix")  
signature(object = "RGList", value = "ANY")  
signature(object = "SnpSet", value = "matrix")

---

 featureNames-methods    *Methods for Function featureNames*


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**Description**

Methods for function featureNames for objects of class EList and RGList

**Methods**

signature(x = "EList") featureNames for EList object

signature(x = "RGList") featureNames for RGList object

---

 lvs                                    *Least Variant Set selection and Normalization Function(s)*


---

**Description**

Selects the Least Variant Set of mircoRNAs, according to the chosen proportion of miRNAs expected not to vary between arrays. Then performs normalization.

**Usage**

```
lvs(RG,RA,ref,proportion=0.7,df=3,method=c("joint","rlm"),
    cov.formula=c("weighted","asymptotic"),
    spar=NULL,normalize.method=c("vsn","smooth.spline","mixed"),
    summarize.args=NULL,stratify=TRUE,n.strata=3,
    level=c("mir","probe"),Atransf=c("sqrt","log"),keep.iset=FALSE,c1Name,
    verbose=FALSE,...)
## S3 method for class 'RGList'
lvs(RG,RA,ref,proportion=0.7,df=3,method=c("joint","rlm"),
    cov.formula=c("weighted","asymptotic"),
    spar=NULL,normalize.method=c("vsn","smooth.spline","mixed"),
    summarize.args=NULL,stratify=TRUE,n.strata=3,
    level=c("mir","probe"),Atransf=c("sqrt","log"),
    keep.iset=FALSE,c1Name,verbose=FALSE,...)
## S3 method for class 'EList'
lvs(RG,RA,ref,proportion=0.7,df=3,method=c("joint","rlm"),
    cov.formula=c("weighted","asymptotic"),
    spar=NULL,normalize.method=c("vsn","smooth.spline","mixed"),
    summarize.args=NULL,stratify=TRUE,n.strata=3,
    level=c("mir","probe"),Atransf=c("sqrt","log"),keep.iset=FALSE,c1Name,
    verbose=FALSE,...)
```

**Arguments**

RG	an object of class EList or RGList
RA	a list containing components residual standard deviations, chi-square statistics and array effects. It can be computed by estVC. If not provided it will be computed (slower),
proportion	the proportion below which miRNAs are expected not to vary between arrays. Default is set to 0.7.
ref	reference array to be used for normalization. Default is set to mean of array effects across samples.
df	the desired equivalent number of degrees of freedom (trace of the smooth matrix) in smoothing spline.
method	character string specifying the estimating algorithm to be used. Choices are "joint" and "rlm".
cov.formula	character string specifying the covariance formula to be used. Choices are "weighted" and "asymptotic".
spar	smoothing parameter, typically in (0,1].
normalize.method	character string specifying the normalization method to be used. Choices are "smooth.spline" and "vsn".
summarize.args	a named list containing components from argument of summarize.
stratify	logical, if TRUE selection of least variant set will be stratified by expression level.
n.strata	integer giving the number of strata.
level	character string specifying the normalization performed at miRNA level or probe-level.
Atransf	Which transformation to use for Array Effect
keep.iset	return the LVS ids
clName	Cluster object. See estVC.
verbose	Verbose computation
...	...

**Details**

lvs works by first identifying least variant set (LVS) with the smallest array-to-array variation. The total information extracted from probe-level intensity data of all samples is modeled as a function of array and probe effect in order to select the reference set for normalization. If the residual variances and array effects are available, lvs runs faster because the step of robust linear modeling has already been done.

Once the LVS miRNAs are identified, the normalization is performed using vsn or smooth.spline.

**Value**

An object of the same class as RG.

G	matrix containing the normalized intensities for each array with miRNAs as rows and arrays as columns.
Gb	matrix containing the background intensities for each array with probes as rows and arrays as columns.
targets	data frame with column <code>FileName</code> giving the names of the files read, with column <code>Sample</code> giving the names of the sample.
genes	data frame containing annotation information about the probes, for examples miRNA names and IDs and positions on the array.
source	character string giving the image analysis program name.
preprocessing	list with components <code>Background</code> , <code>Normalization</code> , <code>is.log</code> , <code>Summarization</code> indicate which pre-processing step has been done.

**Author(s)**

Stefano Calza <stefano.calza@biostatistics.it>, Suo Chen and Yudi Pawitan.

**References**

Calza et al., 'Normalization of oligonucleotide arrays based on the least variant set of genes' (2008, BMCBioinformatics).

