Package ‘puma’

April 10, 2019

Type Package

Title Propagating Uncertainty in Microarray Analysis (including Affymetrix transitional 3’ arrays and exon arrays and Human Transcriptome Array 2.0)

Version 3.24.0

Date 2015-7-29

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Depends R (>= 3.2.0), oligo (>= 1.32.0), graphics, grDevices, methods, stats, utils, mclust, oligoClasses

Imports Biobase (>= 2.5.5), affy (>= 1.46.0), affyio, oligoClasses

Suggests pumadata, affydata, snow, limma, ROCR, annotate

Description Most analyses of Affymetrix GeneChip data (including transitional 3’ arrays and exon arrays and Human Transcriptome Array 2.0) are based on point estimates of expression levels and ignore the uncertainty of such estimates. By propagating uncertainty to downstream analyses we can improve results from microarray analyses. For the first time, the puma package makes a suite of uncertainty propagation methods available to a general audience. In addition to calculate gene expression from Affymetrix 3’ arrays, puma also provides methods to process exon arrays and produces gene and isoform expression for alternative splicing study. puma also offers improvements in terms of scope and speed of execution over previously available uncertainty propagation methods. Included are summarisation, differential expression detection, clustering and PCA methods, together with useful plotting functions.

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biocViews Microarray, OneChannel, Preprocessing, DifferentialExpression, Clustering, ExonArray, GeneExpression, mRNAMicroarray, ChipOnChip, AlternativeSplicing, DifferentialSplicing, Bayesian, TwoChannel, DataImport, HTA2.0

URL http://umber.sbs.man.ac.uk/resources/puma

NeedsCompilation yes

git_url https://git.bioconductor.org/packages/puma

git_branch RELEASE_3_8

git_last_commit aeac592

git_last_commit_date 2018-10-30

Date/Publication 2019-04-09
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Description

Most analyses of Affymetrix GeneChip data (including traditional 3' arrays and exon arrays) are based on point estimates of expression levels and ignore the uncertainty of such estimates. By propagating uncertainty to downstream analyses we can improve results from microarray analyses. For the first time, the puma package makes a suite of uncertainty propagation methods available to a general audience. In addition to calculate gene expression from Affymetrix 3' arrays, puma also provides methods to process exon arrays and produces gene and isoform expression for alternative splicing study. puma also offers improvements in terms of scope and speed of execution over previously available uncertainty propagation methods. Included are summarisation, differential expression detection, clustering and PCA methods, together with useful plotting functions.

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<tr>
<td>Version:</td>
<td>3.4.3</td>
</tr>
<tr>
<td>Date:</td>
<td>2013-11-04</td>
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<tr>
<td>License:</td>
<td>LGPL excluding donlp2</td>
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For details of using the package please refer to the Vignette

Author(s)

Richard Pearson, Xuejun Liu, Guido Sanguinetti, Marta Milo, Neil D. Lawrence, Magnus Rattray, Li Zhang

Maintainer: Richard Pearson <richard.pearson@postgrad.manchester.ac.uk>, Li Zhang <leo.zhang@nuaa.edu.cn>

References


**Examples**

```r
# Next 4 lines commented out to save time in package checks, and saved version used
# if (require(affydata)) {
# data(Dilution)
# eset_mmgmos <- mmgmos(Dilution)
# }

data(eset_mmgmos)
pumapca_mmgmos <- pumaPCA(eset_mmgmos)
plot(pumapca_mmgmos)
eset_mmgmos_100 <- eset_mmgmos[1:100,]
eset_comb <- pumaComb(eset_mmgmos_100)
eset_combImproved <- pumaCombImproved(eset_mmgmos_100)
esetDE <- pumaDE(eset_comb)
esetDEImproved <- pumaDE(eset_combImproved)
```

**Description**

This function calculates the combined signal for each condition from replicates using Bayesian models. The inputs are gene expression levels and the probe-level standard deviation associated with expression measurement for each gene on each chip. The outputs include gene expression levels and standard deviation for each condition. This function was originally part of the pplr package. Although this function can be called directly, it is recommended to use the pumaComb function instead, which can work directly on ExpressionSet objects, and can automatically determine which arrays are replicates.

*bcomb*

Combining replicates for each condition
Usage

bcomb(e, se, replicates, method=c("map","em"),
       gsnorm=FALSE, nsample=1000, eps=1.0e-6)

Arguments

e          a data frame containing the expression level for each gene on each chip.
se         a data frame containing the standard deviation of gene expression levels.
replicates a vector indicating which chip belongs to which condition.
method     character specifying the method algorithm used.
gsnorm     logical specifying whether do global scaling normalisation or not.
nsample    integer. The number of sampling in parameter estimation.
eps        a numeric, optimisation parameter.

Details

Each element in replicate represents the condition of the chip which is in the same column order as
in the expression and standard deviation matrix files.

Method "map" uses MAP of a hierarchical Bayesian model with Gamma prior on the between-
replicate variance (Gelman et.al. p.285) and shares the same variance across conditions. This
method is fast and suitable for the case where there are many conditions.

Method "em" uses variational inference of the same hierarchical Bayesian model as in method
"map" but with conjugate prior on between-replicate variance and shares the variance across condi-
tions.

The parameter nsample should be large enough to ensure stable parameter estimates. Should be at
least 1000.

Value

The result is a data frame with components named 'M1', 'M2', and so on, which represent the mean
expression values for condition 1, condition 2, and so on. It also has components named 'Std1',
'Std2', and so on, which represent the standard deviation of the gene expression values for condition
1, condition 2, and so on.

Author(s)

Xuejun Liu, Marta Milo, Neil D. Lawrence, Magnus Rattray

References

1995.

See Also

Related methods pumaComb, mmgmos and pplr
Examples

```r
data(exampleE)
data(exampleStd)
r<-bcomb(exampleE,exampleStd,replicates=c(1,1,1,2,2,2),method="map")
```

calcAUC

Calculate Area Under Curve (AUC) for a standard ROC plot.

Description

Calculates the AUC values for one or more ROC plots.

Usage

```r
calcAUC(scores, truthValues, includedProbesets = 1:length(truthValues))
```

Arguments

- `scores`: A vector of scores. This could be, e.g. one of the columns of the statistics of a DEResult object.
- `truthValues`: A boolean vector indicating which scores are True Positives.
- `includedProbesets`: A vector of indices indicating which scores (and truthValues) are to be used in the calculation. The default is to use all, but a subset can be used if, for example, you only want a subset of the probesets which are not True Positives to be treated as False Positives.

Value

A single number which is the AUC value.

Author(s)

Richard D. Pearson

See Also

Related methods `plotROC` and `numFP`.

Examples

```r
class1a <- rnorm(1000,0.2,0.1)
class2a <- rnorm(1000,0.6,0.2)
class1b <- rnorm(1000,0.3,0.1)
class2b <- rnorm(1000,0.5,0.2)
scores_a <- c(class1a, class2a)
scores_b <- c(class1b, class2b)
classElts <- c(rep(FALSE,1000), rep(TRUE,1000))
print(calcAUC(scores_a, classElts))
print(calcAUC(scores_b, classElts))
```
**calculateFC**  
*Calculate differential expression between conditions using FC*

**Description**
Automatically creates design and contrast matrices if not specified. This function is useful for comparing fold change results with those of other differential expression (DE) methods such as `pumaDE`.

**Usage**
```r
calculateFC(
  eset,
  design.matrix = createDesignMatrix(eset),
  contrast.matrix = createContrastMatrix(eset)
)
```

**Arguments**
- `eset` An object of class `ExpressionSet`
- `design.matrix` A design matrix
- `contrast.matrix` A contrast matrix

**Details**
The `eset` argument must be supplied, and must be a valid `ExpressionSet` object. Design and contrast matrices can be supplied, but if not, default matrices will be used. These should usually be sufficient for most analyses.

**Value**
An object of class `DEResult`.

**Author(s)**
Richard D. Pearson

**See Also**
Related methods `pumaDE, calculateLimma, calculateTtest, createDesignMatrix` and `createContrastMatrix` and class `DEResult`

**Examples**
```r
if (require(affydata)) {
  data(Dilution)
  eset_rma <- affy::rma(Dilution)
  # Next line used so eset_rma only has information about the liver factor
  # The scanner factor will thus be ignored, and the two arrays of each level
  # of the liver factor will be treated as replicates
  pData(eset_rma) <- pData(eset_rma)[,1, drop=FALSE]
```
calculateLimma

Calculate differential expression between conditions using limma

Description

Runs a default analysis using the limma package. Automatically creates design and contrast matrices if not specified. This function is useful for comparing limma results with those of other differential expression (DE) methods such as pumaDE.

Usage

calculateLimma(
  eset,
  design.matrix = createDesignMatrix(eset),
  contrast.matrix = createContrastMatrix(eset)
)

Arguments

eset An object of class ExpressionSet
design.matrix A design matrix
contrast.matrix A contrast matrix

Details

The eset argument must be supplied, and must be a valid ExpressionSet object. Design and contrast matrices can be supplied, but if not, default matrices will be used. These should usually be sufficient for most analyses.

Value

An object of class DEResult.

Author(s)

Richard D. Pearson

See Also

Related methods pumaDE, calculateTtest, calculateFC, createDesignMatrix and createContrastMatrix and class DEResult
**calculateTtest**

**Examples**

```r
if (require(affydata)) {
  data(Dilution)
  eset_rma <- affy::rma(Dilution)
  # Next line used so eset_rma only has information about the liver factor
  # The scanner factor will thus be ignored, and the two arrays of each level
  # of the liver factor will be treated as replicates
  pData(eset_rma) <- pData(eset_rma)[,1, drop=FALSE]
  limmaRes <- calculateLimma(eset_rma)
  topGeneIDs(limmaRes, numberOfGenes=6)
  plotErrorBars(eset_rma, topGenes(limmaRes))
}
```

---

**calculateTtest**

*Calculate differential expression between conditions using T-test*

**Description**

Automatically creates design and contrast matrices if not specified. This function is useful for comparing T-test results with those of other differential expression (DE) methods such as `pumaDE`.

**Usage**

```r
calculateTtest(
eset
  , design.matrix = createDesignMatrix(eset)
  , contrast.matrix = createContrastMatrix(eset)
)
```

**Arguments**

- `eset`: An object of class `ExpressionSet`
- `design.matrix`: A design matrix
- `contrast.matrix`: A contrast matrix

**Details**

The `eset` argument must be supplied, and must be a valid `ExpressionSet` object. Design and contrast matrices can be supplied, but if not, default matrices will be used. These should usually be sufficient for most analyses.

**Value**

An object of class `DEResult`.

**Author(s)**

Richard D. Pearson
See Also

Related methods `pumaDE`, `calculateLimma`, `calculateFC`, `createDesignMatrix` and `createContrastMatrix` and class `DEResult`.

Examples

```r
eset_test <- new("ExpressionSet", exprs=matrix(rnorm(400,8,2),100,4))
pData(eset_test) <- data.frame("class"=c("A", "A", "B", "B"))
TtestRes <- calculateTtest(eset_test)
plotErrorBars(eset_test, topGenes(TtestRes))
```

---

### Clust.exampleE

**The example data of the mean gene expression levels**

**Description**

This data is an artificial example of the mean gene expression levels.

**Usage**

```r
data(Clust.exampleE)
```

**Format**

A 700x20 matrix including 700 genes and 20 chips. Every 100 genes belong to one cluster from the first gene. There are 7 clusters.

**Source**


**See Also**

`Clust.exampleStd`

---

### Clust.exampleStd

**The example data of the standard deviation for gene expression levels**

**Description**

This data is an artificial example of the standard deviation for gene expression levels.

**Usage**

```r
data(Clust.exampleStd)
```

**Format**

A 700x20 matrix including 700 genes and 20 chips. Every 100 genes belong to one cluster from the first gene. There are 7 true clusters.
clusterApplyLBDots

Source

See Also
Clust.exampleE

clusterApplyLBDots  

clusterApplyLB with dots to indicate progress

Description
This is basically the clusterApplyLB function from the snow package, but with dots displayed to indicate progress.

Usage
clusterApplyLBDots(cl, x, fun, ...)

Arguments
cl  
cluster object
x  
array
fun  
function or character string naming a function
...  
additional arguments to pass to standard function

Author(s)
Richard D. Pearson (modified from original snow function)

clusterNormE  

Zero-centered normalisation

Description
This function normalise the data vector to have zero mean.

Usage
clusterNormE(x)

Arguments
x  
a vector which contains gene expression level on log2 scale.

Details
Vector x is related to a gene and each element is related to a chip.
Description
This function adjusts the variance of the gene expression according to the zero-centered normalisation.

Usage
clusterNormVar(x)

Arguments
x  a vector which contains the variance of gene expression level on log2 scale.

Details
Vector x is related to a gene and each element is related to a chip.

Value
The return vector is in the same format as the input x.

Author(s)
Xuejun Liu, Magnus Rattray

See Also
See Also as pumaClust and pumaClustii

Examples
data(Clust.exampleE)
Clust.exampleE.centered<-t(apply(Clust.exampleE, 1, clusterNormE))

data(Clust.exampleE)
data(Clust.exampleStd)
Clust.exampleVar<-Clust.exampleStd^2
Clust.exampleStd.centered<-t(apply(cbind(Clust.exampleE,Clust.exampleVar), 1, clusterNormVar))
Clustii.exampleE

**The example data of the mean gene expression levels**

**Description**
This data is an artificial example of the mean gene expression levels generated by package *mmgmos*.

**Usage**
```r
data(Clustii.exampleE)
```

**Format**
A 600x80 matrix including 600 genes and 20 conditions. Each condition has 4 replicates. Every 100 genes belong to one cluster from the first gene. There are 6 clusters.

**Source**

**See Also**
- Clustii.exampleStd

---

Clustii.exampleStd

**The example data of the standard deviation for gene expression levels**

**Description**
This data is an artificial example of the standard deviation for gene expression levels generated by package *mmgmos*.

**Usage**
```r
data(Clustii.exampleStd)
```

**Format**
A 600x80 matrix including 600 genes and 20 conditions. Each condition has 4 replicates. Every 100 genes belong to one cluster from the first gene. There are 6 clusters.

**Source**

**See Also**
- Clustii.exampleE
compareLimmapumaDE

Compare pumaDE with a default Limma model

Description

This function compares the identification of differentially expressed (DE) genes using the pumaDE function and the limma package.

