CALIB

October 5, 2010

ParameterList-class

Class "ParameterList" - List to store all the parameters

Description

A simple list-based class for storing all the model parameters of a batch of microarray.

Objects from the Class

Objects can be created by calls of the form new("ParameterList", parameter) where parameter is a list. In the CALIB package, ParameterList objects are normally generated by function estimateParameter.

List Components

Objects should contain the following list components:

MuS: Spot parameter. Mean of spot capacity.

Ka: Hybridization constant.

P1: Respective slope of the linear dye saturation function. It is different for different dyes.

P2: Respective intercept of the linear dye saturation function. It is different for different dyes

SigmaA: Standard deviation of additive error. It is different for different dyes.

SigmaM: Standard deviation of multiplicative error.

SigmaS: Spot parameter. Standard deviation of spot capacity.

SpotError: numeric matrix containing spot error of all external control spikes on arrays

Method: boolean values indicating the way to calculate the measured intensities. It contains two subfields which are both logical value: BC and Area. BC indicates whether background corrected measured intensities are used. Area indicates whether spot area is used to calculate measured intensities.

ErrorModel: a character to represent the distribution of spot capacity. "L" means spot capacity follows log normal distribution. "N" means spot capacity follows normal distribution.

genes: data.frame containing information on spikes spotted on the arrays. Should include a character column Name containing names for all the spikes.

See reference for more detailed explaination for these list components.

RG

Extends

```
Class "list", from data part. Class "vector", by class "list".
```

Methods

This class inherits directly from class List. However since it represents the parameters of the calibration model for arrays, it makes on sense to run functions like dim, dimnames, or merge on this class.

Therefore, only some operations appropriate for list will work on objects of this class. ParameterList objects can be cbind and show in a compact way.

ParameterList objects are used on functions such as normalizeData or on some other data visualization functions like plotSpikeHI in the CALIB package.

Author(s)

Hui Zhao

References

Engelen, K., Naudts, B., DeMoor, B., Marchal, K. (2006) A calibration method for estimating absolute expression levels from microarray data. Bioinformatics 22: 1251-1258.

RG

Experiment Data: RGList_CALIB Example

Description

This RGList_CALIB object represents microarray data of two arrays.

It is generated by function read.rg from raw data files.

Usage

data(RG)

Format

RG is an RGList_CALIB object containing the following list components: \\$R,\\$G,\\$Rb,\\$Gb, \\$RArea,\\$GArea,\\$GArea,\\$source and \\$genes. It represents two microarrays and 19749 clones.

Source

This comes from a publicly availbe dataset, consisting 14 hybridizations. From this RG, two out of these fourteen are chosen. The experiment design of these two are color flip. Except for cDNA probes, external control spikes are also spotted on the arrays. There are 192 ratio controls, 480 calibration controls,24 negative controls and 72 utility controls.

For more information, see the reference.

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References

Engelen, K., Naudts, B., DeMoor, B., Marchal, K. (2006) A calibration method for estimating absolute expression levels from microarray data. Bioinformatics 22: 1251-1258.

Hilson,P,et al. (2004) Versatile gene-specific sequence tags for Arabidopsis functional genomics: transcript profiling and reverse genetics applications. Genome Res. 14, 2176-2189.

Examples

data (RG)

RGList CALIB-class Red, Green Intensity List - Class

Description

A simple list-based class for storing red and green channel foreground and background intensities for a batch of spotted microarrays. It is a extension of the RGList in the LIMMA package.

Objects from the Class

Objects can be created by calls of the form new ("RGList_CALIB", RG), where RG is a list. In the CALIB package, RGList_CALIB objects are normally generated by read.rg.

List Components

objects should contain the following list components:

R: numeric matrix containing the red(Cy5) foreground intensities. Rows correspond to spots and columns to arrays.

G: numeric matrix containing the green(Cy3) foreground intensities.

Rb: numeric matrix containing the red(Cy5) background intensities.

Gb: numeric matrix containing the green(Cy3) background intensities.

RArea: numeric matrix containing the red(Cy5) spot areas.

GArea: numeric matrix containing the green(Cy3) spot areas.

Optional components include:

weights: numeric matrix containing relative spot quality weights. Should be non-negative.

printer: list containing information on the process used to print the spots on the arrays. See read.rg.

genes: data.frame containing information on the genes spotted on the arrays. Should include a character column Name containing names for the genes or controls.

targets: data.frame containing information on the target RNA samples. Should include factor or character columns Cy3 and Cy5 specifying which RNA was hybridized to each array.

other: list containing numeric matrices of other spot-specific information.

All of the matrices should have the same dimensions. The row dimension of targets should match the column dimension of the matrices.

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Extends

Class "list", from data part. Class "LargeDataObject", directly. Class "vector", by class "list".

Methods

This class inherits directly from class List so any operation appropriate for lists will work on objects of this class. In addition, RGList_CALIB objects can be subsetted, combined and merge. RGList_CALIB objects will return dimensions and hence functions such as dim, dimnames, nrow and ncol are defined. RGList_CALIB also inherit a show method from the virtual class LagerDataObject, which means that RGList_CALIB objects will print in a compact way.

In the CALIB package, RGList_CALIB objects are mainly for storing microarray data and they are used to pass microarray data into functions such as <code>estimateParameter</code> or <code>normalizeData</code>.

Author(s)

Hui Zhao

References

RGList in the limma package

See Also

RGList and LargeDataObject in limma packge.

SpikeList-class

Class "SpikeList" - Spike Intensity and Concentration List

Description

A simple list-based class for storing red and green channel foreground and background intensities, spot area and concentrations for external control spike on spotted microarray.

