Package ‘GeoDiff’

March 22, 2024

Type Package

Title Count model based differential expression and normalization on GeoMx RNA data

Version 1.8.0

Description A series of statistical models using count generating distributions for background modelling, feature and sample QC, normalization and differential expression analysis on GeoMx RNA data. The application of these methods are demonstrated by example data analysis vignette.

Imports Matrix, robust, plyr, lme4, Rcpp (>= 1.0.4.6), withr, methods, graphics, stats, testthat, GeomxTools, NanoStringNCTools

LinkingTo Rcpp, RcppArmadillo, roptim

License MIT + file LICENSE

URL https://github.com/Nanostring-Biostats/GeoDiff

BugReports https://github.com/Nanostring-Biostats/GeoDiff

Encoding UTF-8

Suggests knitr, rmarkdown, dplyr

VignetteBuilder knitr

Depends R (>= 4.1.0), Biobase

RoxygenNote 7.1.2

biocViews GeneExpression, DifferentialExpression, Normalization

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

git_branch RELEASE_3_18

git_last_commit 924bdcc

git_last_commit_date 2023-10-24

Repository Bioconductor 3.18

Date/Publication 2024-03-22

Author Nicole Ortogero [cre], Lei Yang [aut], Zhi Yang [aut]

Maintainer Nicole Ortogero <nortogero@nanostring.com>
### aggreprobe

**Description**

Generate aggregated counts of probes for the same target, based on their score test results or correlation.

**Usage**

```r
aggreprobe(object, ...)
```

## S4 method for signature 'NanoStringGeoMxSet'

```r
aggreprobe(
  object,
  split,
  use = c("score", "cor", "both"),
  corcutoff = 0.85,
  ...
)
```
aggreprobe

## S4 method for signature 'matrix'
aggreprobe(
    object,
    probenames,
    featurenames,
    negmod,
    use = c("score", "cor", "both"),
    corcutoff = 0.85,
    ...
)

Arguments

- **object**: matrix of probes
- **...**: additional argument list that might be used
- **split**: indicator variable on whether it is for multiple slides (Yes, TRUE; No, FALSE)
- **use**: the method to determine outliers including score, cor, and both
- **corcutoff**: the cutoff value for correlation
- **probenames**: vector of names of probe
- **featurenames**: vector of names of features each probe corresponding to
- **negmod**: Poisson Background model object for negative probes

Value

- remain, the list of remaining probes of targets
- probenum, numerical vector of probe numbers of targets
- featuremat, the matrix of features

Examples

data("demoData")
demoData <- aggreprobe(demoData, use = "cor")
BGScoreTest

Testing for features above the background

Description
Testing for features above the background using Poisson background model as reference

Usage

BGScoreTest(object, ...)

## S4 method for signature 'NanoStringGeoMxSet'
BGScoreTest(
  object,
  split = FALSE,
  adj = 1,
  removeoutlier = FALSE,
  useprior = FALSE
)

## S4 method for signature 'matrix'
BGScoreTest(
  object,
  BGmod,
  adj = 1,
  probenum,
  removeoutlier = FALSE,
  useprior = FALSE
)

Arguments

object count matrix with features in rows and samples in columns
...
additional argument list that might be used
split indicator variable on whether it is for multiple slides (Yes, TRUE; No, FALSE)
adj adjustment factor for the number of feature in each gene, default =1 i.e. each
target only consists of one probe
removeoutlier whether to remove outlier
useprior whether to use the prior that the expression level of background follows a Beta
distribution, leading to a more conservative test
BGmod a list of sizefact, sizefact, and countmat
probenum a vector of numbers of probes in each gene
**Value**

a valid GeoMx S4 object including the following items

- pvalues - Background score test pvalues, in featureData
- scores - Background score test statistics, in featureData

If split is TRUE, a valid GeoMx S4 object including the following items

- pvalues_XX - Background score test pvalues vector, column name (denoted as XX) the same as slide names, in featureData
- scores_XX - Background score test statistics vector, column name (denoted as XX) the same as slide names, in featureData

A list of following items

- pvalues - Background score test pvalues
- scores - Background score test statistics

**Examples**

data(demoData)
demoData <- fitPoisBG(demoData, size_scale = "sum")
demoData <- aggreprobe(demoData, use = "cor")
demoData <- BGScoreTest(demoData, adj = 1, useprior = FALSE)
demoData <- fitPoisBG(demoData, size_scale = "sum", groupvar = "slide name")
demoData <- BGScoreTest(demoData, adj = 1, useprior = TRUE, split = TRUE)
coefNBth