Usage

```r
compareLimmapumaDE(
  eset_mmgmos,
  eset_comb = NULL,
  eset_other = eset_mmgmos,
  limmaRes = calculateLimma(eset_other),
  pumaDERes = pumaDE(eset_comb),
  contrastMatrix = createContrastMatrix(eset_mmgmos),
  numberToCompareForContrasts = 3,
  numberToCompareForVenn = 100,
  plotContrasts = TRUE,
  contrastsFilename = NULL,
  plotOther = FALSE,
  otherFilename = "other",
  plotBcombContrasts = FALSE,
  bcombContrastsFilename = "bcomb_contrasts",
  plotVenn = FALSE,
  vennFilename = "venn.pdf",
  showTopMatches = FALSE,
  returnResults = FALSE)
```

Arguments

- **eset_mmgmos**: An object of class `ExpressionSet`, that includes both expression levels as well as standard errors of the expression levels. This will often have been created using `mmgmos`, but might also have been created by `mgmos`, or any other method capable of providing standard errors.

- **eset_comb**: An object of class `ExpressionSet`, includes both expression levels as well as standard errors of the expression levels for each unique condition in an experiment (i.e. created from combining the information from each replicate). This will usually have been created using `pumaComb`.

- **eset_other**: An object of class `ExpressionSet`, that includes expression levels , and may optionally also include standard errors of the expression levels. This is used for comparison with eset_mmgmos, and might have been created by any summarisation method, e.g. `rma`.

- **limmaRes**: A list with two elements, usually created using the function `calculateLimma`. The first element is a matrix of p-values. Each column represent one contrast. Within each column the p-values are ordered. The second element is a matrix of row numbers, which can be used to map p-values back to probe sets. If not supplied this will be automatically created from eset_other.
pumaDERes  A list with two elements, usually created using the function pumaDE. The first element is a matrix of PPLR values. Each column represent one contrast. Within each column the PPLR values are ordered. The second element is a matrix of row numbers, which can be used to map PPLR values back to probe sets. If not supplied this will be automatically created from eset_comb.

contrastMatrix  A contrast matrix. If not supplied this will be created from eset_mmgmos

numberToCompareForContrasts  An integer specifying the number of most differentially expressed probe sets (genes) that will be used in comparison charts.

numberToCompareForVenn  An integer specifying the number of most differentially expressed probe sets (genes) that will be used for comparison in the Venn diagram.

plotContrasts  A boolean specifying whether or not to plot the most differentially expressed probe sets (genes) for each contrast for the eset_mmgmos ExpressionSet.

contrastsFilename  A character string specifying a file name stem for the PDF files which will be created to hold the contrast plots for the eset_mmgmos ExpressionSet. The actually filenames will have the name of the contrast appended to this stem.

plotOther  A boolean specifying whether or not to plot the most differentially expressed probe sets (genes) for each contrast for the eset_other ExpressionSet.

otherFilename  A character string specifying a file name stem for the PDF files which will be created to hold the contrast plots for the eset_other ExpressionSet. The actually filenames will have the name of the contrast appended to this stem.

plotBcombContrasts  A boolean specifying whether or not to plot the most differentially expressed probe sets (genes) for each contrast for the eset_comb ExpressionSet.

bcombContrastsFilename  A character string specifying a file name stem for the PDF files which will be created to hold the contrast plots for the eset_comb ExpressionSet. The actually filenames will have the name of the contrast appended to this stem.

plotVenn  A boolean specifying whether or not to plot a Venn diagram showing the overlap in the most differentially expressed probe sets (genes) as identified from the two different methods being compared.

vennFilename  A character string specifying the filename for the PDF file which will hold the Venn diagram showing the overlap in the most differentially expressed probe sets (genes) as identified from the two different methods being compared.

showTopMatches  A boolean specifying whether or not to show the probe sets which are deemed most likely to be differentially expressed.

returnResults  A boolean specifying whether or not to return a list containing results generated.

Value

The main outputs from this function are a number of PDF files.

The function only returns results if returnResults=TRUE

Author(s)

Richard D. Pearson
createContrastMatrix

Automatically create a contrast matrix from an ExpressionSet and optional design matrix

Description

To appear

Usage

createContrastMatrix(eset, design=NULL)

Arguments

eset An object of class ExpressionSet.
design A design matrix

Details

The puma package has been designed to be as easy to use as possible, while not compromising on power and flexibility. One of the most difficult tasks for many users, particularly those new to microarray analysis, or statistical analysis in general, is setting up design and contrast matrices. The puma package will automatically create such matrices, and we believe the way this is done will suffice for most users’ needs.

It is important to recognise that the automatic creation of design and contrast matrices will only happen if appropriate information about the levels of each factor is available for each array in the experimental design. This data should be held in an AnnotatedDataFrame class. The easiest way of doing this is to ensure that the AnnotatedDataFrame object holding the raw CEL file data has an appropriate phenoData slot. This information will then be passed through to any ExpressionSet object created, for example through the use of mmgmos. The phenoData slot of an ExpressionSet object can also be manipulated directly if necessary.

Design and contrast matrices are dependent on the experimental design. The simplest experimental designs have just one factor, and hence the phenoData slot will have a matrix with just one column. In this case, each unique value in that column will be treated as a distinct level of the factor, and hence pumaComb will group arrays according to these levels. If there are just two levels of the factor, e.g. A and B, the contrast matrix will also be very simple, with the only contrast of interest being A vs B. For factors with more than two levels, a contrast matrix will be created which reflects all possible combinations of levels. For example, if we have three levels A, B and C, the contrasts of interest will be A vs B, A vs C and B vs C. In addition, if the others argument is set to TRUE, the following additional contrasts will be created: A vs other (i.e. A vs B & C), B vs other and C vs other. Note that these additional contrasts are experimental, and not currently recommended for use in calculating differential expression.

If we now consider the case of two or more factors, things become more complicated. There are now two cases to be considered: factorial experiments, and non-factorial experiments. A factorial experiment is one where all the combinations of the levels of each factor are tested by at least one array (though ideally we would have a number of biological replicates for each combination of
factor levels). The estrogen case study from the package vignette is an example of a factorial experiment.

A non-factorial experiment is one where at least one combination of levels is not tested. If we treat the example used in the `puma-package` help page as a two-factor experiment (with factors “level” and “batch”), we can see that this is not a factorial experiment as we have no array to test the conditions “level=ten” and “batch=B”. We will treat the factorial and non-factorial cases separately in the following sections.

**Factorial experiments**

For factorial experiments, the design matrix will use all columns from the `phenoData` slot. This will mean that `pumaComb` will group arrays according to a combination of the levels of all the factors.

**Non-factorial designs**

For non-factorial designed experiments, we will simply ignore columns (right to left) from the `phenoData` slot until we have a factorial design or a single factor. We can see this in the example used in the `puma-package` help page. Here we have ignored the “batch” factor, and modelled the experiment as a single-factor experiment (with that single factor being “level”).

**Value**

The result is a matrix. See the code below for an example.

**Author(s)**

Richard D. Pearson

**See Also**

Related methods `createDesignMatrix` and `pumaDE`

**Examples**

# This is a simple example based on a real data set. Note that this is an "unbalanced" design, the "level" factor
# has two levels, but we only have two arrays for the "ten" condition. Also note that the second factor, "batch" is not used in the design or contrast matrices, as we don't have every combination of the levels of "level" and "batch" (there is no array for level=twenty and batch=B).

# Next 4 lines commented out to save time in package checks, and saved version used
# if (require(afydata)) {
# data(Dilution)
# eset_mmgmos <- mmgmos(Dilution)
# }

data(eset_mmgmos)
createContrastMatrix(eset_mmgmos)

# The following shows a set of 15 synthetic data sets with increasing complexity. We first create the data sets:

# single 2-level factor
eset1 <- new("ExpressionSet", exprs=matrix(0,100,4))
pData(eset1) <- data.frame("class"=c(1,1,2,2))

# single 2-level factor - unbalanced design
eset2 <- new("ExpressionSet", exprs=matrix(0,100,4))
pData(eset2) <- data.frame("class"=c(1,2,2,2))

# single 3-level factor
eset3 <- new("ExpressionSet", exprs=matrix(0,100,6))
pData(eset3) <- data.frame("class"=c(1,1,2,2,3,3))
# single 4-level factor
eset4 <- new("ExpressionSet", exprs=matrix(0,100,8))
pData(eset4) <- data.frame("class"=c(1,1,2,3,4))

# 2x2 factorial
eset5 <- new("ExpressionSet", exprs=matrix(0,100,8))
pData(eset5) <- data.frame("fac1"=c("a","a","a","a","b","b","b","b"), "fac2"=c(1,1,2,2,1,1,2,2))

# 2x2 factorial - unbalanced design
eset6 <- new("ExpressionSet", exprs=matrix(0,100,8))
pData(eset6) <- data.frame("fac1"=c("a","a","a","b","b","b","b","b"), "fac2"=c(1,2,1,2,1,2,1,2))

# 3x2 factorial
eset7 <- new("ExpressionSet", exprs=matrix(0,100,12))
pData(eset7) <- data.frame("fac1"=c("a","a","a","a","b","b","b","b","c","c","c","c"), "fac2"=c(1,1,2,2,1,1,2,2,1,1,2,2))

# 2x2x2 factorial
eset9 <- new("ExpressionSet", exprs=matrix(0,100,12))
pData(eset9) <- data.frame("fac1"=c("a","a","a","a","a","a","b","b","b","b","b","b"), "fac2"=c(1,1,1,1,2,2,1,1,2,2,1,1), "fac3"=c("X","Y","X","Y","X","Y","X","Y","X","Y","X","Y"))

# 3x2x2 factorial
eset10 <- new("ExpressionSet", exprs=matrix(0,100,12))
pData(eset10) <- data.frame("fac1"=c("a","a","a","a","a","a","a","a","b","b","b","b","b","b"), "fac2"=c(1,1,2,2,1,1,2,2,1,1,2,2,1,1), "fac3"=c("X","Y","X","Y","X","Y","X","Y","X","Y","X","Y"))

# 3x2x2 factorial
eset11 <- new("ExpressionSet", exprs=matrix(0,100,18))
pData(eset11) <- data.frame("fac1"=c("a","a","a","a","a","a","b","b","b","b","b","b","b","b","b"), "fac2"=c(1,1,2,2,3,3,1,1,2,2,3,3,1,1,2,2,3,3), "fac3"=c("X","Y","X","Y", "X","Y","X","Y","X","Y","X","Y","X","Y","X","Y","X","Y"))

# 2x2x2x2 factorial
eset13 <- new("ExpressionSet", exprs=matrix(0,100,16))
pData(eset13) <- data.frame("fac1"=c("a","a","a","a","a","a","a","a","a","a","a","a","b","b","b","b"), "fac2"=c(0,0,0,0,1,1,1,1,0,0,0,0,1,1,1,1), "fac3"=c(2,2,3,3,2,2,3,3,2,2,3,3,2,2,3,3), "fac4"=c("X","Y","X","Y","X","Y","X","Y","X","Y","X","Y","X","Y","X","Y"))
# "Un-analysable" data set - all arrays are from the same class
eset14 <- new("ExpressionSet", exprs=matrix(0,100,4))
pData(eset14) <- data.frame("class"=c(1,1,1,1))

# "Non-factorial" data set - there are no arrays for fac1="b" and fac2=2. In this case only the first factor (fac1) is used.
eset15 <- new("ExpressionSet", exprs=matrix(0,100,6))
pData(eset15) <- data.frame("fac1"=c("a","a","a","a","b","b"), "fac2"=c(1,1,2,2,1,1))

createContrastMatrix(eset1)
createContrastMatrix(eset2)
createContrastMatrix(eset3)
createContrastMatrix(eset4)
createContrastMatrix(eset5)
createContrastMatrix(eset6)
createContrastMatrix(eset7)
createContrastMatrix(eset8)
createContrastMatrix(eset9)
# For the last 4 data sets, the contrast matrices get pretty big, so we'll just show the names of each contrast.
colnames(createContrastMatrix(eset10))
colnames(createContrastMatrix(eset11))
# Note that the number of contrasts can rapidly get very large for multi-factorial experiments!
colnames(createContrastMatrix(eset12))
colnames(createContrastMatrix(eset13))
# For this final data set, note that the puma package does not currently create interaction terms for data sets with 4 or more factors.
colnames(createContrastMatrix(eset13))

# "Un-analysable" data set - all arrays are from the same class - gives an error. Note that we've commented this out so that we don't get errors which would make the package fail the Bioconductor checks!
# createContrastMatrix(eset14)
# "Non-factorial" data set - there are no arrays for fac1="b" and fac2=2. In this case only the first factor (fac1) is used.
createContrastMatrix(eset15)

createDesignMatrix

Automatically create a design matrix from an ExpressionSet

Description

Automatically create a design matrix from an ExpressionSet.

Usage

createDesignMatrix(eset)

Arguments

eset An object of class ExpressionSet.

Details

The puma package has been designed to be as easy to use as possible, while not compromising on power and flexibility. One of the most difficult tasks for many users, particularly those new to microarray analysis, or statistical analysis in general, is setting up design and contrast matrices. The puma package will automatically create such matrices, and we believe the way this is done will suffice for most users’ needs.
createDesignMatrix

It is important to recognise that the automatic creation of design and contrast matrices will only happen if appropriate information about the levels of each factor is available for each array in the experimental design. This data should be held in an AnnotatedDataFrame class. The easiest way of doing this is to ensure that the AnnotatedDataFrame object holding the raw CEL file data has an appropriate phenoData slot. This information will then be passed through to any ExpressionSet object created, for example through the use of mmgmos. The phenoData slot of an ExpressionSet object can also be manipulated directly if necessary.

Design and contrast matrices are dependent on the experimental design. The simplest experimental designs have just one factor, and hence the phenoData slot will have a matrix with just one column. In this case, each unique value in that column will be treated as a distinct level of the factor, and hence pumaComb will group arrays according to these levels. If there are just two levels of the factor, e.g. A and B, the contrast matrix will also be very simple, with the only contrast of interest being A vs B. For factors with more than two levels, a contrast matrix will be created which reflects all possible combinations of levels. For example, if we have three levels A, B and C, the contrasts of interest will be A vs B, A vs C and B vs C.

If we now consider the case of two or more factors, things become more complicated. There are now two cases to be considered: factorial experiments, and non-factorial experiments. A factorial experiment is one where all the combinations of the levels of each factor are tested by at least one array (though ideally we would have a number of biological replicates for each combination of factor levels). The estrogen case study from the package vignette is an example of a factorial experiment.