**See Also**

[estVC](#), [summarize](#)

**Examples**

```
## Not run:

# Starting from an Elist object called MIR
data("MIR-spike-in")
AA <- estVC(MIR,method="joint")
bb <- lvs(MIR,RA=AA,level="probe")

##It can also run with object RA missing, but taking longer time
cc <- lvs(MIR)

## End(Not run)
```

---

MIR-spike-in

*Data example*


---

### Description

Data from a micro-RNA spike-in experiment, extracted from scanned images using Agilent Feature Extraction Software.

### Usage

```
data("MIR-spike-in")
data("MIR_RA")
```

### Details

This dataset is derived from a library of synthetic RNA sequences, corresponding to human mature miRNAs as well as in-house miRNAs with particularly similar sequences hybridized on an Agilent Human miRNA Microarray 2.0. Data consist of a total of 799 miRNA species (excluding control features) for 4 samples organized in two groups A and B.

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

- MIR\G: 'gMeanSignal'
- MIR\Gb: 'gProcessedSignal'
- MIR\gBGMedianSignal: 'gBGMedianSignal'
- MIR\targets 'targets'
- MIR\Row 'Row'
- MIR\Col 'Column'
- MIR\ProbeUID 'Probe ID'
- MIR\genes\ControlType 'FLAG to specify the sort of feature'
- MIR\genes\ProbeName 'Probe Name'
- MIR\genes\GeneName 'microRNA Name'
- MIR\genes\SystematicName 'microRNA Name'
- MIR\genes\Description 'Description (not used)'

MIR\_RA holds an object of class RA obtained from using estVC on the example data.

### Author(s)

Stefano Calza

### References

Willenbrock H, Salomon J, Barken KIMB, Nielsen FC, Litman T. 2009. Quantitative miRNA expression analysis: Comparing microarrays with next-generation sequencing. *RNA* 15: 2028-2034.

Data available from Genome Expression Omnibus (GEO) database under the series accession number GSE14511

### See Also

read.mir, estVC



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plot-method	<i>Plot of Residual Variance and Array Effect</i>
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---

**Description**

Plots results from estVC

**Usage**

```
## S4 method for signature 'RA,ANY'  
plot(x,Atransf=c("both","sqrt","log"), abline=c("none","rq"),df=3,proportion=.7,  
      col="black",col.rq="red")
```

**Arguments**

x	An object of class RA resulting from estVC.
Atransf	Transformation to apply at Array Effect
abline	Add a line to the plot representing a quantile fit
df	Degrees of freedom of the quantile regression
proportion	Quantile to fit
col	Color for plotting points
col.rq	Color for plotting quantile line

**Author(s)**

Stefano Calza <stefano.calza@biostatistics.it>, Suo Chen and Yudi Pawitan.

**References**

Calza et al., 'Normalization of oligonucleotide arrays based on the least variant set of genes', (2008, BMCBioinformatics); Pawitan, Y. 'In All Likelihood: Statistical Modeling and Inference Using Likelihood', (2001, Oxford University Press); Huber, P. J., 'Robust estimation of a location parameter', (1964, Annuas of Mathematical Statistics).

**See Also**

[estVC,rq](#)

**Examples**

```
## Not run:  
  
# Starting from an EList object called MIR  
data("MIR-spike-in")  
AA <- estVC(MIR,method="joint")  
plot(AA)  
  
## End(Not run)
```

---

preproc-methods      *Methods for Function preproc*

---

**Description**

Methods for function preproc for objects of class EList and RGList

**Methods**

signature(x = "EList") preproc for EList object

signature(x = "RGList") preproc for RGList object

---

preproc<--methods      *Methods for Function preproc*

---

**Description**

Methods for function preproc<- for objects of class EList and RGList

**Methods**

signature(x = "EList") preproc for EList object

signature(x = "RGList") preproc for RGList object

---

probeNames-methods      *Methods for Function probeNames*

---

**Description**

Methods for function probeNames for objects of class EList and RGList

**Methods**

signature(x = "EList") probeNames for EList object

signature(x = "RGList") probeNames for RGList object

---

read.mir	<i>Read in miRNA Data from Agilent Feature Extractiion Output Files</i>
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---

### Description

Reads intensity data from a set of one-color microarray image analysis output files.