Arguments

object: count matrix with features in rows and samples in columns
...
BGmod: fitted background model, multiple slides case
adj: adjustment factor for the number of probes in each feature, default = 1 i.e. each target only consists of one probe
probenum: a vector of numbers of probes in each gene
removeoutlier: whether to remove outlier
useprior: whether to use the prior that the expression level of background follows the Beta distribution, leading to a more conservative test

Value

a list of following items

- pvalues: Background score test pvalues matrix, columns the same as slide names
- scores_sp: Background score test statistics matrix, columns the same as slide names

coefNBth

Generate list of Wald test inference results on model coefficients

Description

Generate list of Wald test inference results including parameter estimation and p value

Usage

coeffNBth(object, ...)

# S4 method for signature 'list'
coeffNBth(object, fullpara = FALSE)

Arguments

object: DE model, output by fitNBthDE or fitNBthmDE
...
fullpara: whether to generate results on all parameters

Value

- estimate, coefficients estimate
- wald_stat, Wald test statistics
- p_value, p value of Wald test
- se, standard error
contrastNBth

Examples

```r
data(NBthmDEmod2)
coeff <- coefNBth(NBthmDEmod2)
```

```r
contrastNBth(NBthmDEmod2)
```

### Description

Generate list of Wald test inference results including contrast estimation and p value

### Usage

```r
contrastNBth(object, ...)
```

```r
## S4 method for signature 'list'
contrastNBth(
    object,
    test = c("two-sided", ">", "<"),
    method = diag(1, ncol(object$X)),
    baseline = rep(0, ncol(method))
)
```

### Arguments

- **object**: DE model, output by fitNBthDE or fitNBthmDE
- **...**: additional argument list that might be used
- **test**: type of statistical test, choose from c("two-sided", ">", "<")
- **method**: contrasts methods, only matrix of contrast vector is allowed for now, default=diag(1, ncol(object$X)), i.e. testing the regression coefficients
- **baseline**: testing baseline, default=0.

### Value

- `estimate`, contrasts estimate
- `wald_stat`, Wald test statistics
- `p_value`, p value of Wald test
- `se`, standard error

### Examples

```r
data(NBthmDEmod2)
coeff <- contrastNBth(NBthmDEmod2)
```
**demoData**

*A demo dataset for GeoMx Cancer Transcriptome Atlas (CTA) panel*

**Description**

A demo dataset contains 88 ROIs and 8707 features

**Usage**

```r
data(demoData)
```

**Format**

A NanoStringGeoMxSet S4 object with 8707 features and 88 samples

**Examples**

```r
data(demoData)
```

---

**DENBth**

*Generate DE table using the inference list generated by coefNBth or contrastNBth*

**Description**

Generate DE table using the inference list generated by coefNBth or contrastNBth

**Usage**

```r
DENBth(object, ...) ## S4 method for signature 'list'
DENBth(object, variable, NAto1 = TRUE, padj = TRUE, adj_method = "BH")
```

**Arguments**

- `object`: inference list from coefNBth or contrastNBth
- `variable`: additional argument list that might be used
- `NAto1`: whether to replace NA in pvalue by 1
- `p.adj`: whether to adjust p value
- `padj_method`: p value adjustment method, default="BH"
Value

DEtab, DE table

Examples

data(NBthmDEmod2)
coef <- coefNBth(NBthmDEmod2)
DEtab <- DNBth(coef, variable = "regiontubule")

Description

Perform diagnosis on Poisson background model

Usage

diagPoisBG(object, ...)

## S4 method for signature 'NanoStringGeoMxSet'
diagPoisBG(
  object,
  split = FALSE,
  padj = FALSE,
  padj_method = "BH",
  cutoff = 1e-06,
  generate_ppplot = TRUE
)

## S4 method for signature 'list'
diagPoisBG(
  object,
  padj = FALSE,
  padj_method = "BH",
  cutoff = 1e-06,
  generate_ppplot = TRUE
)

Arguments

object a list of sizefact, featfact, countmat, or id (if it is for multiple slides)
... additional argument list that might be used
split indicator variable on whether it is for multiple slides (Yes, TRUE; No, FALSE)
padj whether to adjust p value for outlier detection, default = TRUE
padj_method  p value adjustment method, default = "BH"
cutoff  p value(or adjusted p value) cutoff to determine outliers
generate_ppplot  whether to generate ppplot, default = TRUE