Objects from the Class

Objects can be created by calls of the form new("SpikeList", spike) where spike is a list. In the CALIB package, SpikeList objects are normally generated by function read.spike.

List Components

Objects should contain the following list components:

- R: numeric matrix containing the red(Cy5) foreground intensities of all external control spikes on arrays. Rows correspond to spikes and columns to arrays.
- G: numeric matrix containing the green(Cy3) foreground intensities of all external control spikes on arrays.
- Rb: numeric matrix containing the red(Cy5) background intensities of all external control spikes on arrays.
- Gb: numeric matrix containing the green(Cy3) background intensities of all external control spikes on arrays.

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RArea: numeric matrix containing the red(Cy5) spot areas of all external control spikes on arrays.

GArea: numeric matrix containing the green(Cy3) spot areas of all external control spikes on arrays.

RConc: numeric matrix containing the red(Cy5) known concentrations of all external control spikes on arrays.

GConc: numeric matrix containing the green(Cy3) know concentrations of all external control spikes on arrays.

genes: data.frame containing information on spikes spotted on the arrays. Should include a character column Name containing names for all the spikes.

All of the matrices should have the same dimensions.

Extends

```
Class "list", from data part. Class "vector", by class "list".
```

Methods

This class inherits directly from class List, so any operation appropriate for lists will work on objects of this class. In addition, SpikeList objects can be subsetted, combined and merged. SpikeList objects will return dimensions and hence functions such as dim, dimnames, nrow and ncol are defined. Generic method show is applied on SpikeList, so SpikeList will print in a compact way.

SpikeList objects are used on functions such as estimateParameter or on some other data visualization functions like plotSpikeHI in the CALIB package.

Author(s)

Hui Zhao

References

the limma package

See Also

```
RGList_CALIB.
RGList in limma package.
```

adjustP2

Adjust model parameter P2

Description

Adjust the calibration model parameter P2 according to the measured intensities of all clones spotted on the array.

Usage

```
adjustP2(RG, parameter, arrayindex = arrayindex, colorindex = colorindex)
```

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Arguments

RG an RGList object.

parameter a ParameterList object.

arrayindex integer vector specifying the index of the arrays of whose the parameter P2

needed to be adjusted.

colorindex integer vector specifying which color needed to be adjusted.

Details

RG is an RGList_CALIB object which contains all the experimental data. parameter is the return result of function estimateParameter. arrayindex is an integer vector. It gives the index of the arrays whose P2 is needed to be adjusted. colorindex is an integer vector. It gives the color needed to be adjusted. 1 means red P2 and 2 means green P2.

Value

It returns a ParameterList object with a adjusted P2 compared to the input argument parameter.

Note

The user should decide on which array and which color the adjustment is needed. Therefore it is important to specify the right array index and color index. There is no check on this in the function.

Author(s)

Hui Zhao

Examples

```
# load data: RG and parameter:
data(RG)
data(parameter)
# adjust P2
parameter_new <- adjustP2(RG,parameter,arrayindex=c(1,2),colorindex=c(2,2))</pre>
```

calibReadMe View CALIB readme file

Description

Finds the location of the CALIB readme file and optionally opens it.

Usage

```
calibReadMe(view = TRUE)
```

Arguments

view logical, TRUE means open the readme file and FALSE means finds out the loca-

tion of the file only.

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Details

The function vignette ("limma") will find the short CALIB vignette which describes how to obtain the CALIB readme file. The readme file is not a true vignette because it is not automatically generated using 'Sweave' during the package build process. This means that it cannot be found using vignette, hence the need for this special function.

If the operating system is other than Windows, then the PDF viewer used is the one given by Sys.getenv("R_PDFVIEWER"). The PDF viewer can be changed using Sys.putenv("R_PDFVIEWER").

Value

Character string giving the file location.

Author(s)

Hui Zhao

References

limmaUsersGuide in the limma package

Examples

```
calibReadMe(view=FALSE)
```

cbind

Combine RGList_CALIB, SpikeList or ParameterList objects

Description

Combine a series of RGList_CALIB objects or a series of SpikeList objects or a series of ParameterList objects.

Usage

```
## S3 method for class 'RGList\_CALIB':
cbind(..., deparse.level = 1)
## S3 method for class 'RGList\_CALIB':
rbind(..., deparse.level = 1)
```

Arguments

```
... RGList_CALIB objects, SpikeList objects or ParameterList objects deparse.level see cbind in base package.
```

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Details

cbind combines data objects assuming the same gene lists but different arrays. rbind combines data objects assuming equivalent arrays, i.e., the same RNA targets, but different genes.

For ParameterList objects, only chind is available, because it makes no sense to rhind parameter.

For RGList_CALIB objects and SpikeList objects, cbind and rbind are both available. For cbind, the matrices of expression data from the individual objects are cbinded. The data frames of target information, if they exist, are rbinded. The combined data object will preserve any additional components or attributes found in the first object to be combined. For rbind, the matrices of expression data are rbinded while the target information, in any, is unchanged.

Value

```
An RGList_CAILB, a SpikeList or
```

a ParameterList object holding data from all the arrays and all genes from the individual objects.