### Usage

```
read.mir(files = NULL, path = NULL, ext = NULL, annotation=NULL, names = NULL, columns = list(E="gMedianS
other.columns = NULL, read.bg = TRUE, wt.fun = NULL, verbose = TRUE, sep = "\t", quote = "",
remove.ctrl=TRUE, ...)
```

### Arguments

files	data frame with column FileName giving the names of the files read, with column Sample giving the names of the samples.
path	character string giving the directory containing the files. The default is the current working directory.
ext	character string giving optional extension to be added to each file name
annotation	character vector of names of columns containing annotation information about the probes
names	character vector of names to be associated with each array as column name. Defaults to removeExt(files).
columns	character vector of names of the required columns.
other.columns	character vector of names of additional required columns to be read.
read.bg	logical, TRUE to indicate background corrected values are read.
wt.fun	function to calculate spot quality weights
verbose	logical, TRUE to report each time a file is read
sep	the field separator character. Values on each line of the file are separated by this character.
quote	character string of characters to be treated as quote marks
remove.ctrl	logical, if TRUE control probes will not be read
...	any other arguments are passed to read.table

### Details

This is the main data input function for the LVSmRNA package for one-color microRNA data. It extracts the green channel intensities from a series of files, produced by Agilent Feature Extractiion software, and assembles them into the components of one list. Data from some other image analysis programs can be read if the appropriate column names containing the intensities are specified

using the `columns` argument. (This will work if the column names are unique and if there are no incomplete rows in the file after the last line of data. Header lines are ok, if appropriately skipped.)

The argument `files` should be a matrix with two columns at least. One column should contain the names of the samples and the other column should contain names of files containing intensity data.

The argument `other.columns` allows arbitrary columns of the image analysis output files to be reserved in the data object. These become matrices in the 'other' component.

### Value

An EList object.

<code>G</code>	matrix containing the intensities for each array with probes as rows and arrays as columns.
<code>Gb</code>	matrix containing the background intensities for each array with probes as rows and arrays as columns.
<code>targets</code>	data frame with column <code>FileName</code> giving the names of the files read, with column <code>Sample</code> giving the names of the sample.
<code>genes</code>	data frame containing annotation information about the probes, for examples miRNA names and IDs and positions on the array.
<code>source</code>	character string giving the image analysis program name.
<code>preprocessing</code>	list with components <code>Background</code> , <code>Normalization</code> , <code>is.log</code> , <code>Summarization</code> indicate which pre-processing step has been done.

### Note

All image analysis files being read are assumed to contain data for the same genelist in the same order. No checking is done to confirm that this is true. Probe annotation information is read from one of the files only.

### Author(s)

Stefano Calza <stefano.calza@biostatistics.it>, Suo Chen and Yudi Pawitan.

### See Also

`read.mir` is based on "[read.table](#)" in the base package and modified from "[read.maimages](#)" in the [limma](#) package.

### Examples

```
# Read all intensity files from current working directory
## Not run:
dir.files <- system.file("extdata", package="LVSmirna")
taqman.data <- read.table(file.path(dir.files, "Comparison_Array.txt"), header=TRUE, as.is=TRUE)
MIR <- read.mir(taqman.data)

## End(Not run)
```

**Description**

Fit a linear model by robust regression using the Huber estimator.

**Usage**

```
RLM(formula, maxit=20, k=1.345, data, model=TRUE, na.action,
method=c("joint", "rlm"), x=TRUE, y=TRUE,
offset, cov.formula=c("weighted", "asymptotic"), start=NULL, ...)
```

**Arguments**

formula	a formula of the form $y \sim x_1 + x_2 + \dots$
maxit	the limit on the number of IWLS iterations.
k	tuning constant used for Huber proposal 2 scale estimation.
data	data frame from which variables specified in formula are preferentially to be taken.
model	should the model frame be returned in the object?
na.action	A function to specify the action to be taken if NAs are found. The 'factory-fresh' default action in R is <code>na.omit</code> , and can be changed by <a href="#">options</a> .
method	currently, <code>method="rlm"</code> and <code>"joint"</code> are supported.
x	should the model frame be returned in the object?
y	should the model matrix be returned in the object?
offset	numeric of length n. This can be used to specify an a priori known component to be included in the linear predictor during fitting.
cov.formula	are the methods to compute covariance matrix, currently either <code>weighted</code> or <code>asymptotic</code> .
start	vector containing starting values for the parameters in the predictor.
...	...

**Details**

Fitting is done by iterated re-weighted least squares (IWLS). This customized version of robust linear model deal with wild outliers using log link in joint modelling heterogeneous variance of covariates.

**Value**

An object of class "RLM" inheriting from "lm".

**Author(s)**

Stefano Calza <stefano.calza@biostatistics.it>, Suo Chen and Yudi Pawitan.

**References**

Pawitan, Y. 'In All Likelihood: Statistical Modeling and Inference Using Likelihood', (2001, Oxford University Press); Huber, P. J. , Robust Statistics, (1981. Wiley).