Value

a valid S4 object

• lowtail - A matrix of lower tail probability, in assay slot
• uptail - A matrix of upper tail probability, in assay slot
• disper (or disper_sp if non single-valued groupvar is provided) - dispersion parameter in experimentData
• low_outlier - A matrix to indicate lower outliers (0:False, 1:True) in assay slot
• upper_outlier - A matrix to indicate upper outliers (0:False, 1:True) in assay slot

a list of following items

• lowtail - A matrix of lower tail probability
• uptail - A matrix of upper tail probability
• disper - dispersion parameter
• outlier - A list of coordinates of lower and upper outliers

Examples

data(demoData)
demoData <- fitPoisBG(demoData, size_scale = "sum")
demoData <- diagPoisBG(demoData)
Biobase::notes(demoData)$disper
demoData <- fitPoisBG(demoData, groupvar = "slide name")
demoData <- diagPoisBG(demoData, split = TRUE)
Biobase::notes(demoData)$disper_sp

fitNBth

Negative Binomial threshold model

Description

Estimate the signal size factor for features above the background.
Estimate the signal size factor for features above the background.
fitNBth

Usage

fitNBth(object, ...)

## S4 method for signature 'NanoStringGeoMxSet'
fitNBth(
  object,
  split = TRUE,
  features_high = NULL,
  sizefact_BG = NULL,
  sizefact_start = sizefact_BG,
  size_scale = c("sum", "first"),
  threshold_start = NULL,
  threshold_fix = FALSE,
  tol = 1e-07,
  iterations = 8,
  start_para = c(threshold_start, 0.5),
  lower_sizefact = 0,
  lower_threshold = threshold_start/5
)

## S4 method for signature 'matrix'
fitNBth(
  object,
  features_high,
  probenum,
  sizefact_BG,
  sizefact_start = sizefact_BG,
  size_scale = c("sum", "first"),
  threshold_start,
  threshold_fix = FALSE,
  tol = 1e-07,
  iterations = 8,
  start_para = c(threshold_start, 1),
  lower_sizefact = 0,
  lower_threshold = threshold_start/5
)

Arguments

object count matrix with features in rows and samples in columns

... additional argument list that might be used

split indicator variable on whether it is for multiple slides (Yes, TRUE; No, FALSE)

features_high subset of features which are well above the background

sizefact_BG size factors for the background

sizefact_start initial value for size factors

size_scale method to scale the sizefact, sum(sizefact)=1 when size_scale="sum", sizefact[1]=1 when size_scale="first"
threshold_start
  initial value for threshold

threshold_fix
  whether to fix the threshold, default=FALSE

tol
  tolerance to determine convergence, default=1e-3

iterations
  maximum iterations to be run, default=5

start_para
  starting values for parameter estimation, default=c(threshold_start, 1)

lower_sizefact
  lower limit for sizefact, default=0

lower_threshold
  lower limit for threshold

probenum
  a vector of numbers of probes in each gene

Value

a valid GeoMx S4 object

- para0 = "NA", in experimentData
- para, estimated parameters, "signal" "r" in rows and features in columns, in featureData
- sizefact, estimated size factor, in phenoData
- preci1 = "NA", in experimentData
- conv0 = "NA", in experimentData
- conv = "NA", in experimentData
- Im = "NA", in experimentData
- features_high, a vector of indicators, in featureData (0: No; 1: Yes; NA: not included in features_high)
- features_all = "NA", in experimentData
- threshold, estimated threshold, when threshold_fix, equals to threshold_start, in experimentData

a list of following items, some items are place holders = NA

- para0 = NA,
- para, estimated parameters, "signal" "r" in rows and features in columns
- sizefact, estimated size factor
- preci1 = NA
- conv0 = NA
- conv = NA
- Im = NA
- features_high = features_high
- features_all = NA
- threshold, estimated threshold, when threshold_fix, equals to threshold_start
Examples

```r
library(Biobase)
library(dplyr)
data(demoData)
demoData <- fitPoisBG(demoData, size_scale = "sum")
demoData <- aggreprobe(demoData, use = "cor")
demoData <- BGScoreTest(demoData)
thmean <- 1 * mean(fData(demoData)$featfact, na.rm = TRUE)
demo_pos <- demoData[which(!fData(demoData)$CodeClass == "Negative"), ]
demo_neg <- demoData[which(fData(demoData)$CodeClass == "Negative"), ]
scl_scores <- fData(demo_pos)[, "scores"]
names(scl_scores) <- fData(demo_pos)[, "TargetName"]
features_high <- ((scl_scores > quantile(scl_scores, probs = 0.4)) &
    (scl_scores < quantile(scl_scores, probs = 0.95))) |> 
    which() |> 
    names()
set.seed(123)
features_high <- sample(features_high, 100)
demoData <- fitNBth(demoData,
    features_high = features_high,
    sizefact_BG = demo_neg$sizefact,
    threshold_start = thmean,
    iterations = 5,
    start_para = c(200, 1),
    lower_sizefact = 0,
    lower_threshold = 100,
    tol = 1e-8)
```