Author(s)

Hui Zhao

References

cbind in limma package

See Also

```
cbind in the base package cbind in the limma package
```

Examples

```
R1 <- G1 <- matrix(1:8,4,2)
rownames(R1) <- rownames(G1) <- c("g1", "g2", "g3", "g4")
colnames(R1) <- colnames(G1) <- c("a1", "a2")
RG1 <- new("RGList_CALIB", list(R=R1, G=G1))

R2 <- G2 <- matrix(9:16,4,2)
rownames(R2) <- rownames(G2) <- c("g1", "g2", "g3", "g4")
colnames(R2) <- colnames(G2) <- c("a3", "a4")
RG2 <- new("RGList_CALIB", list(R=R2, G=G2))

RG <- cbind(RG1, RG2)
```

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dim

Retrieve the Dimensions of an RGList_CALIB or SpikeList object

Description

Retrieve the number of rows (genes) and columns (arrays) for an RGList_CALIB or SpikeList

Usage

```
## S3 method for class 'RGList\_CALIB':
dim(x)
## S3 method for class 'RGList\_CALIB':
length(x)
```

Arguments

Х

an object of class RGList_CALIB or SpikeList.

Details

Microarray data objects share many analogies with ordinary matrices in which the rows correspond to spots or genes and the columns to arrays. These methods allow one to extract the size of microarray data objects in the same way that one would do for ordinary matrices.

A consequence is that row and column commands nrow(x), ncol(x) and so on also work.

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)

Hui Zhao

References

dim in limma package

See Also

```
dim in the base package
dim in the limma package
```

Examples

```
# for RGList_CALIB
R <- G <- matrix(1:8,4,2)
rownames(R) <- rownames(G) <- c("g1","g2","g3","g4")
colnames(R) <- colnames(G) <- c("a1","a2")
RG <- new("RGList_CALIB",list(R=R,G=G))</pre>
```

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```
dim(RG)

# for SpikeList
SR <- SG <- matrix(1:8,4,2)
rownames(SR) <- rownames(SG) <- c("s1","s2","s3","s4")
colnames(SR) <- colnames(SG) <- c("a1","a2")
spike <- new("SpikeList",list(R=SR,G=SG))
dim(spike)</pre>
```

dimnames

Retrieve the Dimension Names of an RGList_CALIB or SpikeList object.

Description

Retrieve the the dimension names of an RGList_CALIB object or an SpikeList object

Usage

```
## S3 method for class 'RGList\_CALIB':
dimnames(x)
```

Arguments

v

an object of class RGList_CALIB or SpikeList.

Details

The dimension names of a microarry object or a spike object are the same as those of the most important matrix component of that object.

A consequence is that row and column command rownames and colnames also work.

Value

Either NULL or a list of length 2. If the value is a list, its componets are either NULL or a character vector with the length of the appropriate dimension of x. If the list component is not NULL, the first field of the list indicates rownames and the second field indicates colnames.

Author(s)

Hui Zhao

References

dimnames in limma package

See Also

```
dimnames in the base package dimnames in the limma package
```

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Examples

```
# for RGList_CALIB
R <- G <- matrix(1:8,4,2)
rownames(R) <- rownames(G) <- c("g1", "g2", "g3", "g4")
colnames(R) <- colnames(G) <- c("a1", "a2")
RG <- new("RGList_CALIB", list(R=R,G=G))
dimnames(RG)
# for SpikeList
SR <- SG <- matrix(1:8,4,2)
rownames(SR) <- rownames(SG) <- c("s1", "s2", "s3", "s4")
colnames(SR) <- colnames(SG) <- c("a1", "a2")
spike <- new("SpikeList", list(R=SR,G=SG))
dimnames(spike)</pre>
```

estimateParameter Estimate model parameter from spikes

a SpikeList object.

Description

Estimate the calibration model parameters according to the known concentration and the measured intensities of external control spikes on each array.

Usage

```
estimateParameter(spike, RG, bc = FALSE, area = TRUE, errormodel = "M")
```

Arguments

spike

ppike	u opinelise object.
RG	a RGList_CALIB object.
bc	a logical value. ${\tt TRUE}$ means background corrected measured intensities are used. Default is ${\tt FALSE}.$
area	a logical value. TRUE means spot area is used to calculate measured intensities. Namly, measured intensities are calculated by foreground intensities(or background corrected intensities, if bc is ${\tt TRUE}$) multiply spot area. ${\tt FALSE}$ means spot area is not used. Default is ${\tt TRUE}$.
errormodel	a character to indicate the distribution of spot capacity. "A" means spot capacity is additive. "M" means spot capacity is multiplicative. Default is "M".

Details

This function estimates calibration model parameters. In this function, the model parameters are estimated separately for each microarray, based on the measured intensities of the external control spikes and their known concentration in the hybridization solution. It accepts spike measured intensities and concentration from spike argument, which is an object of SpikeList class.

It supports different ways to calculate the measured intensities. Arguments bc and area are logical and their combinations is used for specifying four differents ways. bc indicates using background

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correction or not. area indicates multiplying spot area or not. The default value of these two arguments are bc = FALSE and area = TRUE.

The argument errormodel is to specify the distribution of spot capacity of each array. The spot capacity is either additive or multiplicative. Whichever distribution is more appropriate will depend largely on the type of microarray slide and spotting procedure used. The spot parameters mus and sigmas can be considered equal for all measurements of a single array.

The argument RG is for calculating the maximum intensity of each array. These maximum intensities are used to estimate the upper saturation level of each array.

More details please refer to the reference literature.

Value

An ParameterList object containing the components:

MuS	matrix containing MuS for each array.
Ka	matrix containing Ka for each array.
P1	matrix containing P1 of each dye for each array.
P2	matrix containing P2 of each dye for each array.
SigmaA	matrix containing sigma additive for each array.
SigmaM	matrix containing sigma multiplicative for each array.
SigmaS	matrix containing sigma spoterror for each array.
SpotError	matrix containing the spot error of each spot for each array.
Method	boolean values indicating the way to calculate the measured intensities.
ErrorModel	character "M" or "A" to indicate the type of spot capacity distribution.

Author(s)

Hui Zhao

References

Engelen, K., Naudts, B., DeMoor, B., Marchal, K. (2006) A calibration method for estimating absolute expression levels from microarray data. Bioinformatics 22: 1251-1258.

Examples