**See Also**

RLM is modified from "[rlm](#)" in the MASS, "[rlmFit](#)"

**Examples**

```
set.seed(133)
n <- 9
p <- 3
X <- matrix(rnorm(n * p), n,p)
y <- rnorm(n)

fit <- RLM(y~X-1) #no intercept
```

---

 rlmFit

---

*Fitter Functions for Robust Linear Models*


---

**Description**

These are the basic computing engines called by [RLM](#) used to fit robust linear models. These should not be used directly unless by experienced users.

**Usage**

```
rlmFit(x, y, maxit=20L, k=1.345, offset=NULL,method=c("joint","rlm"),
cov.formula=c("weighted","asymptotic"),start=NULL, error.limit=0.01)
```

**Arguments**

x	design matrix of dimension n * p.
y	vector of observations of length n, or a matrix with n rows.
maxit	the limit on the number of IWLS iterations.
k	tuning constant used for Huber proposal 2 scale estimation.
offset	numeric of length n. This can be used to specify an a priori known component to be included in the linear predictor during fitting.
method	currently, only method="rlm.fit" is supported.

cov.formula are the methods to compute covariance matrix, currently either weighted or asymptotic.

start vector containing starting values for the parameter estimates.

error.limit the convergence criteria during iterative estimation.

**Value**

a list with components

coefficients p vector

Std.Error p vector

t.value p vector

cov.matrix matrix of dimension p\*p

res.SD value of residual standard deviation

...

**Author(s)**

Stefano Calza <stefano.calza@biostatistics.it>, Suo Chen and Yudi Pawitan.

**References**

Yudi Pawitan: In All Likelihood: Statistical modeling and inference using likelihood. Oxford University Press. 2001.

**See Also**

[RLM](#) which you should use for robust linear regression usually.

**Examples**

```
set.seed(133)
n <- 9
p <- 3
X <- matrix(rnorm(n * p), n,p) #no intercept
y <- rnorm(n)

RLM.fit <- rlmFit (x=X, y=y)
```

---

sampleNames-methods    *Methods for Function sampleNames*

---

### Description

Methods for function sampleNames for objects of class EList and RGList

### Methods

signature(x = "EList") sampleNames for EList object  
signature(x = "RGList") sampleNames for RGList object

---

summarize                    *LVSmiRNA Summarization Function(s) for microRNA Microarray*

---

### Description

Summarize microRNA microarray data objects.

### Usage

```
summarize(object, ...)
## S3 method for class 'EList'
summarize(object,RA,remove.ctrl=FALSE,is.log=!is.null(object$preprocessing$Normalization),
method=c("rlm","medianpolish","mean"),verbose=FALSE,make.exprs=FALSE,...)
## S3 method for class 'RGList'
summarize(object,RA,remove.ctrl=FALSE,is.log=!is.null(object$preprocessing$Normalization),
method=c("rlm","medianpolish","mean"),verbose=FALSE,make.exprs=FALSE,...)
```

### Arguments

object	an object for which a summary is desired.
RA	an object from estVC.
remove.ctrl	logical, indicating whether to remove control probes.
is.log	Are data already logged?
method	currently, method "medianpolish","mean" and "rlm" are supported.
verbose	More output
make.exprs	Should the output be and exprSet object?
...	...

### Details

For multi-probe, multi-replicate microarray, intensities need to be summarized into a single expression value for each miRNA. The data objects are summarized as if they were lists.



**Value**

An Elist object containing components as follows:

G	matrix containing the summarized intensities for each array with miRNAs as rows and arrays as columns.
Gb	matrix containing the background intensities for each array with probes as rows and arrays as columns.
targets	data frame with column <code>FileName</code> giving the names of the files read, with column <code>Sample</code> giving the names of the sample.
genes	data frame containing annotation information about the probes, for examples gene names and IDs and positions on the array.
source	character string giving the image analysis program name.
preprocessing	list with components <code>Background</code> , <code>Normalization</code> , <code>is.log</code> , <code>Summarization</code> indicate which pre-processing step has been done.

**Author(s)**

Stefano Calza <stefano.calza@biostatistics.it>, Suo Chen and Yudi Pawitan.

**References**

Irizarry et al., 'Exploration, normalization, and summaries of high density oligonucleotide array probe level data', (2003a, Biostatistics); Huber, P. J., 'Robust estimation of a location parameter', (1964, Annals of Mathematical Statistics)

**See Also**

[lvs](#), [estVC](#)

**Examples**

```
## Not run:

data("MIR-spike-in")
AA <- estVC(MIR,method="joint")
dd <- summarize(MIR,RA=AA,method="rlm")

##summarization methods other than rlm, object RA is not required
dd1 <- summarize(MIR,method="medianpolish")
dd2 <- summarize(MIR,method="mean")

## End(Not run)
```

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