A non-factorial experiment is one where at least one combination of levels is not tested. If we treat the example used in the puma-package help page as a two-factor experiment (with factors “level” and “batch”), we can see that this is not a factorial experiment as we have no array to test the conditions “level=ten” and “batch=B”. We will treat the factorial and non-factorial cases separately in the following sections.

Factorial experiments
For factorial experiments, the design matrix will use all columns from the phenoData slot. This will mean that pumaComb will group arrays according to a combination of the levels of all the factors.

Non-factorial designs
For non-factorial designed experiments, we will simply ignore columns (right to left) from the phenoData slot until we have a factorial design or a single factor. We can see this in the example used in the puma-package help page. Here we have ignored the “batch” factor, and modelled the experiment as a single-factor experiment (with that single factor being “level”).

Value
The result is a matrix. See the code below for an example.

Author(s)
Richard D. Pearson

See Also
Related methods createContrastMatrix, pumaComb, pumaDE and pumaCombImproved

Examples
# This is a simple example based on a real data set. Note that this is an "unbalanced" design, the "level" factor...
# The following shows a set of 15 synthetic data sets with increasing complexity. We first create the data sets

eiset1 <- new("ExpressionSet", exprs=matrix(0,100,4))
pData(eset1) <- data.frame("class"=c(1,1,2,2))

# single 2-level factor

eiset2 <- new("ExpressionSet", exprs=matrix(0,100,4))
pData(eset2) <- data.frame("class"=c(1,2,2,2))

# single 3-level factor

eiset3 <- new("ExpressionSet", exprs=matrix(0,100,6))
pData(eset3) <- data.frame("class"=c(1,1,2,2,3,3))

# single 4-level factor

eiset4 <- new("ExpressionSet", exprs=matrix(0,100,8))
pData(eset4) <- data.frame("class"=c(1,1,2,2,3,3,4,4))

# 2x2 factorial

eiset5 <- new("ExpressionSet", exprs=matrix(0,100,8))
pData(eset5) <- data.frame("fac1"="a", "fac2"="c(1,2,2,1,1,2,2)"

# 2x2 factorial - unbalanced design

eiset6 <- new("ExpressionSet", exprs=matrix(0,100,10))
pData(eset6) <- data.frame("fac1"="a", "fac2"="c(1,2,2,1,1,2,2,2)"

# 3x2 factorial

eiset7 <- new("ExpressionSet", exprs=matrix(0,100,12))
pData(eset7) <- data.frame("fac1"="a", "fac2"="c(1,1,2,2,3,3,1,1,2,2,3,3)"

# 2x2x2 factorial

eiset8 <- new("ExpressionSet", exprs=matrix(0,100,8))
pData(eset8) <- data.frame("fac1"="a", "fac2"="c(1,1,2,2,1,1,2,2)"

# 3x2x2 factorial

eiset9 <- new("ExpressionSet", exprs=matrix(0,100,8))
pData(eset9) <- data.frame("fac1"="a", "fac2"="c(1,1,2,2,1,1,2,2)"

# 3x3x2 factorial

eiset10 <- new("ExpressionSet", exprs=matrix(0,100,12))
pData(eset10) <- data.frame("fac1"="a", "fac2"="c(1,1,2,2,1,1,2,2)"

# The following shows a set of 15 synthetic data sets with increasing complexity. We first create the data sets
createDesignMatrix

# 3x2x2 factorial
eset11 <- new("ExpressionSet", exprs=matrix(0,100,12))
pData(eset11) <- data.frame("factor1"=c("a","a","a","a","a","a","b","b","b","b","b","b"), "factor2"=c(1,2,2,3,3,1,1,2,2,3,3), "factor3"=c("X","Y","X","Y","X","Y","X","Y","X","Y","X","Y") )

# 3x2x2 factorial
eset12 <- new("ExpressionSet", exprs=matrix(0,100,18))
pData(eset12) <- data.frame("factor1"=c("a","a","a","a","a","a","a","a","a","b","b","b","b","b","b","b","b"), "factor2"=c(1,2,3,3,1,1,2,2,3,3,1,1,2,2,3,3), "factor3"=c("X","Y","X","Y","X","Y","X","Y","X","Y","X","Y") )

# Un-analysable" data set - all arrays are from the same class
eset14 <- new("ExpressionSet", exprs=matrix(0,100,4))
pData(eset14) <- data.frame("
    class"=c(1,1,1,1))

# "Non-factorial" data set - there are no arrays for fac1="b" and fac2=2. In this case only the first factor (fac1) is used.
eset15 <- new("ExpressionSet", exprs=matrix(0,100,6))
pData(eset15) <- data.frame("factor1"=c("a","a","a","a","b","b"), "factor2"=c(1,1,2,2,1,1))

# "pseudo 2 factor" data set - second factor is informative
eset16 <- new("ExpressionSet", exprs=matrix(0,100,8))
pData(eset16) <- data.frame("factor1"=c("a","a","b","b"), "factor2"=c(1,1,1,1))

# "pseudo 2 factor" data set - first factor is informative
eset17 <- new("ExpressionSet", exprs=matrix(0,100,8))
pData(eset17) <- data.frame("factor1"=c("a","a","a","a"), "factor2"=c(1,1,2,2))

# "pseudo 3 factor" data set - first factor is uninformative so actually a 2x2 factorial
eset18 <- new("ExpressionSet", exprs=matrix(0,100,8))
pData(eset18) <- data.frame("factor1"=c("a","a","a","a","a","a","a","a"), "factor2"=c(1,1,2,2,1,1,2,2))

# "pseudo 3 factor" data set - first and third factors are uninformative so actually a single factor
eset19 <- new("ExpressionSet", exprs=matrix(0,100,8))
pData(eset19) <- data.frame("factor1"=c("a","a","a","a","a","a","a","a"), "factor2"=c(1,1,2,2,1,1,2,2))

createDesignMatrix(eset1)
createDesignMatrix(eset2)
createDesignMatrix(eset3)
createDesignMatrix(eset4)
create_eset_r

createDesignMatrix(eset5)
createDesignMatrix(eset6)
createDesignMatrix(eset7)
createDesignMatrix(eset8)
createDesignMatrix(eset9)
createDesignMatrix(eset10)
createDesignMatrix(eset11)
createDesignMatrix(eset12)
createDesignMatrix(eset13)

# "Un-analysable" data set - all arrays are from the same class - gives an error. Note that we've commented this out so that we don't get errors which would make the package fail the Bioconductor checks!
createDesignMatrix(eset14)

# "Non-factorial" data set - there are no arrays for fac1="b" and fac2=2. In this case only the first factor (fac1) is used.
createDesignMatrix(eset15)

---

create_eset_r

Create an ExpressionSet from a PPLR matrix

Description

This is really an internal function called from pumaComb. It is used to create an ExpressionSet object from the output of the bcomb function (which was originally part of the pplr package. Don’t worry about it!

Usage

create_eset_r(
  eset,
  r,
  design.matrix=createDesignMatrix(eset)
)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>eset</td>
<td>An object of class ExpressionSet. The phenotype information from this is used as the phenotype information of the returned object</td>
</tr>
<tr>
<td>r</td>
<td>A data frame with components named 'M1', 'M2', and so on, which represent the mean expression values for condition 1, condition 2, and so on. It also has components named 'Std1', 'Std2', and so on, which represent the standard deviation of the gene expression values for condition 1, condition 2, and so on. This type of data frame is output by function bcomb and hcomb</td>
</tr>
<tr>
<td>design.matrix</td>
<td>A design matrix.</td>
</tr>
</tbody>
</table>

Value

An object of class ExpressionSet.

Author(s)

Richard D. Pearson
See Also

Related methods bcomb, hcomb, pumaComb and pumaCombImproved

### Description

Class to contain and describe results of a differential expression (DE) analysis. The main components are statistic which hold the results of any statistic (e.g. p-values, PPLR values, etc.), and FC which hold the fold changes.

### Creating Objects

DEResult objects will generally be created using one of the functions pumaDE, calculateLimma, calculateFC or calculateTtest.

Objects can also be created from scratch:

```r
new("DEResult")
new("DEResult",statistic=matrix() ,FC=matrix() ,statisticDescription="unknown" ,DEMethod="unknown" )
```

### Slots

- **statistic**: Object of class "matrix" holding the statistics returned by the DE method.
- **FC**: Object of class "matrix" holding the fold changes returned by the DE method.
- **statisticDescription**: A text description of the contents of the statistic slot.
- **DEMethod**: A string indicating which DE method was used to create the object.

### Methods

Class-specific methods.

- `statistic(DEResult), statistic(DEResult,matrix)<-` Access and set the statistic slot.
- `FC(DEResult), FC(DEResult,matrix)<-` Access and set the FC slot.
- `statisticDescription(DEResult), statisticDescription(DEResult,character)<-` Access and set the statisticDescription slot.
- `DEMethod(DEResult), DEMethod(DEResult,character)<-` Access and set the DEMethod slot.
- `pLikeValues(object, contrast=1, direction="either")` Access the statistics of an object of class DEResult, converted to "p-like values". If the object holds information on more than one contrast, only the values of the statistic for contrast number contrast are given. Direction can be "either" (meaning we want order genes by probability of being either up- or down-regulated), "up" (meaning we want to order genes by probability of being up-regulated), or "down" (meaning we want to order genes by probability of being down-regulated). "p-like values" are defined as values between 0 and 1, where 0 identifies the highest probability of being differentially expressed, and 1 identifies the lowest probability of being differentially expressed. We use this so that we can easily compare results from methods that provide true p-values (e.g. calculateLimma) and methods methods that do not provide p-values (e.g. pumaDE). For objects created using pumaDE, this returns 1-PPLR if the direction is "up", PPLR
if direction is "down", and 1-abs(2*(PPLR-0.5)) if direction is "either". For objects created using calculateLimma or calculateTtest, this returns the p-value if direction is "either", 
((p-1 * sign(FC))/2)+ 0.5, if the direction is "up", and 
((1-p * sign(FC))/2)+ 0.5 if the direction is "down". For all other methods, this returns the rank of the appropriate statistic, scaled to lie between 0 and 1. contrast will be returned.

topGenes(object, numberOfGenes=1, contrast=1, direction="either") Returns the index numbers (row numbers) of the genes determined to be most likely to be differentially expressed. numberOfGenes specifies the number of genes to be returned by the function. If the object holds information on more than one contrast, only the values of the statistic for contrast number contrast are given. Direction can be "either" (meaning we want order genes by probability of being either up- or down-regulated), "up" (meaning we want to order genes by probability of being up-ragulated), or "down" (meaning we want to order genes by probability of being down-regulated). Note that genes are ordered by "p-like values" (see pLikeValues).

object is an object of class DEResult.

topGeneIDs(object, numberOfGenes=1, contrast=1, direction="either") Returns the Affy IDs (row names) of the genes determined to be most likely to be differentially expressed. numberOfGenes specifies the number of genes to be returned by the function. If the object holds information on more than one contrast, only the values of the statistic for contrast number contrast are given. Direction can be "either" (meaning we want order genes by probability of being either up- or down-regulated), "up" (meaning we want to order genes by probability of being up-ragulated), or "down" (meaning we want to order genes by probability of being down-regulated). Note that genes are ordered by "p-like values" (see pLikeValues).

object is an object of class DEResult.

numberOfProbesets(object) Returns the number of probesets (number of rows) in an object of class DEResult. This method is synonymous with numberOfGenes.

numberOfGenes(object) Returns the number of probesets (number of rows) in an object of class DEResult. This method is synonymous with numberOfProbesets.

numberOfContrasts(object) Returns the number of contrasts (number of columns) in an object of class DEResult.

write.reslts(object) signature(x = "DEResult"): writes the statistics and related fold changes (FCs) to files. It takes the same arguments as write.table. The argument "file" does not need to set any extension. The different file marks and extension "csv" will be added automatically. The default file name is "tmp". In the final results, statistics are in the file "tmp\_statistics.csv", and FCs are in "tmp\_FCs.csv" respectively.

Standard generic methods:

show(object) Informatively display object contents.

Author(s)

Richard D. Pearson

See Also

Related methods pumaDE, calculateLimma, calculateFC or calculateTtest.

Examples

## Create an example DEResult object
# Next 4 lines commented out to save time in package checks, and saved version used
# if (require(affydata)) {
erfc

The complementary error function

Description

This function calculates the complementary error function of an input x.

Usage

erfc(x)

Arguments

x  
a numeric, the input.

Details

erfc is implemented using the function qnorm.

Value

The return is a numeric.
An example ExpressionSet created from the Dilution data with mmgmos

This data is created by applying mmgmos to the Dilution AffyBatch object from the affydata package.

Usage
data(eset_mmgmos)

Format
An object of class ExpressionSet.

Source
see Dilution

The example data of the mean gene expression levels

This data is an artificial example of the mean gene expression levels from golden spike-in data set in Choe et al. (2005).

Usage
data(exampleE)

Format
A 200x6 matrix including 200 genes and 6 chips. The first 3 chips are replicates for C condition and the last 3 chips are replicates for S condition.
Source


See Also

typp

The example data of the standard deviation for gene expression levels

Description

This data is an artificial example of the standard deviation for gene expression levels from golden spike-in data set in Choe et al. (2005).

Usage

data(exampleStd)

Format

A 200x6 matrix including 200 genes and 6 chips. The first 3 chips are replicates for C condition and the last 3 chips are replicates for S condition.

Source


See Also

typp

exprReslt-class

Class exprReslt

Description

This is a class representation for Affymetrix GeneChip probe level data. The main component are the intensities, estimated expression levels and the confidence of expression levels from multiple arrays of the same CDF type. In extends ExpressionSet.

Objects from the Class

Objects can be created by calls of the form `new("exprReslt", ...)`. 
**exprReslt-class**

**Slots**

- **prcfive**: Object of class "matrix" representing the 5 percentile of the observed expression levels. This is a matrix with columns representing patients or cases and rows representing genes.

- **prctwfive**: Object of class "matrix" representing the 25 percentile of the observed expression levels. This is a matrix with columns representing patients or cases and rows representing genes.

- **prcfifty**: Object of class "matrix" representing the 50 percentile of the observed expression levels. This is a matrix with columns representing patients or cases and rows representing genes.

- **prcsevfive**: Object of class "matrix" representing the 75 percentile of the observed expression levels. This is a matrix with columns representing patients or cases and rows representing genes.

- **prcninfive**: Object of class "matrix" representing the 95 percentile of the observed expression levels. This is a matrix with columns representing patients or cases and rows representing genes.

- **phenoData**: Object of class "phenoData" inherited from `ExpressionSet`.