```
# load data: RG and spike
data(RG)
data(spike)

# for the measured itensities, take the default bc=FALSE and area=TRUE.
# use multiplicative spot error model
parameter <- estimateParameter(spike, RG)</pre>
```

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getColClasses

Construct colClasses vector for use within read.rg function

Description

Construct a colClasses vector, an argument in read.table used in read.rg

Usage

```
getColClasses(cols,...)
```

Arguments

a character vector of all columns to search against
 accepts any character lists, character vectors or weight functions to match wanted columns against cols

Details

This is an internally called function by read.rg to create a colClasses vector used in read.table for fast loading of only required columns in read.rg

Value

Character. A named vector of classes to assumed for the columns. Possible values are NA (when type.convert is used), NULL (when the column is skipped).

Author(s)

Hui Zhao

References

getColClasses in limma package

Examples

```
allcnames <- c("Block","Column","Row","Name","ID", "F635 Mean","F532 Mean","Flag","Autof]
Annotation <- c("Block","Column")
Columns <- list(R="F635 Mean",G="F532 Mean")
getColClasses(allcnames, Annotation, Columns)</pre>
```

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merge

Merge RGList_CALIB or SpikeList objects

Description

Merge two RGList_CALIB objects or two SpikeList objects in possibly irregular order.

Usage

```
## S3 method for class 'RGList\_CALIB':
merge(x,y,...)
```

Arguments

```
x an RGList_CALIB object or an SpikeList object.
```

y corresponding RGList_CALIB object or SpikeList object. Has the same genes or spikes as x, possibly in a different order, but with different arrays.

.. other arguments can be used in merge in the base packge.

Details

```
RGList_CALIB and SpikeList
```

objects are list objects containing numeric matrices with the same dimensions. The RGLists_CALIB or SpikeLists are merged by merging each of the components by row names or, if there are no row names, by IDs in the genes component. Unlike when using cbind, row names are not required to be in the same order or to be unique. In the case of repeated row names, the order of the rows with repeated names in preserved. This means that the first occurrence of each name in x is matched with the first occurrence of the same name in y. The final vector of row names is the same as in x.

Value

An merged object of the same class as x and y with the same components as x. Components matrices have the same row names as in x but columns from y as well as x.

Note

If the RGList_CALIB or SpikeList objects contain the same number of genes or spikes in the same order then the appropriate function to combine them is cbind rather than merge.

Author(s)

Hui Zhao

References

```
merge in limma package
```

See Also

```
merge in the base package merge in the limma package
```

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Examples

```
R1 <- G1 <- matrix(1:8,4,2)
rownames(R1) <- rownames(G1) <- c("g1","g1","g2","g3")
colnames(R1) <- colnames(G1) <- c("a1","a2")
RG1 <- new("RGList_CALIB",list(R=R1,G=G1))

R2 <- G2 <- matrix(9:16,4,2)
rownames(R2) <- rownames(G2) <- c("g2","g3","g1","g1")
colnames(R2) <- colnames(G2) <- c("a3","a4")
RG2 <- new("RGList_CALIB",list(R=R2,G=G2))

RG12 <- merge(RG1,RG2)
RG21 <- merge(RG2,RG1)
```

normalizeData

Normalization: estimation of absolute expression levels

Description

estimates absolute expression levels for each combination of a gene and a tested biological condition.

Usage

Arguments

RG	an RGList_CALIB object
parameter	a ParameterList object
array	integer vector specifying the index of the arrays. Has length equal to two times of the number of arrays.
condition	integer vector specifying the index of the conditions. Has length equal to two times of the number of arrays.
dye	integer vector specifying the index of the dyes. Has length equal to two times of the number of arrays.
cloneid	string vector specifying the clone ids of the clones to be normalized. If missing, normalize all the clones.
idcol	string specifying the column name of clone ids in the genes field of RG.

Details

This function estimates absolute expression levels for each combination of a gene and a tested biological condition from the measured intensity. It accepts measured intensities from RG.

The argument parameter is an object of ParameterList. The function accepts model parameters from this argument.

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By using this function, for each combination of a gene and a tested biological condition, a single absolute expression level fo target is estimated. Therefore, specifying the design of experiment is necessary. Namely, the design of array, condition and dye is needed. The three arguments array, condition and dye are three numeric vector to indicate the design of array, condition and dye respectively. How to specify these three arguments refer to the example below.

The function is able to not only estimate all the genes on the slides but also estimate any gene on the slides seperately. The argument cloneid accepts the clone ids of which the genes are interested by the user. If cloneid argument is missing, the function will estimate all the genes on the slides. In order to match clone id in the RG, column name which indicates clone ids in RG\\$genes should be specified by argument idcol.

Value

a numeric matix containing the absolute expression levels. Columns indicate different conditions and rows indicate different genes.

Warning

The function doesn't allow missing clone id. So please check before run the function.

Note

The main calculation part in this function is done by c++ code.

Author(s)

Hui Zhao

References

Engelen, K., Naudts, B., DeMoor, B., Marchal, K. (2006) A calibration method for estimating absolute expression levels from microarray data. Bioinformatics 22: 1251-1258.

Examples

```
# load data: RG and parameter
data(RG)
data(parameter)

# define design matrix: two arrays, two condition and color-flip design
array <- c(1,1,2,2)
condition <- c(1,2,2,1)
dye <- c(1,2,1,2)