---

**fitNBthDE**

*Negative Binomial threshold model for differential expression analysis*

**Description**

Negative Binomial threshold model for differential expression analysis

**Usage**

```r
fitNBthDE(object, ...)  
```
fitNBthDE(form, annot, object, probenum, features_high, features_all, sizefact_start, sizefact_BG, threshold_mean, preci2 = 10000, lower_threshold = 0.01, prior_type = c("contrast", "equal"), sizefactrec = TRUE, size_scale = c("sum", "first"), sizescalebythreshold = FALSE, iterations = 2, covrob = FALSE, precilcon = 1/25, cutoff = 10, confac = 1)

Arguments

object count matrix with features in rows and samples in columns

... additional argument list that might be used

form model formula

split indicator variable on whether it is for multiple slides (Yes, TRUE; No, FALSE)

ROI_s_high ROIs with high expressions defined based on featfact and featfact
features_high               subset of features which are well above the background
features_all                full list of features
sizefact_start             initial value for size factors
sizefact_BG                size factor for background
threshold_mean             average threshold level
preci2                     precision for the background, default=10000
lower_threshold            lower limit for the threshold, default=0.01
prior_type                 empirical bayes prior type, choose from c("contrast", "equal")
sizefactrec                whether to recalculate sizefact, default=TRUE
size_scale                 method to scale the sizefact, sum(sizefact)=1 when size_scale="sum", sizefact[1]=1 when size_scale="first"

Value

a list of

- X, design matrix
- para0, estimated parameters for the first iteration, including regression coefficients, r and threshold in rows and features in columns
- para, estimated parameters, including regression coefficients, r and threshold in rows and features in columns
- sizefact, estimated sizefact
- sizefact0, estimated sizefact in iter=1
- preci1, precision matrix for regression coefficients estimated in iter=1
- Im0, Information matrix of parameters in iter=1
- Im, Information matrix of parameters in iter=2
- conv0, vector of convergence for iter=1, 0 converged, 1 not converged
- conv, vector of convergence for iter=2, 0 converged, 1 not converged
- features_high, same as the input features_high
- `features_all`, same as the input `features_all`

a list of

- `X`, design matrix
- `para0`, estimated parameters for the first iteration, including regression coefficients, r and threshold in rows and features in columns
- `para`, estimated parameters, including regression coefficients, r and threshold in rows and features in columns
- `sizefact`, estimated sizefact
- `sizefact0`, estimated sizefact in iter=1
- `preci1`, precision matrix for regression coefficients estimated in iter=1
- `Im0`, Information matrix of parameters in iter=1
- `Im`, Information matrix of parameters in iter=2
- `conv0`, vector of convergence for iter=1, 0 converged, 1 not converged
- `conv`, vector of convergence for iter=2, 0 converged, 1 not converged
- `features_high`, same as the input `features_high`
- `features_all`, same as the input `features_all`

**Examples**

```r
library(Biobase)
library(dplyr)
data(demoData)
demoData <- demoData[, c(1:5, 33:37)]
demoData <- fitPoisBG(demoData, size_scale = "sum")
demoData <- aggreprobe(demoData, use = "cor")
demoData <- BGScoreTest(demoData)
demoData$slidename <- substr(demoData["slide name"], 12, 17)
theta <- 1 * mean(fData(demoData)$featfact, na.rm = TRUE)
demo_pos <- demoData[which(!fData(demoData)$CodeClass == "Negative"), ]
demo_neg <- demoData[which(fData(demoData)$CodeClass == "Negative"), ]
scl1_scores <- fData(demo_pos)[, "scores"]
names(scl1_scores) <- fData(demo_pos)[, "TargetName"]
features_high <- ((scl1_scores > quantile(scl1_scores, probs = 0.4)) &
(scl1_scores < quantile(scl1_scores, probs = 0.95))) |> names()
set.seed(123)
demoData <- fitNBth(demoData,
features_high = features_high,
sizefact_BG = demo_neg$sizefact,
threshold_start = theta,
iterations = 5,
start_para = c(200, 1),
lower_sizefact = 0,
lower_threshold = 100,
tol = 1e-8)
```
fitNBthmDE

Negative Binomial threshold mixed model for differential expression analysis

Usage

fitNBthmDE(object, ...)