- **annotation**: A character string identifying the annotation that may be used for the `ExpressionSet` instance.

**Extends**

Class "ExpressionSet", directly.

**Methods**

- **se.exprs** signature(object = "exprReslt"): obtains the standard error of the estimated expression levels.

- **se.exprs<-** signature(object = "exprReslt"): replaces the standard error of the estimated expression levels.

- **prcfifty** signature(object = "exprReslt"): obtains the 50 percentile of the estimated expression levels.

- **prcfifty<-** signature(object = "exprReslt"): replaces the 50 percentile of the estimated expression levels.

- **prcfive** signature(object = "exprReslt"): obtains the 5 percentile of the estimated expression levels.

- **prcfive<-** signature(object = "exprReslt"): replaces the 5 percentile of the estimated expression levels.

- **prcninfive** signature(object = "exprReslt"): obtains the 95 percentile of the estimated expression levels.

- **prcninfive<-** signature(object = "exprReslt"): replaces the 95 percentile of the estimated expression levels.

- **prcsevfive** signature(object = "exprReslt"): obtains the 75 percentile of the estimated expression levels.

- **prcsevfive<-** signature(object = "exprReslt"): replaces the 75 percentile of the estimated expression levels.

- **prctwfive** signature(object = "exprReslt"): obtains the 25 percentile of the estimated expression levels.

- **prctwfive<-** signature(object = "exprReslt"): replaces the 25 percentile of the estimated expression levels.
show signature(object = "exprReslt"): renders information about the exprReslt in a concise way on stdout.

write.results signature(x = "exprReslt"): writes the expression levels and related confidences to files. It takes the same arguments as write.table. The argument "file" does not need to set any extension. The different file marks and extension "csv" will be added automatically. The default file name is "tmp". In the final results, expression levels are in the file "tmp\_exprs.csv", standard deviations in "tmp\_se.csv", 5 percentiles in "tmp\_prctile5.csv", likewise, 25, 50, 75 and 95 percentiles in "tmp\_prctile25.csv", "tmp\_prctile50.csv", "tmp\_prctile75.csv" and "tmp\_prctile95.csv" respectively.

Author(s)
Xuejun Liu, Magnus Rattray, Marta Milo, Neil D. Lawrence, Richard D. Pearson

See Also
Related method mmgmos and related class ExpressionSet.

Examples
```r
## load example data from package affydata
# Next 4 lines commented out to save time in package checks, and saved version used
# if (require(affydata)) {
# data(Dilution)
# eset_mmgmos <- mmgmos(Dilution)
# }

data(eset_mmgmos)
## save the expression results into files
write.results(eset_mmgmos, file="example")
```

---

**gmhta**

*Compute gene and transcript expression values and standard deviations from hta2.0 CEL Files*

**Description**

This function converts an object of FeatureSet into an object of class exprReslt using the gamma model for hta2.0 chips. This function obtains confidence of measures, standard deviation and 5, 25, 50, 75 and 95 percentiles, as well as the estimated expression levels.

**Usage**

```r
gmhta(
  object, 
  background=FALSE,
  gsnorm=c("median", "none", "mean", "meanlog"),
  savepar=FALSE,
  eps=1.0e-6,
  addConstant = 0,
  cl=NULL,
  BatchFold=10
)
```
**gmhta**

### Arguments

- **object**: an object of `FeatureSet`
- **background**: Logical value. If `TRUE`, perform background correction before applying `gmhta`.
- **gsnorm**: character. specifying the algorithm of global scaling normalisation.
- **savepar**: Logical value. If `TRUE` the estimated parameters of the model are saved in file `par\_gmhta.txt`
- **eps**: Optimisation termination criteria.
- **addConstant**: numeric. This is an experimental feature and should not generally be changed from the default value.
- **cl**: This function can be parallelised by setting parameter `cl`. For more details, please refer to the vignette.
- **BatchFold**: we divide tasks into `BatchFold*n` jobs where `n` is the number of cluster nodes. The first `n` jobs are placed on the `n` nodes. When the first job is completed, the next job is placed on the available node. This continues until all jobs are completed. The default value is ten. The user also can change the value according to the number of cluster nodes `n`. We suggest that for bigger `n` BatchFold should be smaller.

### Details

The obtained expression measures are in log base 2 scale. Using the known relationships between genes, transcripts and probes, we propose a gamma model for hta2.0 data to calculate transcript and gene expression levels. The algorithms of global scaling normalisation can be one of "median", "none", "mean", "meanlog", "mean" and "meanlog" are mean-centered normalisation on raw scale and log scale respectively, and "median" is median-centered normalisation. "none" will result in no global scaling normalisation being applied. This function can be parallelised by setting parameter `cl`. For more details, please refer to the vignette.

### Value

A list of two object of class `exprResult`.

### Author(s)

Xuejun Liu, WuJun Zhang, Zhenzhu gao, Magnus Rattray

### References


### See Also

Related class `exprResult-class`
### Examples

```r
# The following scripts show the use of the method.
#library(puma)
## load CEL files
# object<-read.celfiles("celnames")
eset<-gmhta(object,gsnorm="none",cl=cl)
```

---

**gmoExon**

**Compute gene and transcript expression values and standard deviations from exon CEL Files**

**Description**

This function converts an object of *FeatureSet* into an object of class *exprResult* using the gamma model for exon chips. This function obtains confidence of measures, standard deviation and 5, 25, 50, 75 and 95 percentiles, as well as the estimated expression levels.

**Usage**

```r
gmoExon(
  object,
  exontype = c("Human", "Mouse", "Rat"),
  background=FALSE,
  gsnorm=c("median", "none", "mean", "meanlog"),
  savepar=FALSE,
  eps=1.0e-6,
  addConstant = 0,
  cl=NULL,
  BatchFold=10
)
```

**Arguments**

- **object**: an object of *FeatureSet*
- **exontype**: character. specifying the type of exon chip.
- **background**: Logical value. If TRUE, perform background correction before applying gmoExon.
- **gsnorm**: character. specifying the algorithm of global scaling normalisation.
- **savepar**: Logical value. If TRUE the estimated parameters of the model are saved in file `par\_gmoExon.txt`
- **eps**: Optimisation termination criteria.
- **addConstant**: numeric. This is an experimental feature and should not generally be changed from the default value.
- **cl**: This function can be parallelised by setting parameter cl. For more details, please refer to the vignette.
- **BatchFold**: we divide tasks into BatchFold*n jobs where n is the number of cluster nodes. The first n jobs are placed on the n nodes. When the first job is completed, the next job is placed on the available node. This continues until all jobs are completed. The default value is ten. The user also can change the value according to the number of cluster nodes n. We suggest that for bigger n BatchFold should be smaller.
Details
The obtained expression measures are in log base 2 scale. Using the known relationships between
genes, transcripts and probes, we propose a gamma model for exon array data to calculate transcript
and gene expression levels. The algorithms of global scaling normalisation can be one of "median",
"none", "mean", "meanlog". "mean" and "meanlog" are mean-centered normalisation on raw scale
and log scale respectively, and "median" is median-centered normalisation. "none" will result in no
global scaling normalisation being applied. This function can be parallelised by setting parameter
cl. For more details, please refer to the vignette.

Value
A list of two object of class exprResult.

Author(s)
Xuejun Liu, Zhenzhu gao, Magnus Rattray, Marta Milo, Neil D. Lawrence

References
probe-level analysis across multiple chips, Bioinformatics, 21:3637-3644.
for summarising oligonucleotide gene expression data, technical report available upon request.
Milo,M., Fazeli,A., Niranjan,M. and Lawrence,N.D. (2003) A probabilistic model for the extrac-
tion of expression levels from oligonucleotide arrays, Biochemical Society Transactions, 31: 1510-
1512.
Peter Spellucci. DONLP2 code and accompanying documentation. Electronically available via
http://plato.la.asu.edu/donlp2.html
Risueno A, Fontanillo C, Dinger ME, De Las Rivas J. GATExplorer: genomic and transcriptomic
explorer; mapping expression probes to gene loci, transcripts, exons and ncRNAs. BMC Bioinfor-
matics.2010.

See Also
Related class exprResult-class

Examples
## The following scripts show the use of the method.
## load CEL files
# celFiles<-c("SR20070419HEX01.CEL", "SR20070419HEX02.CEL","SR20070419HEX06.CEL","SR20070419HEX07.CEL)
#oligo_object.exon<-read.celfiles(celFiles);

## use method gmoExon to calculate the expression levels and related confidence
## of the measures for the example data
#eset_gmoExon<-gmoExon(oligo_object.exon,exontype="Human",gsnorm="none",cl=cl)
Combining replicates for each condition with the true gene expression

Description

This function calculates the combined (from replicates) signal for each condition using Bayesian models, which are added a hidden variable to represent the true expression for each gene on each chip. The inputs are gene expression levels and the probe-level standard deviations associated with expression measurements for each gene on each chip. The outputs include gene expression levels and standard deviation for each condition.

Usage

```
hcmb(e, se, replicates, max_num=c(200,500,1000),gsnorm=FALSE, eps=1.0e-6)
```

Arguments

- `e`: a data frame containing the expression level for each gene on each chip.
- `se`: a data frame containing the standard deviation of gene expression levels.
- `replicates`: a vector indicating which chip belongs to which condition.
- `max_num`: integer. The maximum number of iterations controls the convergence.
- `gsnorm`: logical specifying whether do global scaling normalisation or not.
- `eps`: a numeric, optimisation parameter.

Details

Each element in replicate represents the condition of the chip which is in the same column order as in the expression and standard deviation matrix files.

The `max_num` is used to control the maximum number of the iterations in the EM algorithm. The best value of the `max_num` is from 200 to 1000, and should be set 200 at least. The default value is 200.

Value

The result is a data frame with components named 'M1', 'M2', and so on, which represent the mean expression values for condition 1, condition 2, and so on. It also has components named 'Std1', 'Std2', and so on, which represent the standard deviation of the gene expression values for condition 1, condition 2, and so on.

Author(s)

Li Zhang, Xuejun Liu

References

See Also

Related method `pumaCombImproved`, `mmgmos` and `pplr`.

Examples

```r
data(exampleE)
data(exampleStd)
r <- hcomb(exampleE, exampleStd, replicates = c(1, 1, 2, 2))
```

---

### hgu95aphis

*Estimated parameters of the distribution of phi*

<table>
<thead>
<tr>
<th></th>
<th>Estimated parameters of the distribution of phi</th>
</tr>
</thead>
<tbody>
<tr>
<td>hgu95aphis</td>
<td>0.171 -1.341 0.653</td>
</tr>
</tbody>
</table>

**Description**

The pre-estimated parameters of log normal distribution of $\phi$, which is the fraction of specific signal binding to mismatch probe.

**Usage**

```r
data(hgu95aphis)
```

**Format**

- The format is: num [1:3] 0.171 -1.341 0.653

**Details**

- The current values of hgu95aphis are estimated from Affymetrix spike-in data sets. It was loaded in the method "mmgmos".
- hgu95aphis[1:3] is respectively the mode, mean and variance of the log normal distribution of $\phi$, and hgu95aphis[1] is also the initial value of $\phi$ in the model optimisation.

---

### igmoExon

*Separately Compute gene and transcript expression values and standard deviations from exon CEL Files by the conditions.*

**Description**

The principle of this function is as same as the function gmoExon. This function separately calculates gene expression values by the conditions and then combined every condition’s results, and normalises them finally.
Usage

```r
igmoExon(
  cel.path,
  SampleNameTable,
  exontype = c("Human", "Mouse", "Rat"),
  background=FALSE,
  gsnorm=c("median", "none", "mean", "meanlog"),
  savepar=FALSE,
  eps=1e-6,
  addConstant = 0,
  condition=c("Yes", "No"),
  cl=NULL,
  BatchFold=10)
```

Arguments

cel.path  The directory where you put the CEL files.
SampleNameTable  It is a tab-separated table with two columns, ordered by "Celnames", "Condition"
exontype  character. specifying the type of exon chip.
background  Logical value. If TRUE, perform background correction before applying gmoExon.
savepar  Logical value. If TRUE the estimated parameters of the model are saved in file `par\_gmoExon.txt`
eps  Optimisation termination criteria.
addConstant  numeric. This is an experimental feature and should not generally be changed from the default value.
gsnorm  character. specifying the algorithm of global scaling normalisation.
condition  Yes or No. "Yes" means the `igmoExon` function separately calculates gene expression values by the conditions and then combined every condition’s results, and normalises them finally. "No" means the `igmoExon` calculates the gene expression values as same as the `gmoExon` function.
cl  This function can be parallelised by setting parameter cl. For more details, please refer to the vignette.
BatchFold  we divide tasks into BatchFold*n jobs where n is the number of cluster nodes. The first n jobs are placed on the n nodes. When the first job is completed, the next job is placed on the available node. This continues until all jobs are completed. The default value is ten. The user also can change the value according to the number of cluster nodes n. We suggest that for bigger n BatchFold should be smaller.

Details

The obtained expression measures are in log base 2 scale. Using the known relationships between genes, transcripts and probes, we propose a gamma model for exon array data to calculate transcript and gene expression levels. The algorithms of global scaling normalisation can be one of "median", "none", "mean", "meanlog", "mean" and "meanlog" are mean-centered normalisation on raw scale and log scale respectively, and "median" is median-centered normalisation. "none" will result in no global scaling normalisation being applied.
Value

A list of two objects of class `exprReslt`.

Author(s)

Xuejun Liu, Zhenzhu Gao, Magnus Rattray, Marta Milo, Neil D. Lawrence

References


Peter Spellucci. DONLP2 code and accompanying documentation. Electronically available via http://plato.la.asu.edu/donlp2.html


See Also

Related class `exprReslt-class`

Examples

```r
## The following scripts show the use of the method.
## load CEL files
# cel.path<-cel.path;
# SampleNameTable<="SampleNameTable"
# eset_igmoExon<-igmoExon(cel.path="cel.path"
# , SampleNameTable="SampleNameTable"
# , exontype="Human"
# , gsnorm="none", condition="Yes", cl=cl)
```

justmgMOS

**Compute mgmos Directly from CEL Files**

Description

This function converts CEL files into an `exprReslt` using mgmos.
justmgMOS

Usage

justmgMOS(..., filenames=character(0),
    widget=getOption("BioC")$affy$use.widgets,
    compress=getOption("BioC")$affy$compress.cel,
    celfile.path=getwd(),
    sampleNames=NULL,
    phenoData=NULL,
    description=NULL,
    notes="",
    background=TRUE, gsnorm=c("median", "none", "mean", "meanlog"), savepar=FALSE, eps=1.0e-6)

just.mgmos(..., filenames=character(0),
    phenoData=new("AnnotatedDataFrame"),
    description=NULL,
    notes="",
    compress=getOption("BioC")$affy$compress.cel,
    background=TRUE, gsnorm=c("median", "none", "mean", "meanlog"), savepar=FALSE, eps=1.0e-6)

Arguments

... file names separated by comma.
filenames file names in a character vector.
widget a logical specifying if widgets should be used.
compress are the CEL files compressed?
celfile.path a character denoting the path where cel files locate.
sampleNames a character vector of sample names to be used in the FeatureSet.
phenoData an AnnotatedDataFrame object.
description a MIAME object.
notes notes.
background Logical value. If TRUE, then perform background correction before applying mgmos.
gsnorm character. specifying the algorithm of global scaling normalisation.
savepar Logical value. If TRUE, then the estimated parameters of the model are saved in file par\_mgmos.txt and phi\_mgmos.txt.
eps Optimisation termination criteria.