# specify clone-id column
idcol <- "CLONE_ID"

#data <- normalizeData(RG,parameter,array=array,condition=condition,dye=dye,idcol=idcol)

## only normalize a group of genes
cloneid_interested <- c("250001", "250002", "250003", "250004", "250005")
data <- normalizeData(RG,parameter,array=array,condition=condition,dye=dye,cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=cloneid=c
```

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normdata

Example of normalized data

Description

Normalized data of data set RG. It is generated by function normalizeData in the CALIB package by using calibration model parameter parameter.

Usage

```
data (normdata)
```

Format

This is a numeric matrix. Row corresponds to unique clone on the arrays and column corresponds to the two different conditions.

References

```
dataset RG.
dataset parameter.
```

Engelen, K., Naudts, B., DeMoor, B., Marchal, K. (2006) A calibration method for estimating absolute expression levels from microarray data. Bioinformatics 22: 1251-1258.

Hilson,P.,et al. (2004) Versatile gene-specific sequence tags for Arabidopsis functional genomics: transcript profiling and reverse genetics applications. Genome Res. 14, 2176-2189.

Examples

```
data(normdata)
plotNormalizedData(normdata,condition = c(1,2))
```

parameter

Calibration Model parameter: ParameterList Example

Description

```
This ParameterList object represents calibration model parameters of two arrays. It was estimated from the dataset spike by the function <code>estimateParameter</code> in the CALIB package.
```

Usage

```
data(parameter)
```

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Format

parameter is a ParameterList object containing the following list components:\MuS,\R, \\$P1,\P2,\SigmaA,\SigmaM,\SigmaS,\SpotError,\Method with two subfields \Method\BC and \Method\Area, \ErrorModel and \genes. Among these parameters,P1,P2 and SigamA are different for different dyes.

References

dataset spike.

Engelen, K., Naudts, B., DeMoor, B., Marchal, K. (2006) A calibration method for estimating absolute expression levels from microarray data. Bioinformatics 22: 1251-1258.

Hilson,P,et al. (2004) Versatile gene-specific sequence tags for Arabidopsis functional genomics: transcript profiling and reverse genetics applications. Genome Res. 14, 2176-2189.

Examples

```
data(parameter)
```

plotNormalizedData plot estimated absolute levels of two conditions

Description

plot estimated absolute levels of any two user-specified conditions. The values in the plot are in log scale.

Usage

Arguments

data	matrix containing estimated absolute levels. columns are conditions and rows are genes.
condition	integer vector giving the two conditions to be plotted.
xlab	a title for the x axis.
ylab	a title for the y axis.
main	an overall title for the plot.
xlim	the x limits (min,max) of the plot.
ylim	the y limits of the plot.
pch	an integer code for one of a set of plotting characters or symbols for the spike data set. Default is 19.
cex	a numerical value giving the amount by which points should be scaled relative to the default. Default is 0.2.
col	the color of the points. Default is black.

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```
diag a logical value. Add diagonal on the plot if it is TRUE. Default is TRUE.

diagcol the color of the diagonal. Default is blue.

diaglwd the width of the diagonal. Default is 1.5.

other graphical parameters can be used in function plot.
```

Details

The function polts estimated absolute expression levels of two conditions. It accepts expression levels from the argument 'data', which should have the same data format as the output value of the function normalizeData.

The two conditions to be plotted should be specified by the argument condition. The condition should be a numeric vector with length two and it should be subset of condition vector of the design matrix. see function NormalizeData.

see other graphic functions for the other arguments.

Value

A plot is created on the current graphics device.

Author(s)

Hui Zhao

Examples

```
# load data: normalized data
data(normdata)

# specify the two conditions to be plotted.
cond <- c(1,2)

# use the default values for other parameters.
plotNormalizedData(normdata,condition = cond)</pre>
```

plotSpikeCI

plot spike concentration vs measured intensity

Description

plot spike known concentration and measured intensity of one array.

Usage

```
plotSpikeCI(spike, parameter,array = 1, bc = FALSE, area = TRUE,
    meanpoint = TRUE,xlab = "log(Concentration)",
    ylab = "log(Intensity)", main = colnames(spike$R)[array],
    onlycalib = TRUE, xlim = NULL, ylim = NULL, pch = 19,
    cex = 0.2, meanpch = 21, meancex = 1, lwd = 1.5,
    cy5col = "red", cy3col = "green", ...)
```

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Arguments

spike a SpikeList object.
parameter a ParameterList object.

array integer giving the array to be plotted.

bc a logical value. TRUE means background corrected measured intensities are

used. Default is FALSE.

area a logical value. TRUE means spot area is used to calculate measured intensities.

Namly, measured intensities are calculated by foreground intensities(or background corrected intensities, if bc is TRUE) multiply spot area. FALSE means

spot area is not used. Default is TRUE.

meanpoint a logical value. TRUE is to show meanpoint of measured intensities with the

same concentration on the plot. FALSE means not show.

xlab a title for the x axis. ylab a title for the y axis.

main an overall title for the plot.

onlycalib a logical value. TRUE means only the calibration controls are on the plot.

FALSE means to plot all the spikes

xlim the x limits (min,max) of the plot.

ylim the y limits of the plot.

pch a integer code for one of plotting characters or symbols for the spike data set.

Default is 21.

cex a numerical value giving the amount by which the points which indicate spike

data set should be scaled relative to the default. Default is 0.4.

meanpch a integer code for one of plotting characters or symbols for the meanpoints.