## S4 method for signature 'NanoStringGeoMxSet'

fitNBthmDE(
  object,
  form,
  split,
  ROIs_high = NULL,
  features_all = NULL,
  sizefact = NULL,
  sizefact_BG = NULL,
  preci1,
  threshold_mean = NULL,
  preci2 = 10000,
sizescalebythreshold = TRUE,
controlRandom = list()
)

## S4 method for signature 'matrix'
fitNBthmDE(
  form,
  annot,
  object,
  probenum = rep(1, NROW(object)),
  features_all,
  sizefact,
  sizefact_BG,
  preci1,
  threshold_mean = NULL,
  preci2 = 10000,
  sizescalebythreshold = TRUE,
  controlRandom = list()
)

Arguments

object       count matrix with features in rows and samples in columns
...          additional argument list that might be used
form         model formula
split        indicator variable on whether it is for multiple slides (Yes, TRUE; No, FALSE)
ROIs_high    ROIs with high expressions defined based on featfact and featfact
features_all vector of all features to be run
sizefact     size factor
sizefact_BG  size factor for background
preci1       precision matrix for regression coefficients
threshold_mean average background level
preci2       precision for the background, default=10000
sizescalebythreshold whether to scale the size factor, default=TRUE
controlRandom list of random effect control parameters
annot        annotations files with variables in the formula
probenum     a vector of numbers of probes in each gene, default = rep(1, NROW(object))

Value

a list with parameter estimation #'

• X, design matrix for fixed effect
• Z, design matrix for random effect
• rt, random effect terms
• para0, =NA
• para, estimated parameters, including regression coefficients, r and threshold in rows and features in columns
• sizefact, same as input sizefact
• sizefact0, NA
• preci1, input precision matrix for regression coefficients
• Im0, NA
• Im, Information matrix of parameters
• conv0, NA
• conv, vector of convergence, 0 converged, 1 not converged
• features_high, NA
• features_all, same as the input features_all
• theta, list of estimated random effect parameters
• MAP random effect

A list with parameter estimation #'

• X, design matrix for fixed effect
• Z, design matrix for random effect
• rt, random effect terms
• para0, =NA
• para, estimated parameters, including regression coefficients, r and threshold in rows and features in columns
• sizefact, same as input sizefact
• sizefact0, NA
• preci1, input precision matrix for regression coefficients
• Im0, NA
• Im, Information matrix of parameters
• conv0, NA
• conv, vector of convergence, 0 converged, 1 not converged
• features_high, NA
• features_all, same as the input features_all
• theta, list of estimated random effect parameters (for relative covariance matrix)
• varcov, list of estimated variance covariance parameter estimation
• MAP random effect
Examples

```r
library(Biobase)
library(dplyr)
data(demoData)
demoData <- demoData[, c(1:5, 33:37)]
demoData <- fitPoisBG(demoData, size_scale = "sum")
demoData <- aggreprobe(demoData, use = "cor")
demoData <- BGScoreTest(demoData)
demoData$slidename <- substr(demoData[['slide name']], 12, 17)

thmean <- 1 * mean(fData(demoData)$featfact, na.rm = TRUE)
demo_pos <- demoData[which(!fData(demoData)$CodeClass == "Negative"),]
demo_neg <- demoData[which(fData(demoData)$CodeClass == "Negative"),]
sc1_scores <- fData(demo_pos)$scores
names(sc1_scores) <- fData(demo_pos)$TargetName

features_high <- ((sc1_scores > quantile(sc1_scores, probs = 0.4)) &
  (sc1_scores < quantile(sc1_scores, probs = 0.95))) |
  names()

set.seed(123)
demoData <- fitNBth(demoData,
  features_high = features_high,
  sizefact_BG = demo_neg$sizefact,
  threshold_start = thmean,
  iterations = 5,
  start_para = c(200, 1),
  lower_sizefact = 0,
  lower_threshold = 100,
  tol = 1e-8)

ROIs_high <- sampleNames(demoData)[which(demoData$sizefact_fitNBth * thmean > 2)]
features_all <- rownames(demo_pos)

pData(demoData)$group <- c(rep(1, 5), rep(2, 5))

NBthDEmod2 <- fitNBthDE(form = ~group,
  split = FALSE,
  object = demoData,
  ROIs_high = ROIs_high,
  features_high = features_high,
  features_all = features_all,
  sizefact_start = demoData[, ROIs_high][['sizefact_fitNBth']],
  sizefact_BG = demoData[, ROIs_high][['sizefact']],
  threshold_mean = notes(demoData)["threshold"],
  preci2=10000,
  prior_type="contrast",
  covrob=FALSE,
  precilcon=1/25,
  sizescalebythreshold=TRUE)

set.seed(123)
NBthDEmod1 <- fitNBthmDE(
  form = ~ group + (1 | `slide name`),
  split = FALSE,
  object = demoData,
  ROIs_high = ROIs_high,
  features_high = features_high,
  features_all = features_all,
  sizefact_start = demoData[, ROIs_high][['sizefact_fitNBth']],
  sizefact_BG = demoData[, ROIs_high][['sizefact']],
  threshold_mean = notes(demoData)["threshold"],
  preci2=10000,
  prior_type="contrast",
  covrob=TRUE,
  precilcon=1/25,
  sizescalebythreshold=TRUE)
```
fitPoisBG