Details

This method should require much less RAM than the conventional method of first creating an FeatureSet and then running mgmos.

Note that this expression measure is given to you in log base 2 scale. This differs from most of the other expression measure methods.

The algorithms of global scaling normalisation can be one of "median", "none", "mean", "meanlog". "mean" and "meanlog" are mean-centered normalisation on raw scale and log scale respectively, and "median" is median-centered normalisation. "none" will result in no global scaling normalisation being applied.
Value

An exprReslt.

See Also

Related class exprReslt-class and related method mgmos

justmmgMOS  
Compute mmgmos Directly from CEL Files

Description

This function converts CEL files into an exprReslt using mmgmos.

Usage

justmmgMOS(..., filenames=character(0),
        widget=getOption("BioC")$affy$use.widgets,
        compress=getOption("BioC")$affy$compress.cel,
        celfile.path=getwd(),
        sampleNames=NULL,
        phenoData=NULL,
        description=NULL,
        notes="",
        background=TRUE, gsnorm=c("median", "none", "mean", "meanlog"), savepar=FALSE, eps=1.0e-6)

just.mmgmos(..., filenames=character(0),
        phenoData=new("AnnotatedDataFrame"),
        description=NULL,
        notes="",
        compress=getOption("BioC")$affy$compress.cel,
        background=TRUE, gsnorm=c("median", "none", "mean", "meanlog"), savepar=FALSE, eps=1.0e-6)

Arguments

...  file names separated by comma.
filenames file names in a character vector.
widget a logical specifying if widgets should be used.
compress are the CEL files compressed?
celfile.path a character denoting the path where cel files locate.
sampleNames a character vector of sample names to be used in the FeatureSet.
phenoData an AnnotatedDataFrame object.
description a MIAME object
notes notes
background Logical value. If TRUE, then perform background correction before applying mmgmos.
gsnorm character. specifying the algorithm of global scaling normalisation.
savepar Logical value. If TRUE, the the estimated parameters of the model are saved in file par\_mmgmos.txt and phi\_mmgmos.txt.
eps Optimisation termination criteria.
This function can be used to add legends to plots. This is almost identical to the legend function, accept it has an extra parameter, seg.len which allows the user to change the lengths of lines shown in legends.

Usage

```r
legend2(x, y = NULL, legend, fill = NULL, col = par("col"),
  lty, lwd, pch, angle = 45, density = NULL, bty = "o", bg = par("bg"),
  box.lwd = par("lwd"), box.lty = par("lty"), pt.bg = NA, cex = 1,
  pt.cex = cex, pt.lwd = lwd, xjust = 0, yjust = 1, x.intersp = 1,
  y.intersp = 1, adj = c(0, 0.5), text.width = NULL, text.col = par("col"),
  merge = do.lines && has.pch, trace = FALSE, plot = TRUE,
  ncol = 1, horiz = FALSE, title = NULL, inset = 0, seg.len = 2)
```

Arguments

- `x, y` the x and y co-ordinates to be used to position the legend. They can be specified by keyword or in any way which is accepted by `xy.coords`. See Details.
- `legend` a character or `expression` vector. of length ≥ 1 to appear in the legend. Other objects will be coerced by `as.graphicsAnnot`.
- `fill` if specified, this argument will cause boxes filled with the specified colors (or shaded in the specified colors) to appear beside the legend text.
- `col` the color of points or lines appearing in the legend.
- `lty, lwd` the line types and widths for lines appearing in the legend. One of these two must be specified for line drawing.
Legend2

Arguments x, y, legend are interpreted in a non-standard way to allow the coordinates to be specified via one or two arguments. If legend is missing and y is not numeric, it is assumed that the second argument is intended to be legend and that the first argument specifies the coordinates. The coordinates can be specified in any which is accepted by xy.coords. If this gives the coordinates of one point, it is used as the top-left coordinate of the rectangle containing the legend.
If it gives the coordinates of two points, these specify opposite corners of the rectangle (either pair of corners, in any order).

The location may also be specified by setting `x` to a single keyword from the list "bottomright", "bottom", "bottomleft", "left", "topleft", "top", "topright", "right" and "center". This places the legend on the inside of the plot frame at the given location. Partial argument matching is used. The optional `inset` argument specifies how far the legend is inset from the plot margins. If a single value is given, it is used for both margins; if two values are given, the first is used for `x`-distance, the second for `y`-distance.

"Attribute" arguments such as `col`, `pch`, `lty`, etc, are recycled if necessary. `merge` is not.

Points are drawn after lines in order that they can cover the line with their background color `pt.bg`, if applicable.

See the examples for how to right-justify labels.

**Value**

A list with list components

- **rect**
  - a list with components
    - `w`, `h` positive numbers giving width and height of the legend’s box.
    - `left`, `top` x and y coordinates of upper left corner of the box.

- **text**
  - a list with components
    - `x`, `y` numeric vectors of length `length(legend)`, giving the x and y coordinates of the legend’s text(s).

returned invisibly.

**Author(s)**

Richard Pearson (modified from original `graphics` package function.)

**References**


**See Also**

`legend`

**Examples**

```r
x <- seq(-pi, pi, len = 65)
plot(x, sin(x), type = "l", ylim = c(-1.2, 1.8), col = 3, lty = 2)
points(x, cos(x), pch = 3, col = 4)
lines(x, tan(x), type = "b", lty = 1, pch = 4, col = 6)
title("legend(..., lty = c(2, -1, 1), pch = c(-1,3,4), merge = TRUE)",
cex.main = 1.1)
legend2(-1, 1.9, c("sin", "cos", "tan"), col = c(3,4,6),
text.col = "green4", lty = c(2, -1, 1), pch = c(-1, 3, 4),
merge = TRUE, bg = "gray90", seg.len=6)
```
license.puma  

Print puma license

Description
This function prints the license under which puma is made available.

Usage
license.puma()

Value
Null.

Author(s)
Richard Pearson (based on the license.cosmo function from the cosmo package)

Examples
license.puma()

matrixDistance  

Calculate distance between two matrices

Description
This calculates the mean Euclidean distance between the rows of two matrices. It is used in the function pumaPCA.

Usage
matrixDistance(
  matrixA,
  matrixB
)

Arguments

matrixA the first matrix
matrixB the second matrix

Value
A numeric giving the mean distance

Author(s)
Richard D. Pearson
See Also

Related class `pumaPCA`

Examples

```
show(matrixDistance(matrix(1,2,2),matrix(2,2,2)))
```

---

### mgmos

**modified gamma Model for Oligonucleotide Signal**

#### Description

This function converts an object of class `FeatureSet` into an object of class `exprResult` using the modified gamma Model for Oligonucleotide Signal (multi-mgMOS). This function obtains confidence of measures, standard deviation and 5, 25, 50, 75 and 95 percentiles, as well as the estimated expression levels.

#### Usage

```r
mgmos(
  object,
  background=FALSE,
  replaceZeroIntensities=TRUE,
  gsnorm=c("median", "none", "mean", "meanlog"),
  savepar=FALSE,
  eps=1.0e-6
)
```

#### Arguments

- **object**: an object of `FeatureSet`
- **background**: Logical value. If TRUE, perform background correction before applying mmgmos.
- **replaceZeroIntensities**: Logical value. If TRUE, replace 0 intensities with 1 before applying mmgmos.
- **gsnorm**: character. specifying the algorithm of global scaling normalisation.
- **savepar**: Logical value. If TRUE the estimated parameters of the model are saved in file `par\_mgmos.txt` and `phi\_mgmos.txt`.
- **eps**: Optimisation termination criteria.

#### Details

The obtained expression measures are in log base 2 scale.

The algorithms of global scaling normalisation can be one of "median", "none", "mean", "meanlog". "mean" and "meanlog" are mean-centered normalisation on raw scale and log scale respectively, and "median" is median-centered normalisation. "none" will result in no global scaling normalisation being applied.

There are 4*n columns in file `par\_mgmos.txt`, n is the number of chips. Every 4 columns are parameters for a chip. Among every 4 columns, the first one is for 'alpha' values, the 2nd one is for 'a' values, The 3rd column is for 'c' and the final column is values for 'd'.
Value
An object of class exprReslt.

Author(s)
Xuejun Liu, Magnus Rattray, Marta Milo, Neil D. Lawrence

References
Peter Spellucci. DONLP2 code and accompanying documentation. Electronically available via http://plato.la.asu.edu/donlp2.html

See Also
Related class exprReslt-class and related method mmmgmos

Examples
## Code commented out to speed up checks
## load example data from package pumadata
# if (require(pumadata)&require(puma)){
#  data(oligo.estrogen)
# use method mgMOS to calculate the expression levels and related confidence
# of the measures for the example data
#  eset<-mgmos(oligo.estrogen,gsnorm="none")
#}

---

### mmmgmos

**Multi-chip modified gamma Model for Oligonucleotide Signal**

**Description**
This function converts an object of class FeatureSet into an object of class exprReslt using the Multi-chip modified gamma Model for Oligonucleotide Signal (multi-mgMOS). This function obtains confidence of measures, standard deviation and 5, 25, 50, 75 and 95 percentiles, as well as the estimated expression levels.

**Usage**

```r
mmmngmos(
  object,
  background=FALSE,
  replaceZeroIntensities=TRUE,
  gsnorm=c("median", "none", "mean", "meanlog")
)```

```
mmgmos

, savepar = FALSE
, eps = 1.0e-6
, addConstant = 0
)

Arguments

object
background
replaceZeroIntensities
gsnorm
savepar
eps
addConstant

an object of FeatureSet
Logical value. If TRUE, perform background correction before applying mmgmos.
Logical value. If TRUE, replace 0 intensities with 1 before applying mmgmos.
character. specifying the algorithm of global scaling normalisation.
Logical value. If TRUE the estimated parameters of the model are saved in file
par\_mmgmos.txt and phi\_mmgmos.txt.
Optimisation termination criteria.
numeric. This is an experimental feature and should not generally be changed
from the default value.

Details

The obtained expression measures are in log base 2 scale.
The algorithms of global scaling normalisation can be one of "median", "none", "mean", "meanlog".
"mean" and "meanlog" are mean-centered normalisation on raw scale and log scale respectively, and
"median" is median-centered normalisation. "none" will result in no global scaling normalisation
being applied.

There are 2*n+2 columns in file par\_mmgmos.txt, n is the number of chips. The first n columns are
'alpha' values for n chips, the next n columns are 'a' values for n chips, column 2*n+1 is 'c' values
and the final column is values for 'd'. The file phi\_mmgmos.txt keeps the final optimal value of
'phi'.

Value

An object of class exprResult.

Author(s)

Xuejun Liu, Magnus Rattray, Marta Milo, Neil D. Lawrence

References

probe-level analysis across multiple chips, Bioinformatics 21: 3637-3644.
for summarising oligonucleotide gene expression data, technical report available upon request.
Milo,M., Fazeli,A., Niranjan,M. and Lawrence,N.D. (2003) A probabilistic model for the extrac-
tion of expression levels from oligonucleotide arrays, Biochemical Society Transactions, 31: 1510-
1512.

Peter Spellucci. DONLP2 code and accompanying documentation. Electronically available via
http://plato.la.asu.edu/donlp2.html
normalisation.gs

See Also
Related class exprReslt-class and related method mgmos

Examples
## Code commented out to speed up checks
## load example data from package affydata
# if (require(pumadata)&amp;require(puma)){
# data(oligo.estrogen)
## use method mmgMOS to calculate the expression levels and related confidence
## of the measures for the example data
# eset&lt;-mmgmos(oligo.estrogen,gsnorm="none")
#

data(exampleE)
exE.normalised&lt;-normalisation.gs(exampleE)
data(Clust.exampleE)
Clust.exampleE.normalised&lt;-normalisation.gs(Clust.exampleE)
numFP

Number of False Positives for a given proportion of True Positives.

Description

Often when evaluating a differential expression method, we are interested in how well a classifier performs for very small numbers of false positives. This method gives one way of calculating this, by determining the number of false positives for a set proportion of true positives.

Usage

numFP(scores, truthValues, TPRate = 0.5)

Arguments

scores    A vector of scores. This could be, e.g. one of the columns of the statistics of a DEResult object.
truthValues    A boolean vector indicating which scores are True Positives.
TPRate    A number between 0 and 1 identify the proportion of true positives for which we wish to determine the number of false positives.

Value

An integer giving the number of false positives.

Author(s)

Richard D. Pearson

See Also

Related methods plotROC and calcAUC.

Examples

class1a <- rnorm(1000,0.2,0.1)
class2a <- rnorm(1000,0.6,0.2)
class1b <- rnorm(1000,0.3,0.1)
class2b <- rnorm(1000,0.5,0.2)
scores_a <- c(class1a, class2a)
scores_b <- c(class1b, class2b)
classElts <- c(rep(FALSE,1000), rep(TRUE,1000))
print(numFP(scores_a, classElts))
print(numFP(scores_b, classElts))
**numOfFactorsToUse**

Determine number of factors to use from an ExpressionSet

**Description**

This is really an internal function used to determine how many factors to use in design and contrast matrices.

**Usage**

```r
numOfFactorsToUse(eset)
```

**Arguments**

- `eset`: An object of class `ExpressionSet`.

**Value**

An integer denoting the number of factors to be used.