Default is 21.

meancex a numerical value giving the amount by which the meanpoints should be scaled

relative to the default value. Default is 1.

lwd width of the model curves. Default is 1.5.cy5col color of all symbols for cy5. Default is red.cy3col color of all symbols for cy3. Default is green.

... other graphical parameters can be used in function plot.

Details

The function plots spike concentration and measured intensity of one array, array number is specified by the argument <code>array</code>. It accepts the concentration of given array from the agrument <code>spike</code>, which is a <code>SpikeList</code> object. The measured intensities are calculated from <code>spike</code>. Four different ways can be used to calculate the measured intensities. Arguments <code>bc</code> and <code>area</code> are logical and their combinations are used for specifying the four differents ways. <code>bc</code> indicates using background correction or not. <code>area</code> indicates multipling spot area or not. The default value of these two arguments are <code>bc = FALSE</code> and <code>area = TRUE</code>.

In order to help data visualization, meanpoints and model curve can be added on the plot. And the arguments meanpoint and parameter are correspond to these. The meadians of every group of measured intensities which have the same concentration are shown on the polt if meanpoint is true. Model curves of both dye are shown if the argument parameter is specified after parameter estimation.

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Value

A plot is created on the current graphics device.

Author(s)

Hui Zhao

See Also

```
see graphic functions plot, par
```

Examples

```
# load data: spike
data(spike)

# specify the array to be plotted.
array <- 1

# use the default values for other parameters.
plotSpikeCI(spike, array=array)

# after parameter estimation, the model curves can be shown on the plot.
data(parameter)
plotSpikeCI(spike, parameter, array=array)</pre>
```

plotSpikeHI

plot hybridized target vs intensity

Description

With final parameter setting, plot the amount of hybridized targets and intensities of calibration controls.

Usage

```
plotSpikeHI(spike, parameter, array = 1, xlab = "log(Hybridized)",
    ylab = "log(Intensity)", main = colnames(spike$R)[array],
    xlim = NULL, ylim = NULL, pch = 19, cex = 0.2,
    cy5col = "black", cy3col = "black", noerror = TRUE,
    noepch = 19, noecex = 0.1, noecy5col = "lightpink",
    noecy3col = "lightblue", curve = TRUE, lwd = 1.5,
    curvecy5col = "red", curvecy3col = "green", ...)
```

Arguments

```
spike a SpikeList object.

parameter a ParameterList object.

array integer giving the array to be plotted.

xlab a title for the x axis.

ylab a title for the y axis.
```

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main	an overall title for the plot.
xlim	the x limits (min,max) of the plot.
ylim	the y limits of the plot.
pch	an integer code for one of plotting characters or symbols for the spike data set. Default is 19.
cex	a numerical value giving the amount by which the points which indicate spike data set should be scaled relative to the default. Default is 0.2.
cy5col	color of points for cy5. Default is black.
cy3col	color of points for cy3. Default is black.
noerror	a logical value. If it is TRUE, plot the amount of hybridized targets assuming equal spot capacities. Default is TRUE.
noepch	pch for the points with equal spot capacities. Default is 19.
noecex	cex for the points with equal spot capacities. Default is 0.1.
noecy5col	color for the points with equal spot capacities of cy5. Default is lightpink.
noecy3col	color for the points with equal spot capacities of cy3. Default is lightblue.
curve	a logical value. If it is TRUE, plot final parameter setting. Default is TRUE.
lwd	width of the parameter curves. Default is 1.5.
curvecy5col	color of the parameter curves for cy5. Default is red.
curvecy3col	color of the parameter curves for cy3. Default is green.
	other graphical parameters can be used in function 'plot'.

Details

The function plots hybridized targets vs measured intensities of one array. The argument <code>array</code> gives the array index to be plotted. The function accepts the spike concentrations from the argument <code>spike</code> and the estimated spot error for each spot from the argument <code>parameter</code>. The hyrbidized targets for each spot can be calculated by the following formula: formula.

The argument noerror says whether or not the hybridized targets, which are calculated by the above mentioned formula assuming equal spot capacities, are plotted. If they are plotted, other arguments like noepch,noecex,noecy5col and noecy3col are used to specify the type, the size and the color of the points.

Estimated parameter curves can be shown on the plot. Since model parameters are different for two colors, two parameter curves are expected for one array. The function accepts parameters of both colors from the argument parameter. If the curves are plotted, the arguments lwd, curvecy3col and curvecy5col are used to specify the width and color of the curves.

Details for the graphical parameters can be seen in function plot, points and curve.

Value

A plot is created on the current graphics device.

Author(s)

Hui Zhao

References

Engelen, K., Naudts, B., DeMoor, B., Marchal, K. (2006) A calibration method for estimating absolute expression levels from microarray data. Bioinformatics 22: 1251-1258.

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Examples

```
# load data: spike and parameter
data(spike)
data(parameter)

# specify the array to be plotted.
array <- 1

# use the default values for other parameters
plotSpikeHI(spike,parameter,array=array)</pre>
```

plotSpikeRG

plot spike intensity R vs G

Description

plot red intensity vs green intensity of spikes.

