Package ‘LVSmiRNA’

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Methods

Methods for function boxplot for objects of class EList and RGList

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

Robust Linear Model to Estimate Residual Variance and Array Effect

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)

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


See Also

read.mir, lvs

Examples

```r
## Not run:

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

# Parellel 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)
```
## exprs-methods

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`

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 microRNAs, 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, clName,
    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, clName, 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, clName,
    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 FileName giving the names of the files read, with column Sample giving the names of the sample.

genes

data frame containing annotation information about the probes, for example miRNA names and IDs and positions on the array.

source

character string giving the image analysis program name.

preprocessing

list with components Background, Normalization, is.log, Summarization 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).
### MIR-spike-in

#### See Also

estVC, summarize

#### Examples

```r
## 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)
```

---

### Description

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

### Usage

```r
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$SystematicName`: `miRNA Name`
- `MIR$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

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

See Also

read.mir, estVC

plot-method

Plot of Residual Variance and Array Effect

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


See Also

estVC,rq
## Not run:

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

## End(Not run)

### 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`

### 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`

### 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  

Read in miRNA Data from Agilent Feature Extraction Output Files

Description

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

Usage

read.mir(files=NULL, source="agilent.median", path=NULL, ext=NULL, names=NULL, columns=NULL, other.columns=NULL, annotation=NULL, green.only=TRUE, wt.fun=NULL, verbose=TRUE, sep="\t", quote=NULL, remove.ctrl=TRUE,...)

Arguments

files  character vector giving the names of the files containing image analysis output or, for Imagene data, a character matrix of names of files. Alternatively, it can be a data.frame containing a column called FileName. If omitted, then all files with extension ext in the specified directory will be read in alphabetical order.

source  character string specifying the image analysis program which produced the output files. Choices are "agilent.median", "agilent.mean".

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

names  character vector of names to be associated with each array as column name. Defaults to removeExt(files).

columns  list, or named character vector. For two color data, this should have fields R, G, Rb and Gb giving the column names to be used for red and green foreground and background or, in the case of Imagene data, a list with fields f and b. For single channel data, the fields are usually E and Eb. This argument is optional if source is specified, otherwise it is required.

other.columns  character vector of names of other columns to be read containing spot-specific information

annotation  character vector of names of columns containing annotation information about the probes

green.only  logical, for use with source, should the green (Cy3) channel only be read, or are both red and green required?. Standard Agilent MIR data have only one channel so defaults to TRUE.

wt.fun  function to calculate spot quality weights

verbose  logical, TRUE to report each time a file is read

sep  the field separator 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 LVSmiRNA package for one-color microRNA data. It was originally designed to extract the green channel intensities from a series of files, produced by Agilent Feature Extraction 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 function is a simple wrapper for "read.maimages" in limma package so it shares all its features (though right now the input source is restricted to agilent type file).

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.

G
matrix containing the intensities for each array with probes 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 FileName giving the names of the files read, with column Sample 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 Background, Normalization, is.log, Summarization 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@unibs.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)

---

**RLM**

Robust Fitting of Linear Models

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 ~ x1 + x2 + ...
- `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 `na.omit`, and can be changed by `options`.
- `method`: currently, method="rlm" and "joint" 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 weighted or asymptotic.
- `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

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

coeffecients 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


See Also

RLM which you should use for robust linear regression usually.

Examples

```r
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)
```

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 FileName giving the names of the files read, with column Sample 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 Background, Normalization, is.log, Summarization 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, Annuas of Mathematical Statistics)

**See Also**

`lvs`, `estVC`

**Examples**

```r
## Not run:
data("MIR-spike-in")
AA <- estVC(MIR, method="joint")
dd <- summarize(MIR, RA=AA, method="rlm")

## sumarization 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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