**Author(s)**

Richard D. Pearson

**See Also**

Related methods `createDesignMatrix` and `createContrastMatrix`

**Examples**

```r
# Next 4 lines commented out to save time in package checks, and saved version used
# if (require(affydata)) {
#   data(Dilution)
#   eset_mmgmos <- mmgmos(Dilution)
# }
data(eset_mmgmos)
umOfFactorsToUse(eset_mmgmos)
```

---

**numTP**

Number of True Positives for a given proportion of False Positives.

**Description**

Often when evaluating a differential expression method, we are interested in how well a classifier performs for very small numbers of true positives. This method gives one way of calculating this, by determining the number of true positives for a set proportion of false positives.

**Usage**

```r
numTP(scores, truthValues, FPRate = 0.5)
```
Arguments

scores A vector of scores. This could be, e.g. one of the columns of the statistics of a DEResult object.

truthValues A boolean vector indicating which scores are True Positives.

FPRate A number between 0 and 1 identify the proportion of false positives for which we wish to determine the number of true positives.

Value

An integer giving the number of true positives.

Author(s)

Richard D. Pearson

See Also

Related methods numFP, plotROC and calcAUC.

Examples

class1a <- rnorm(1000,0.2,0.1)
class2a <- rnorm(1000,0.6,0.2)
class1b <- rnorm(1000,0.3,0.1)
class2b <- rnorm(1000,0.5,0.2)
scores_a <- c(class1a, class2a)
scores_b <- c(class1b, class2b)
classElts <- c(rep(FALSE,1000), rep(TRUE,1000))
print(numTP(scores_a, classElts))
print(numTP(scores_b, classElts))

Description

This is the original version of the pplr function as found in the pplr package. This should give exactly the same results as the pplr function. This function is only included for testing purposes and is not intended to be used. It will not be available in future versions of puma.

This function calculates the probability of positive log-ratio (PPLR) between any two specified conditions in the input data, mean and standard deviation of gene expression level for each condition.

Usage

orig_pplr(e, control, experiment)

Arguments

e a data frame containing the mean and standard deviation of gene expression levels for each condition.

control an integer denoting the control condition.

experiment an integer denoting the experiment condition.
Details

The input of 'e' should be a data frame comprising of 2*n components, where n is the number of conditions. The first 1,2,...,n components include the mean of gene expression values for conditions 1,2,...,n, and the n+1, n+2,...,2*n components contain the standard deviation of expression levels for condition 1,2,...,n.

Value

The return is a data frame. The description of the components are below.

<table>
<thead>
<tr>
<th>index</th>
<th>The original row number of genes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cM</td>
<td>The mean expression levels under control condition.</td>
</tr>
<tr>
<td>sM</td>
<td>The mean expression levels under experiment condition.</td>
</tr>
<tr>
<td>cStd</td>
<td>The standard deviation of gene expression levels under control condition.</td>
</tr>
<tr>
<td>sStd</td>
<td>The standard deviation of gene expression levels under experiment condition.</td>
</tr>
<tr>
<td>LRM</td>
<td>The mean log-ratio between control and experiment genes.</td>
</tr>
<tr>
<td>LRStd</td>
<td>The standard deviation of log-ratio between control and experiment genes.</td>
</tr>
<tr>
<td>stat</td>
<td>A statistic value which is -mean/(sqrt(2)*standard deviation).</td>
</tr>
<tr>
<td>PPLR</td>
<td>Probability of positive log-ratio.</td>
</tr>
</tbody>
</table>

Author(s)

Xuejun Liu, Marta Milo, Neil D. Lawrence, Magnus Rattray

References


See Also

Related method bcomb

Examples

data(exampleE)
data(exampleStd)
r<-bcomb(exampleE,exampleStd,replicates=c(1,1,1,2,2,2),method="map")
p<-orig_pplr(r,1,2)
plotErrorBars

Plot method for pumaPCARes objects

Description

This is the method to plot objects of class pumaPCARes. It will produce a scatter plot of two of the principal components.

Usage

```r
## S4 method for signature 'pumaPCARes,missing'
plot(..., firstComponent = 1, secondComponent = 2, useFilenames = FALSE, phenotype = pData(pumaPCARes@phenoData), legend1pos = "topright", legend2pos = "bottomright")
```

Arguments

- `...` Optional graphical parameters to adjust different components of the plot
- `firstComponent` Integer identifying which principal component to plot on the x-axis
- `secondComponent` Integer identifying which principal component to plot on the x-axis
- `useFilenames` Boolean. If TRUE then use filenames as plot points. Otherwise just use points.
- `phenotype` Phenotype information
- `legend1pos` String indicating where to put legend for first factor
- `legend2pos` String indicating where to put legend for second factor

Examples

```r
# Next 4 lines commented out to save time in package checks, and saved version used
# if (require(affydata)) {
#   data(Dilution)
#   eset_mmgmos <- mmgmos(Dilution)
# }
data(eset_mmgmos)
pumapca_mmgmos <- pumaPCA(eset_mmgmos)
plot(pumapca_mmgmos)
```

plotErrorBars

Plot mean expression levels and error bars for one or more probesets

Description

This produces plots of probesets of interest.
Usage

plotErrorBars(
eset
, probesets = if(dim(exprs(eset))[[1]] <= 12) 1:dim(exprs(eset))[[1]] else 1
 , arrays = 1:dim(pData(eset))[[1]] # default is to use all
 , xlab = paste(colnames(pData(eset))[[1:numOfFactorsToUse(eset)]], collapse=":")
 , ylab = "Expression Estimate"
 , xLabels = apply(
     as.matrix(pData(eset)[arrays,1:numOfFactorsToUse(eset)])
     , 1
     , function(mat){paste(mat, collapse=":")}
     
     
   , ylim = NA
   , numOfSEs = qnorm(0.975)
   , globalYlim = FALSE # Not yet implemented!
   , plot_cols = NA
   , plot_rows = NA
   , featureNames = NA
   , showGeneNames = FALSE
   , showErrorBars = if(
       length(assayDataElement(eset,"se.exprs"))==0 ||
       length(assayDataElement(eset,"se.exprs")) == sum(is.na(assayDataElement(eset,"se.exprs")))
     ) FALSE else TRUE
   , plotColours = FALSE
   , log.it = if(max(exprs(eset)) > 32) TRUE else FALSE
   , eset_comb = NULL
   , jitterWidth = NA
   , qtpcrData = NULL
   , ...
)

Arguments

eset An object of class ExpressionSet. This is the main object being plotted.
probesets A vector of integers indicating the probesets to be plotted. These integers refer
to the row numbers of the eset.
arrays A vector of integers indicating the arrays to be shown on plots.
xlab Character string of title to appear on x-axis
ylab Character string of title to appear on y-axis
xLabels Vector of strings for labels of individual points on x-axis.
ylim 2-element numeric vector showing minimum and maximum values for y-axis.
umOfSEs Numeric indicating the scaling for the error bars. The default value give error
bars that include 95% of expected values.
globalYlim Not yet implemented!
plot_cols Integer specifying number of columns for multi-figure plot.
plot_rows Integer specifying number of rows for multi-figure plot.
featureNames A vector of strings for featureNames (Affy IDs). This is an alternative (to the
probesets argument) way of specifying probe sets.
showGeneNames Boolean indicating whether to use Affy IDs as titles for each plot.
showErrorBars  Boolean indicating whether error bars should be shown on plots.
plotColours    A vector of colours to plot.
log.it         Boolean indicating whether expression values should be logged.
eset_comb      An object of class ExpressionSet. This is a secondary object to be plotted on
               the same charts as eset. This should be an object created using pumaComb and
               pumaCombImproved which holds the values created by combining information
               from the replicates of each condition.
jitterWidth    Numeric indicating the x-axis distance between replicates of the same condition.
qtpcrData      A 2-column matrix of qRT-PCR values (or other data to be plotted on the same
               charts).

...            Additional arguments to be passed to plot.

Value

This function has no return value. The output is the plot created.

Author(s)

Richard D. Pearson

Examples

# Next 4 lines commented out to save time in package checks, and saved version used
# if (require(affydata)) {
#  data(Dilution)
#  eset_mmgmos <- mmgmos(Dilution)
# }
data(eset_mmgmos)
plotErrorBars(eset_mmgmos)
plotErrorBars(eset_mmgmos,1:6)

plotHistTwoClasses     Stacked histogram plot of two different classes

Description

Stacked histogram plot of two different classes

Usage

plotHistTwoClasses(
  scores,
  class1Elements,
  class2Elements,
  space=0,
  col=c("white", "grey40"),
  xlab="PPLR",
  ylab="Number of genes",
  ylim=NULL,
  las=0 # axis labels all perpendicular to axes
plotHistTwoClasses

scores A numeric vector of scores (e.g. from the output of pumaDE)
class1Elements Boolean vector, TRUE if element is in first class
class2Elements Boolean vector, TRUE if element is in second class
space Numeric. x-axis distance between bars
col Colours for the two different classes
xlab Title for the x-axis
ylab Title for the y-axis
ylim 2-element numeric vector showing minimum and maximum values for y-axis.
las See par. Default of 0 means axis labels all perpendicular to axes.
legend 2-element string vector giving text to appear in legend for the two classes.
inset See legend
minScore Numeric. Minimum score to plot.
maxScore Numeric. Maximum score to plot.
numOfBars Integer. Number of bars to plot.
main String. Main title for the plot.

Value
This function has no return value. The output is the plot created.

Author(s)
Richard D. Pearson

Examples

class1 <- rnorm(1000,0.2,0.1)
class2 <- rnorm(1000,0.6,0.2)
class1[which(class1<0)] <- 0
class1[which(class1>1)] <- 1
class2[which(class2<0)] <- 0
class2[which(class2>1)] <- 1
scores <- c(class1, class2)
class1elts <- c(rep(TRUE,1000), rep(FALSE,1000))
class2elts <- c(rep(FALSE,1000), rep(TRUE,1000))
plotHistTwoClasses(scores, class1elts, class2elts, ylim=c(0,300))
plotROC  

Receiver Operator Characteristic (ROC) plot

**Description**

Plots a Receiver Operator Characteristic (ROC) curve.

**Usage**

```r
plotROC(
scoresList,
truthValues,
includedProbesets=1:length(truthValues),
legendTitles=1:length(scoresList),
main = "PUMA ROC plot",
lty = 1:length(scoresList),
col = rep(1,length(scoresList)),
lwd = rep(1,length(scoresList)),
yaxisStat = "tpr",
xaxisStat = "fpr",
downsampling = 100,
showLegend = TRUE,
showAUC = TRUE,
,...
)
```

**Arguments**

- **scoresList**  
  A list, each element of which is a numeric vector of scores.

- **truthValues**  
  A boolean vector indicating which scores are True Positives.

- **includedProbesets**  
  A vector of indices indicating which scores (and truthValues) are to be used in the calculation. The default is to use all, but a subset can be used if, for example, you only want a subset of the probesets which are not True Positives to be treated as False Positives.

- **legendTitles**  
  Vector of names to appear in legend.

- **main**  
  Main plot title

- **lty**  
  Line types.

- **col**  
  Colours.

- **lwd**  
  Line widths.

- **yaxisStat**  
  Character string identifying what is to be plotted on the y-axis. The default is "tpr" for True Positive Rate. See `performance` function from `ROCR` package.

- **xaxisStat**  
  Character string identifying what is to be plotted on the x-axis. The default is "fpr" for False Positive Rate. See `performance` function from `ROCR` package.

- **downsampling**  
  See details for `plot.performance` from the `ROCR` package.

- **showLegend**  
  Boolean. Should legend be displayed?

- **showAUC**  
  Boolean. Should AUC values be included in legend?

- **...**  
  Other parameters to be passed to `plot`. 
Description

A plot showing error bars for genes of interest.

Usage

```r
plotWhiskers(
eset
,comparisons=c(1,2)
,sortMethod = c("logRatio", "PPLR")
,numGenes=50
,xlim
,main = "PUMA Whiskers plot"
,highlightedGenes=NULL
)
```

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>eset</code></td>
<td>An object of class <code>ExpressionSet</code>.</td>
</tr>
<tr>
<td><code>comparisons</code></td>
<td>A 2-element integer vector specifying the columns of data to be compared.</td>
</tr>
<tr>
<td><code>sortMethod</code></td>
<td>The method used to sort the genes. &quot;logRatio&quot; is fold change. PPLR is Probability of Positive Log Ratio (as determined by the <code>pumaDE</code> method).</td>
</tr>
<tr>
<td><code>numGenes</code></td>
<td>Integer. Number of probesets to plot.</td>
</tr>
<tr>
<td><code>xlim</code></td>
<td>The x limits of the plot. See <code>plot.default</code>.</td>
</tr>
<tr>
<td><code>main</code></td>
<td>A main title for the plot. See <code>plot.default</code>.</td>
</tr>
<tr>
<td><code>highlightedGenes</code></td>
<td>Row numbers of probesets to highlight with an asterisk.</td>
</tr>
</tbody>
</table>

Examples

```r
class1a <- rnorm(1000,0.2,0.1)
class2a <- rnorm(1000,0.6,0.2)
class1b <- rnorm(1000,0.3,0.1)
class2b <- rnorm(1000,0.5,0.2)
scores_a <- c(class1a, class2a)
scores_b <- c(class1b, class2b)
scores <- list(scores_a, scores_b)
classElts <- c(rep(FALSE,1000), rep(TRUE,1000))
plotROC(scores, classElts)
```
**PMmmgmos**

This function has no return value. The output is the plot created.

**Author(s)**

Richard D. Pearson

**See Also**

Related method `pumaDE`

---

| **PMmmgmos** | **Multi-chip modified gamma Model for Oligonucleotide Signal using only PM probe intensities** |

---

**Description**

This function converts an object of class `FeatureSet` into an object of class `exprResult` using the Multi-chip modified gamma Model for Oligonucleotide Signal (PMmulti-mgMOS). This method uses only PM probe intensities. This function obtains confidence of measures, standard deviation and 5, 25, 50, 75 and 95 percentiles, as well as the estimated expression levels.

**Usage**

```r
PMmmgmos(
  object, background=TRUE, replaceZeroIntensities=TRUE,
  gsnorm=c("median", "none", "mean", "meanlog"), savepar=FALSE,
  eps=1.0e-6, addConstant = 0
)
```

**Arguments**

- `object` an object of `FeatureSet`
- `background` Logical value. If TRUE, perform background correction before applying `PMmmgmos`.
- `replaceZeroIntensities` Logical value. If TRUE, replace 0 intensities with 1 before applying `PMmmgmos`.
- `gsnorm` character. specifying the algorithm of global scaling normalisation.
- `savepar` Logical value. If TRUE the estimated parameters of the model are saved in file `par\_pmmmgmos.txt`.
- `eps` Optimisation termination criteria.
- `addConstant` numeric. This is an experimental feature and should not generally be changed from the default value.
Details

The obtained expression measures are in log base 2 scale.