Usage

```
plotSpikeRG(spike,parameter,RG,array = 1, bc = FALSE, area = TRUE,
    xlab = "log(Rintensity)", ylab = "log(Gintensity)",
    main = colnames(spike$R)[array], onlycalib = FALSE,
    xlim = NULL, ylim = NULL, pch = 19, cex = 0.3, col = "black",
    allpch = 19, allcex = 0.05, allcol = "lightgrey", diag = TRUE,
    diagcol = "grey", diaglwd = 1, curvecol = "blue",
    curvelwd = 1.5, calibtype = 1, adjusttype = 4, ...)
```

Arguments

spike	a SpikeList object.
parameter	a ParameterList object
	If parameter argument is sepcified, model curves are shown on the plot.
RG	a RGList_CALIB object. If parameter argument is specified, this argument is obligated. More description in Detail section.
array	integer giving the array to be plotted.
bc	a logical value. TRUE means background corrected measured intensities are used. Default is ${\tt FALSE}$.
area	a logical value. TRUE means spot area is used to calculate measured intensities. Namly, measured intensities are calculated by foreground intensities(or background corrected intensities, if bc is TRUE) multiply spot area. FALSE means spot area is not used. Default is TRUE.
xlab	a title for the x axis.
ylab	a title for the y axis.
main	an overall title for the plot.
onlycalib	a logical value. TRUE means only the calibration controls are on the plot. FALSE means to plot all the spikes

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xlim	the x limits (min,max) of the plot.
ylim	the y limits (min,max) of the plot.
pch	an integer code for one of a set of plotting characters or symbols for the spike data set. Default is 21.
cex	a numerical value giving the amount by which the points which indicate spike data set should be scaled relative to the default. Default is 0.3.
col	the color of the points indicating spike data set. Default is black.
allpch	an integer code for one of a set of plotting characters or symbols for the spike data set. Default is 19.
allcex	a numerical value giving the amount by which the points which indicate all data set should be scaled relative to the default. Default is 0.05 .
allcol	the color of the points indicating all data set. Default is lightgrey.
diag	a logical value. Add diagonal on the plot if it is TRUE. Default is TRUE.
diagcol	the color of the diagonal. Default is grey.
diaglwd	the width of the diagonal. Default is 1.
curvecol	the color of the model curves. Default is blue.
curvelwd	the width of the model curves for calibration control spikes. Default is 1.5.
calibtype	the line type of the model curves for calibration control spikes. Default is 1.
adjusttype	the line type of the model curves (using parameter after adjustment) for calibration control spikes. Default is 4.
	other graphical parameters can be used in function plot.

Details

The function plots red vs green measured intensities of spikes of one array. The argument array gives the array index to be plotted.

If parameter estimation is done, the model curves can be plotted by giving the argument parameter. And if the argument parameter is specified, the argument RG is obligated. The combination of these two arguments is used to compare how the model fits to the spike and to the whole data set. More details about the usage of this function refers to the readme file of this package.

It supports different ways to calculate the measured intensities. Arguments bc and area are logical and their combinations are used for specifying four differents ways. bc indicates using background correction or not. area indicates multipling spot area or not. The default value of these two arguments are bc = FALSE and area = TRUE.

see other graphic functions for the other arguments.

Value

A plot is created on the current graphics device.

Author(s)

Hui Zhao

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Examples

```
# load data: spike
data(spike)

# specify the array to be plotted.
array <- 1

# use the default values for other arguments
plotSpikeRG(spike,array=array)

# after parameter estimation, the model curves can be shown on the plot.
data(parameter)
data(RG)
plotSpikeRG(spike,parameter,RG)</pre>
```

plotSpikeSpotError plot spot error of spikes.

Description

Plot spot error of spikes. Three types of plots are avaible: histogram, boxplot and density.

Usage

Arguments

parameter a ParameterList object.

array integer giving the array to be plotted.

plottype string giving the type of plot.

width needed for density plot. This exists for compatibility with S; if given, and bw is not, will set bw to width if this is a character string, or to a kernel-dependent multiple of width if this is numeric. Default is 1.

plotnames needed for boxplot. group labels which will be printed under each boxplot.

an overall title for the plot.

other parameters can be used according to the plottype user specified.

Details

The function plots spot error of one array on different types of plots. Three types, which are histogram, boxplot and density function, are available now. The argument plottype is used for giving the plot type. It should be one of the following three types: "hist", "boxplot" and "dens". The argument array gives the array index to be plotted.

The function accepts estimated spot error from the argument parameter.

Value

A plot is created on the current graphics device.

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Author(s)

Hui Zhao

See Also

```
hist, boxplot and plot in the graphics package. density in the stats package.
```

Examples

```
# load data: parameter
data(parameter)

# specify the array to be plotted.
array <- 1

# plot histogram
plotSpikeSpotError(parameter, array=array, plottype="hist")
# plot boxplot
plotSpikeSpotError(parameter, array=array, plottype="boxplot", plotnames=NULL)
# plot density function
plotSpikeSpotError(parameter, array=array, plottype="dens", width=1)</pre>
```

read.rg

Read RGList_CALIB from Image Analysis Output Files

Description

Reads an RGList_CALIB from a series of microarray image analysis output files

rent working directory.

Usage

```
read.rg(files = NULL, source = "generic", path = NULL, ext = NULL,
    names = NULL, columns = NULL, other.columns = NULL,
    annotation = NULL, wt.fun = NULL, verbose = TRUE, sep = "\t",
    quote = NULL, DEBUG = FALSE, ...)
```

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. 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 "generic", "agilent", "arrayvision", "bluefuse", "genepix", "genepix.custom", "genepix.median", "imagene", "quantarray", "scanarrayexpress", "smd.old", "smd", "spot" or "spot.close.open".
path	character string giving the directory containing the files. The default is the cur-

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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 with fields R, G, Rb, Gb, RArea and GArea giving the column names to be

used for red foreground, green foreground, red background, green background, red area and green area respectively. Or, in the case of Imagene data, a list with fields f and b. 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

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

DEBUG a logical value, if TRUE, a series of echo statements will be printed for each

file. Details on the file, skip, and selected columns in a colClasses format for

read.table will be displayed.

any other arguments are passed to read.table.