The algorithms of global scaling normalisation can be one of "median", "none", "mean", "meanlog". "mean" and "meanlog" are mean-centered normalisation on raw scale and log scale respectively, and "median" is median-centered normalisation. "none" will result in no global scaling normalisation being applied.

There are n+2 columns in file par\_pmmmgmos.txt, n is the number of chips. The first n columns are 'alpha' values for n chips, column n+1 is 'c' values and the final column is values for 'd'.

Value

An object of class exprReslt.

Author(s)

Xuejun Liu, Zhenzhu Gao, Magnus Rattray, Marta Milo, Neil D. Lawrence

References


Peter Spellucci. DONLP2 code and accompanying documentation. Electronically available via http://plato.la.asu.edu/donlp2.html

See Also

Related class exprReslt-class and related method mgmos

Examples

## Code commented out to speed up checks
## load example data from package pumadata
#if (require(pumadata)&amp;require(puma)){
  # data(oligo.estrogen)
## use method PMmmgmos to calculate the expression levels and related confidence
## of the measures for the example data
  # eset&lt;-PMmmgmos(oligo.estrogen,gsnorm="none")
#}
**pplr**  
*Probability of positive log-ratio*

### Description

WARNING - this function is generally not expected to be used, but is intended as an internal function. It is included for backwards compatibility with the *pplr* package, but may be deprecated and then hidden in future. Users should generally use *pumaDE* instead.

This function calculates the probability of positive log-ratio (PPLR) between any two specified conditions in the input data, mean and standard deviation of gene expression level for each condition.

### Usage

```r
pplr(e, control, experiment, sorted=TRUE)
```

### Arguments

- **e**
  a data frame containing the mean and standard deviation of gene expression levels for each condition.

- **control**
  an integer denoting the control condition.

- **experiment**
  an integer denoting the experiment condition.

- **sorted**
  Boolean. Should PPLR values be sorted by value? If FALSE, PPLR values are returned in same order as supplied.

### Details

The input of 'e' should be a data frame comprising of 2*n components, where n is the number of conditions. The first 1,2,...,n components include the mean of gene expression values for conditions 1,2,...,n, and the n+1, n+2,...,2*n components contain the standard deviation of expression levels for condition 1,2,...,n.

### Value

The return is a data frame. The description of the components are below.

- **index**
  The original row number of genes.

- **cM**
  The mean expression levels under control condition.

- **sM**
  The mean expression levels under experiment condition.

- **cStd**
  The standard deviation of gene expression levels under control condition.

- **sStd**
  The standard deviation of gene expression levels under experiment condition.

- **LRM**
  The mean log-ratio between control and experiment genes.

- **LRStd**
  The standard deviation of log-ratio between control and experiment genes.

- **stat**
  A statistic value which is -mean/(sqrt(2)*standard deviation).

- **PPLR**
  Probability of positive log-ratio.

### Author(s)

Xuejun Liu, Marta Milo, Neil D. Lawrence, Magnus Rattray
References

See Also
Related methods pumaDE, bcomb and hcomb

Examples
data(exampleE)
data(exampleStd)
r<-bcomb(exampleE,exampleStd,replicates=c(1,1,1,2,2,2),method="map")
p<-pplr(r,1,2)

pplrUnsorted

Return an unsorted matrix of PPLR values

Description
Returns the output from pplr as an unsorted matrix (i.e. sorted according to the original sorting in the original matrix)

Usage
pplrUnsorted(p)

Arguments
p A matrix as output by pplr.

Value
A matrix of PPLR values

Author(s)
Richard D. Pearson

See Also
Related method pplr
pumaClust

Propagate probe-level uncertainty in model-based clustering on gene expression data

Description

This function clusters gene expression using a Gaussian mixture model including probe-level measurement error. The inputs are gene expression levels and the probe-level standard deviation associated with expression measurement for each gene on each chip. The outputs is the clustering results.

Usage

pumaClust(e=NULL, se=NULL, efile=NULL, sefile=NULL, subset=NULL, gsnorm=FALSE, clusters, iter.max=100, nstart=10, eps=1.0e-6, de10=0.01)

Arguments

e either a valid ExpressionSet object, or a data frame containing the expression level for each gene on each chip.
se data frame containing the standard deviation of gene expression levels.
efile character, the name of the file which contains gene expression measurements.
sefile character, the name of the file which contains the standard deviation of gene expression measurements.
subset vector specifying the row number of genes which are clustered on.
gsnorm logical specifying whether do global scaling normalisation or not.
clusters integer, the number of clusters.
iter.max integer, the maximum number of iterations allowed in the parameter initialisation.
nstart integer, the number of random sets chosen in the parameter initialisation.
eps numeric, optimisation parameter.
de10 numeric, optimisation parameter.

Details

The input data is specified either as an ExpressionSet object (in which case se, efile and sefile will be ignored), or by e and se, or by efile and sefile.

Value

The result is a list with components

cluster: vector, containing the membership of clusters for each gene; centers: matrix, the center of each cluster; centersigs: matrix, the center variance of each cluster; likelipergene: matrix, the likelihood of belonging to each cluster for each gene; bic: numeric, the Bayesian Information Criterion score.
pumaClustii

Author(s)
Xuejun Liu, Magnus Rattray

References

See Also
Related method mmgmos and pumaClustii

Examples

data(Clust.exampleE)
data(Clust.exampleStd)
pumaClust.example<-pumaClust(Clust.exampleE,Clust.exampleStd,clusters=7)

pumaClustii

Propagate probe-level uncertainty in robust t mixture clustering on replicated gene expression data

Description
This function clusters gene expression by including uncertainties of gene expression measurements from probe-level analysis models and replicate information into a robust t mixture clustering model. The inputs are gene expression levels and the probe-level standard deviation associated with expression measurement for each gene on each chip. The outputs is the clustering results.

Usage
pumaClustii(e=NULL, se=NULL, efile=NULL, sefile=NULL, subset=NULL, gsnorm=FALSE, mincls, maxcls, conds, reps, verbose=FALSE, eps=1.0e-5, del0=0.01)

Arguments
e data frame containing the expression level for each gene on each chip.
se data frame containing the standard deviation of gene expression levels.
efile character, the name of the file which contains gene expression measurements.
sefile character, the name of the file which contains the standard deviation of gene expression measurements.
subset vector specifying the row number of genes which are clustered on.
gsnorm logical specifying whether do global scaling normalisation or not.
mincls integer, the minimum number of clusters.
maxcls integer, the maximum number of clusters.
conds integer, the number of conditions.
pumaClustii

reps vector, specifying which condition each column of the input data matrix belongs to.
verbose logical value. If 'TRUE' messages about the progress of the function is printed.
eps numeric, optimisation parameter.
de10 numeric, optimisation parameter.

details

The input data is specified either by e and se, or by efile and sefile.

value

The result is a list with components
cluster: vector, containing the membership of clusters for each gene; centers: matrix, the center of each cluster; centersigs: matrix, the center variance of each cluster; likeliper gene: matrix, the likelihood of belonging to each cluster for each gene; optK: numeric, the optimal number of clusters. optF: numeric, the maximised value of target function.

author(s)

Xuejun Liu

references


see also

Related method mmgmos and pumaclust

examples

data(Clustii.exampleE)
data(Clustii.exampleStd)
r<-vector(mode="integer",0)
for (i in c(1:20))
  for (j in c(1:4))
    r<-c(r,i)
cl<-pumaClustii(Clustii.exampleE,Clustii.exampleStd,mincls=6,maxcls=6,conds=20,reps=r,eps=1e-3)
pumaComb

Combining replicates for each condition

Description

This function calculates the combined (from replicates) signal for each condition using Bayesian models. The inputs are gene expression levels and the probe-level standard deviations associated with expression measurements for each gene on each chip. The outputs include gene expression levels and standard deviation for each condition.

Usage

pumaComb(
  eset,
  design.matrix=NULL,
  method="em",
  numOfChunks=1000,
  save_r=FALSE,
  cl=NULL,
  parallelCompute=FALSE
)

Arguments

- **eset**: An object of class ExpressionSet.
- **design.matrix**: A design matrix.
- **method**: Method "map" uses MAP of a hierarchical Bayesion model with Gamma prior on the between-replicate variance (Gelman et.al. p.285) and shares the same variance across conditions. This method is fast and suitable for the case where there are many conditions. Method "em" uses variational inference of the same hierarchical Bayesian model as in method "map" but with conjugate prior on between-replicate variance and shares the variance across conditions. This is generally much slower than "map", but is recommended where there are few conditions (as is usually the case).
- **numOfChunks**: An integer defining how many chunks the data is divided into before processing. There is generally no need to change the default value.
- **save_r**: Will save an internal variable r to a file. Used for debugging purposes.
- **cl**: A "cluster" object. See makeCluster function from snow package for more details (if available).
- **parallelCompute**: Boolean identifying whether processing in parallel should occur.

Details

It is generally recommended that data is normalised prior to using this function. Note that the default behaviour of mmsgmos is to normalise data so this shouldn’t generally be an issue. See the function pumaNormalize for more details on normalisation.
Value

The result is an ExpressionSet object.

Author(s)

Xuejun Liu, Marta Milo, Neil D. Lawrence, Magnus Rattray

References


See Also

Related methods pumaNormalize, bcomb, mmgmos and pumaDE

Examples

# Next 4 lines commented out to save time in package checks, and saved version used
# if (require(affydata)) {
# data(Dilution)
# eset_mmgmos <- mmgmos(Dilution)
# }

data(eset_mmgmos)

# Next line shows that eset_mmgmos has 4 arrays, each of which is a different
# condition (the experimental design is a 2x2 factorial, with both liver and
# scanner factors)
pData(eset_mmgmos)

# Next line shows expression levels of first 3 probe sets
exprs(eset_mmgmos)[1:3,]

# Next line used so eset_mmgmos only has information about the liver factor
# The scanner factor will thus be ignored, and the two arrays of each level
# of the liver factor will be treated as replicates
pData(eset_mmgmos) <- pData(eset_mmgmos)[,1,drop=FALSE]

# To save time we'll just use 100 probe sets for the example
eset_mmgmos_100 <- eset_mmgmos[1:100,]
eset_comb <- pumaComb(eset_mmgmos_100)

# We can see that the resulting ExpressionSet object has just two conditions
# and 1 expression level for each condition
pData(eset_comb)
exprs(eset_comb)[1:3,]
Description

This function calculates the combined (from replicates) signal for each condition using Bayesian models, which are added a hidden variable to represent the true expression for each gene on each chip. The inputs are gene expression levels and the probe-level standard deviations associated with expression measurements for each gene on each chip. The outputs include gene expression levels and standard deviation for each condition.

Usage

```r
pumaCombImproved(
  eset,
  design.matrix=NULL,
  numOfChunks=1000,
  maxOfIterations=200,
  save_r=FALSE,
  cl=NULL,
  parallelCompute=FALSE
)
```

Arguments

- `eset`: An object of class `ExpressionSet`.
- `design.matrix`: A design matrix.
- `numOfChunks`: An integer defining how many chunks the data is divided into before processing. There is generally no need to change the default value.
- `maxOfIterations`: The maximum number of iterations controls the convergence.
- `save_r`: Will save an internal variable `r` to a file. Used for debugging purposes.
- `cl`: A "cluster" object. See `makeCluster` function from `snow` package for more details (if available).
- `parallelCompute`: Boolean identifying whether processing in parallel should occur.

Details

It is generally recommended that data is normalised prior to using this function. Note that the default behaviour of `mmgmos` is to normalise data so this shouldn’t generally be an issue. See the function `pumaNormalize` for more details on normalisation.

The `maxOfIterations` is used to control the maximum number of the iterations in the EM algorithm. You can change the number of `maxOfIterations`, but the best value of the `maxOfIterations` is from 200 to 1000, and should be set 200 at least. The default value is 200.

Value

The result is an `ExpressionSet` object.
Author(s)

Li Zhang, Xuejun Liu

References


See Also

Related methods pumaNormalize, hcomb, mmgmos and pumaDE

Examples

```r
# Next 4 lines commented out to save time in package checks, and saved version used
# if (require(affydata)) {
# data(Dilution)
# eset_mmgmos <- mmgmos(Dilution)
# }
data(eset_mmgmos)

# Next line shows that eset_mmgmos has 4 arrays, each of which is a different
# condition (the experimental design is a 2x2 factorial, with both liver and
# scanner factors)
pData(eset_mmgmos)

# Next line shows that eset_mmgmos has 4 arrays, each of which is a different
# condition (the experimental design is a 2x2 factorial, with both liver and
# scanner factors)
pData(eset_mmgmos)

# Next line shows expression levels of first 3 probe sets
exprs(eset_mmgmos)[1:3,]

# Next line used so eset_mmgmos only has information about the liver factor
# The scanner factor will thus be ignored, and the two arrays of each level
# of the liver factor will be treated as replicates
pData(eset_mmgmos) <- pData(eset_mmgmos)[,1,drop=FALSE]

# To save time we'll just use 100 probe sets for the example
eset_mmgmos_100 <- eset_mmgmos[1:100,]
eset_combimproved <- pumaCombImproved(eset_mmgmos_100)

# We can see that the resulting ExpressionSet object has just two conditions
# and 1 expression level for each condition
pData(eset_combimproved)
exprs(eset_combimproved)[1:3,]
```

pumaDE  

**Calculate differential expression between conditions**

Description

The function generates lists of genes ranked by probability of differential expression (DE). This uses the PPLR method.
Usage

```r
pumaDE(
  eset,
  design.matrix = createDesignMatrix(eset),
  contrast.matrix = createContrastMatrix(eset)
)
```

Arguments

- `eset` An object of class `ExpressionSet`.
- `design.matrix` A design matrix
- `contrast.matrix` A contrast matrix

Details

A separate list of genes will be created for each contrast of interest.

Note that this class returns a `DEResult-class` object. This object contains information on both the PPLR statistic values (which should generally be used to rank genes for differential expression), as well as fold change values (which are generally not recommended for ranking genes, but which might be useful, for example, to use as a filter). To understand more about the object returned see `DEResult-class`, noting that when created a `DEResult` object with the `pumaDE` function, the statistic method should be used to return PPLR values. Also note that the `pLikeValues` method can be used on the returned object to create values which can more readily be compared with p-values returned by other methods such as variants of t-tests (limma, etc.).

While it is possible to run this function on data from individual arrays, it is generally recommended that this function is run on the output of the function `pumaComb` (which combines information from replicates).

Value

An object of class `DEResult-class`.

Author(s)

Richard D. Pearson

See Also

Related methods `calculateLimma`, `calculateFC`, `calculateTtest`, `pumaComb`, `pumaCombImproved`, `mmgmos`, `pplr`, `createDesignMatrix` and `createContrastMatrix`

Examples

```r
# Next 4 lines commented out to save time in package checks, and saved version used
# if (require(affydata)) {
#   data(Dilution)
#   eset_mmgmos <- mmgmos(Dilution)
# }
# data(eset_mmgmos)

# Next line shows that eset_mmgmos has 4 arrays, each of which is a different
# condition (the experimental design is a 2x2 factorial, with both liver and
```
# scanner factors)
pData(eset_mmgmos)

# Next line shows expression levels of first 3 probe sets
exprs(eset_mmgmos)[1:3,]

# Next line used so eset_mmgmos only has information about the liver factor
# The scanner factor will thus be ignored, and the two arrays of each level
# of the liver factor will be treated as replicates
pData(eset_mmgmos) <- pData(eset_mmgmos)[,1,drop=FALSE]

# To save time we'll just use 100 probe sets for the example
eset_mmgmos_100 <- eset_mmgmos[1:100,]
eset_comb <- pumaComb(eset_mmgmos_100)
eset_combimproved <- pumaCombImproved(eset_mmgmos_100)

pumaDEResults <- pumaDE(eset_comb)
pumaDEResults_improved <- pumaDE(eset_combimproved)

topGeneIDs(pumaDEResults,6) # Gives probeset identifiers
topGeneIDs(pumaDEResults_improved,6)
topGenes(pumaDEResults,6) # Gives row numbers
  topGenes(pumaDEResults_improved,6)
statistic(pumaDEResults)[topGenes(pumaDEResults,6),] # PPLR scores of top six genes
statistic(pumaDEResults_improved)[topGenes(pumaDEResults_improved,6),]
FC(pumaDEResults)[topGenes(pumaDEResults,6),] # Fold-change of top six genes
FC(pumaDEResults_improved)[topGenes(pumaDEResults_improved,6),]

---

### pumaDEUnsorted

**Return an unsorted matrix of PPLR values**

**Description**

Returns the output from `pumaDE` as an unsorted matrix (i.e. sorted according to the original sorting in the ExpressionSet)

**Usage**

`pumaDEUnsorted(pp)`

**Arguments**

- `pp`  
  A list as output by `pumaDE`.

**Value**

A matrix of PPLR values

**Author(s)**

Richard D. Pearson

**See Also**

Related method `pumaDE`
pumaFull

Perform a full PUMA analysis

Description

Full analysis including pumaPCA and mmgmos/pumaDE vs rma/limma comparison

Usage

pumaFull (
  ExpressionFeatureSet = NULL
  ,data_dir = getwd()
  ,load_ExpressionFeatureSet = FALSE
  ,calculate_eset = TRUE
  ,calculate_pumaPCAs = TRUE
  ,calculate_bcomb = TRUE
  ,mmgmosComparisons = FALSE
)

Arguments

ExpressionFeatureSet
An object of class FeatureSet.

data_dir
A character string specifying where data files are stored.

load_ExpressionFeatureSet
Boolean. Load a pre-existing ExpressionFeatureSet object? Note that this has to be named "ExpressionFeatureSet.rda" and be in the data_dir directory.

calculate_eset
Boolean. Calculate ExpressionSet from ExpressionFeatureSet object? If FALSE, files named "eset\_mmgmos.rda" and "eset\_rma.rda" must be available in the data_dir directory.

calculate_pumaPCAs
Boolean. Calculate pumaPCA from eset\_mmgmos object? If FALSE, a file named "pumaPCA\_results.rda" must be available in the data_dir directory.

calculate_bcomb
Boolean. Calculate pumaComb from eset\_mmgmos object? If FALSE, files named "eset\_comb.rda" and "eset\_normd\_comb.rda" must be available in the data_dir directory.

mmgmosComparisons
Boolean. If TRUE, will compare mmgmos with default settings, with mmgmos used with background correction.

Value

No return values. Various objects are saved as .rda files during the execution of this function, and various PDF files are created.

Author(s)

Richard D. Pearson
pumaNormalize

See Also
Related methods `pumaDE`, `createDesignMatrix` and `createContrastMatrix`

Examples
```r
## Code commented out to ensure checks run quickly
# if (require(pumadata)) data(oligo.estrogen)
# pumaFull(oligo.estrogen)
```

---

**Description**

This is used to apply a scaling normalization to set of arrays. This normalization can be at the array scale (thus giving all arrays the same mean or median), or at the probeset scale (thus giving all probesets the same mean or median).

It is generally recommended that the default option (median array scaling) is used after running `mmgmos` and before running `pumaComb` and/or `pumaDE`. There are however, situations where this might not be the recommended, for example in time series experiments where it is expected than there will be general up-regulation or down-regulation in overall gene expression levels between time points.

**Usage**

```r
pumaNormalize(
  eset,
  arrayScale = c("median", "none", "mean", "meanlog"),
  probesetScale = c("none", "mean", "median"),
  probesetNormalisation = NULL,
  replicates = list(1:dim(exprs(eset))[[2]])
)
```

**Arguments**

- `eset`: An object of class `ExpressionSet`.
- `arrayScale`: A method of scale normalisation at the array level.
- `probesetScale`: A method of scale normalisation at the probe set level.
- `probesetNormalisation`: If not NULL normalises the expression levels to have zero mean and adjusts the variance of the gene expression according to the zero-centered normalisation.
- `replicates`: List of integer vectors indicating which arrays are replicates.

**Value**

An object of class `ExpressionSet` holding the normalised data.

**Author(s)**

Richard D. Pearson
See Also
Methods `mmgmos`, `pumaComb` and `pumaDE`

Examples

```r
# Next 4 lines commented out to save time in package checks, and saved version used
# if (require(affydata)) {
# data(Dilution)
# eset_mmgmos <- mmgmos(Dilution)
# }
data(eset_mmgmos)
apply(exprs(eset_mmgmos), 2, median)
eset_mmgmos_normd <- pumaNormalize(eset_mmgmos)
apply(exprs(eset_mmgmos_normd), 2, median)
```

---

### `pumaPCA`

#### PUMA Principal Components Analysis

This function carries out principal components analysis (PCA), taking into account not only the expression levels of genes, but also the variability in these expression levels.

The various other `pumaPCA...` functions are called during the execution of `pumaPCA`.

#### Usage

```r
pumaPCA(
  eset,
  latentDim = if (dim(exprs(eset))[2] <= 3)
    dim(exprs(eset))[2] - 1
  else
    3,
  sampleSize = if (dim(exprs(eset))[1] <= 1000)
    dim(exprs(eset))[1]
  else
    1000, ## Set to integer or FALSE for all
  initPCA = TRUE, ## Initialise parameters with PCA
  randomOrder = FALSE, ## Update parameters in random order
  optimMethod = "BFGS", ## ?optim for details of methods
  stoppingCriterion = "deltaW" ## can also be "deltaL"
  tol = 1e-3, ## Stop when delta update < this
  stepChecks = FALSE, ## Check likelihood after each update?
  iterationNumbers = TRUE, ## Show iteration numbers?
  showUpdates = TRUE, ## Show values after each update?
  showTimings = TRUE, ## Show timings after each update?
  showPlot = TRUE, ## Show projection plot after each update?
  maxIters = 500, ## Number of EM iterations.
  transposeData = FALSE, ## Transpose eset matrices?
  returnExpectations = FALSE,
  returnData = FALSE,
  returnFeedback = FALSE
)
```

---

Description

This function carries out principal components analysis (PCA), taking into account not only the expression levels of genes, but also the variability in these expression levels.

The various other `pumaPCA...` functions are called during the execution of `pumaPCA`.

Usage

```r
pumaPCA(
  eset,
  latentDim = if (dim(exprs(eset))[2] <= 3)
    dim(exprs(eset))[2] - 1
  else
    3,
  sampleSize = if (dim(exprs(eset))[1] <= 1000)
    dim(exprs(eset))[1]
  else
    1000, ## Set to integer or FALSE for all
  initPCA = TRUE, ## Initialise parameters with PCA
  randomOrder = FALSE, ## Update parameters in random order
  optimMethod = "BFGS", ## ?optim for details of methods
  stoppingCriterion = "deltaW" ## can also be "deltaL"
  tol = 1e-3, ## Stop when delta update < this
  stepChecks = FALSE, ## Check likelihood after each update?
  iterationNumbers = TRUE, ## Show iteration numbers?
  showUpdates = TRUE, ## Show values after each update?
  showTimings = TRUE, ## Show timings after each update?
  showPlot = TRUE, ## Show projection plot after each update?
  maxIters = 500, ## Number of EM iterations.
  transposeData = FALSE, ## Transpose eset matrices?
  returnExpectations = FALSE,
  returnData = FALSE,
  returnFeedback = FALSE
)
```
Arguments

eset An object of class `ExpressionSet`.
latentDim An integer specifying the number of latent dimensions (kind of like the number of principal components).
sampleSize An integer specifying the number of probesets to sample (default is 1000), or FALSE, meaning use all the data.
initPCA A boolean indicating whether to initialise using standard PCA (the default, and generally quicker and recommended).
randomOrder A boolean indicating whether the parameters should be updated in a random order (this is generally not recommended, and the default is FALSE).
optimMethod See ?optim for details of methods.
stoppingCriterion If set to "deltaW" will stop when W changes by less than tol. If "deltaL" will stop when L (lambda) changes by less than tol.
tol Tolerance value for stoppingCriterion.
stepChecks Boolean. Check likelihood after each update?
iterationNumbers Boolean. Check likelihood after each update?
showUpdates Boolean. Show iteration numbers?
showTimings Boolean. Show values after each update?
showPlot Boolean. Show projection plot after each update?
maxIters Integer. Maximum number of EM iterations.
transposeData Boolean. Transpose eset matrices?
returnExpectations Boolean. Return expectation values?
returnData Boolean. Return expectation data?
returnFeedback Boolean. Return feedback on progress of optimisation?
pumaNormalize Boolean. Normalise data prior to running algorithm (recommended)?

Value

An object of class `pumaPCARes`

Author(s)

Richard D. Pearson

See Also

Related methods `pumaDE`, `createDesignMatrix` and `createContrastMatrix`
pumaPCAExpectations-class

Description

This is a class representation for storing a set of expectations from a pumaPCA model. It is an internal representation and shouldn’t normally be instantiated.

Objects from the Class

Objects can be created by calls of the form `new("pumaPCAExpectations", ...).

Slots

- `x`: Object of class "matrix" representing x
- `xxT`: Object of class "array" representing xT
- `logDetCov`: Object of class "numeric" representing logDetCov

Methods

This class has no methods defined

Author(s)

Richard D. Pearson

See Also

Related method `pumaPCA` and related class `pumaPCARes`.  

Examples

```r
# Next 4 lines commented out to save time in package checks, and saved version used
# if (require(affydata)) {
# data(Dilution)
# eset_mmgmos <- mmgmos(Dilution)
# }

data(eset_mmgmos)
pumapca_mmgmos <- pumaPCA(eset_mmgmos)
plot(pumapca_mmgmos)
```
Class pumaPCAModel

Description
This is a class representation for storing a pumaPCA model. It is an internal representation and shouldn’t normally be instantiated.

Objects from the Class
Objects can be created by calls of the form `new("pumaPCAModel", ...)`.

Slots
- `sigma`: Object of class "numeric" representing sigma
- `m`: Object of class "matrix" representing m
- `Cinv`: Object of class "matrix" representing Cinv
- `W`: Object of class "matrix" representing W
- `mu`: Object of class "matrix" representing mu

Methods
This class has no methods defined

Author(s)
Richard D. Pearson

See Also
Related method `pumaPCA` and related class `pumaPCARes`.

Class pumaPCARes

Description
This is a class representation for storing the outputs of the pumaPCA function. Objects of this class should usually only be created through the `pumaPCA` function.

Objects from the Class
Objects can be created by calls of the form `new("pumaPCARes", ...)`. 
removeUninformativeFactors

Slots

model: Object of class "pumaPCAModel" representing the model parameters
expectations: Object of class "pumaPCAExpectations" representing the model expectations
varY: Object of class "matrix" representing the variance in the expression levels
Y: Object of class "matrix" representing the expression levels
phenoData: Object of class "AnnotatedDataFrame" representing the phenotype information
timeToCompute: Object of class "numeric" representing the time it took pumaPCA to run
numberOfIterations: Object of class "numeric" representing the number of iterations it took pumaPCA to converge
likelihoodHistory: Object of class "list" representing the history of likelihood values while pumaPCA was running
timingHistory: Object of class "list" representing the history of how long each iteration took while pumaPCA was running
modelHistory: Object of class "list" representing the history of how the model was changing while pumaPCA was running
exitReason: Object of class "character" representing the reason pumaPCA halted. Can take the values "Update of Likelihood less than tolerance x", "Update of W less than tolerance x", "Iterations exceeded", "User interrupt", "unknown exit reason"

Methods

plot signature(x="pumaPCARes-class"): plots two principal components on a scatter plot.
write.reslts signature(x = "pumaPCARes-class"): writes the principal components for each array to a file. It takes the same arguments as write.table. The argument "file" does not need to set any extension. The file name and extension "csv" will be added automatically. The default file name is "tmp".

Author(s)

Richard D. Pearson

See Also

Related method pumaPCA and related class pumaPCARes.

removeUninformativeFactors

Remove uninformative factors from the phenotype data of an ExpressionSet

Description

This is really an internal function used to remove uninformative factors from the phenotype data. Uninformative factors here are defined as those which have the same value for all arrays in the ExpressionSet.

Usage

removeUninformativeFactors(eset)
Arguments

eset  
An object of class ExpressionSet.

Value

An ExpressionSet object with the same data as the input, except for a new phenoData slot.

Author(s)

Richard D. Pearson

See Also

Related methods createDesignMatrix and createContrastMatrix

Examples

eset_test <- new("ExpressionSet", exprs=matrix(rnorm(400,8,2),100,4))
pData(eset_test) <- data.frame("informativeFactor"=c("A", "A", "B", "B"), "uninformativeFactor"=c("X","X","X","X"))
eset_test2 <- removeUninformativeFactors(eset_test)
pData(eset_test)
pData(eset_test2)
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