Details

This is the main data input function for CALIB package. It has the similar usage as the read.maimages function in limma package. The output of the function is an RGList_CALIB object. However, there are two more fields - \$RArea and \$GArea than RGList object in limma package. These two fields contain spot area of each color. More details see read.maimages in limma package.

Value

An RGList_CALIB object containing the components

R	matrix	containing	the red	channel	foreground	intensities	for each	spot for each

array.

G matrix containing the green channel foreground intensities for each spot for each

array.

Rb matrix containing the red channel background intensities for each spot for each

array.

Gb matrix containing the green channel background intensities for each spot for

each array.

RArea matrix containing the red spot area for each spot for each array.

GArea matrix containing the green spot area for each spot for each array.

weights spot quality weights, if wt.fun is given

other list containing matrices corresponding to other.columns if given

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genes	data frame containing annotation information about the probes, for example
	gene names and IDs and spatial positions on the array, currently set only if
	source is "agilent", "genepix" or source="imagene" or if the
	annotation argument is set
targets	data frame with column FileName giving the names of the files read
source	character string giving the image analysis program name
printer	list of class PrintLayout, currently set only if source="imagene"

Author(s)

Hui Zhao

References

```
read.maimages in limma package
```

See Also

'read.rg' is based on read.table in the base package

Examples

```
# Read all .gpr files from current working directory.
# files <- dir(pattern="*\.gpr$")
# RG <- read.rg(files, "genepix")</pre>
```

read.spike

Read SpikeList from a RGList_CALIB and Concentration files

Description

Reads a SpikeList from a given RGList_CALIB object and user specified concentration files

Usage

Arguments

RG	a RGList_CALIB object.
file	character string giving the name of the concentration file.
path	character string giving the directory containing the file. Can be omitted if the file is in the current working directory.
ext	character string giving optional extension to be added to each file name.
sep	field separator character.
conccol	list with fields RConc, GConc giving the column names to be used for red and green concentration in the concentration file.

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regexpcol	character vector giving regular expressions in the concentration file.
different	a logical value. TRUE means that different arrays use different spikes. So every array should have one concentration file. FALSE means that different arrays use the same spike and only one concentration file is needed. Default is FALSE.
	any other arguments are passed to read.table.

Details

This is the function to generate SpikeList in the CALIB package. SpikeList contains all the information for the spikes. This function exacts foreground and background intensities and spot area from RGList_CALIB, which is generated from funtion read.rg. Also this funtion reads concentrations for each spike from a user-specified concentration file (or more than one concentration files if different arrays use different spikes).

For the concentration file, it should contain the following columns: regular expression, red and green concentrations and spike type. Spike type should be in the set of Calibration, Ratio and Negative.

See the CALIB User's Guide for the example of this function.

Value

An SpikeList object containing the components

R	matrix containing the red channel foreground intensities for each spot for each array.
G	matrix containing the green channel foreground intensities for each spot for each array.
Rb	matrix containing the red channel background intensities for each spike for each array.
Gb	matrix containing the green channel background intensities for each spike for each array.
RArea	matrix containing the red spot area for each spike for each array.
GArea	matrix containing the green spot area for each spike for each array.
RConc	matrix containing the red concentration for each spike for each array.
GConc	matrix containing the green concentration for each spike for each array.
genes	data frame containing annotation information about the probes, for example, spike types and IDs and spatial positions on the array

Author(s)

Hui Zhao

References

~put references to the literature/web site here ~

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spike

Experiment Data: SpikeList Example

Description

```
This SpikeList object contains all the spikes in dataset

RG.It is generated by the function

read.spike from RG and user-specified concentraion file in the CALIB package.
```

Usage

```
data(spike)
```

Format

spike is an SpikeList object containing the following list components: \\$R,\\$G,\\$Rb,\\$Gb, \\$RArea,\\$GArea,\\$GConc,\\$GConc and \\$genes. It represents two microarrays and 600 spikes, including 480 calibration controls, 96 ratio controls and 24 negative controls.

Source

For the source information, see the introduction of dataset RG.

References

dataset RG.

Engelen, K., Naudts, B., DeMoor, B., Marchal, K. (2006) A calibration method for estimating absolute expression levels from microarray data. Bioinformatics 22: 1251-1258.

Hilson,P.,et al. (2004) Versatile gene-specific sequence tags for Arabidopsis functional genomics: transcript profiling and reverse genetics applications. Genome Res. 14, 2176-2189.

Examples

```
data(spike)
plotSpikeRG(spike,array=1)
```

subsetting

Subset of RGList_CALIB, SpikeList or ParameterList object

Description

Exact a subset of an RGList_CALIB, SpikeList or ParameterList object.

Usage

```
## S3 method for class 'RGList\_CALIB':
object[i, j, ...]
```

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Arguments

```
object an object of class RGList_CALIB or SpikeList.

i, j subscripts. i is the subscripts of the spots and j is the subscripts of the arrays.

not used
```

Details

i,j may take any values acceptable for the matrix components of object.

Value

An object of the same class as object containing the data with specified subset of spots and arrays.

Author(s)

Hui Zhao

References

```
subsetting in limma package
```

See Also

```
subsetting in the limma package
```

Examples

```
# for RGList_CALIB
R <- G <- matrix(1:8,4,2)
rownames(R) <- rownames(G) <- c("g1", "g2", "g3", "g4")
colnames(R) <- colnames(G) <- c("a1", "a2")
RG <- new("RGList_CALIB", list(R=R,G=G))

RG[1:2,]
RG[1:2,1:2]</pre>
```

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