Estimate Poisson background model for either single slide or multiple slides

Description

Estimate Poisson background model for either single slide or multiple slides

Estimate Poisson background model:

Usage

fitPoisBG(object, ...)  

## S4 method for signature 'NanoStringGeoMxSet'
fitPoisBG(
  object,
  groupvar = NULL,
  iterations = 10,
  tol = 0.001,
  size_scale = c("sum", "first"),
  ...
)

## S4 method for signature 'matrix'
fitPoisBG(object, iterations = 10, tol = 0.001, size_scale = c("sum", "first"))

Arguments

object count matrix with features in rows and samples in columns

... additional argument list that might be used

groupvar the group variable name for slide

iterations maximum iterations to be run, default=10

tol tolerance to determine convergence, default = 1e-3

size_scale method to scale the sizefact, sum(sizefact)=1 when size_scale="sum", sizefact[1]=1 when size_scale="first"
Value

a valid GeoMx S4 object if split is FALSE

- sizefact - estimated size factor in phenoData
- featfact - estimated feature factor in featureData

a valid GeoMx S4 object if split is TRUE,

- sizefact - estimated size factor in phenoData
- featfact_XX - estimated feature factor vector, column name (denoted as XX) the same as the slide id, in featureData for each unique slide
- fitPoisBG_sp_var - the column name for slide, in experimentData

a list of following items

- sizefact - estimated size factor
- featfact - estimated feature factor
- countmat - the input count matrix

Examples

data(demoData)
demoData <- fitPoisBG(demoData, size_scale = "sum")
data(demoData)
demoData <- fitPoisBG(demoData, groupvar = "slide name", size_scale = "sum")

Description

Estimate Poisson background model for multiple slides:

Usage

fitPoisBG_sp(object, ...)

# S4 method for signature 'matrix'
fitPoisBG_sp(
  object,
  id,
  iterations = 10,
  tol = 0.001,
  size_scale = c("sum", "first")
)
**Arguments**

- `object` count matrix with features in rows and samples in columns
- `...` additional argument list that might be used
- `id` character vector same size as sample size representing slide names of each sample
- `iterations` maximum iterations to be run, default=10
- `tol` tolerance to determine convergence, default = 1e-3
- `size_scale` method to scale the sizefact, sum(sizefact)=1 when size_scale="sum", sizefact[1]=1 when size_scale="first"

**Value**

a list of following items

- `sizefact` - estimated size factor
- `featfact` - estimated feature factor matrix, column names the same as the slide id
- `countmat` - the input count matrix
- `id` - the input id

---

**fitPoisthNorm**

*Poisson threshold model based normalization-log2 transformation for single slide or for multiple slides*

---

**Description**

Poisson threshold model based normalization-log2 transformation for single slide or for multiple slides

**Usage**

```r
fitPoisthNorm(object, ...)
```

```r
## S4 method for signature 'NanoStringGeoMxSet'
fitPoisthNorm(
  object,
  split = FALSE,
  ROIs_high = NULL,
  features_high = NULL,
  features_all = NULL,
  sizefact_start = NULL,
  sizefact_BG = NULL,
  threshold_mean = NULL,
  preci2 = 10000,
  iterations = 2,
)```
prior_type = c("contrast", "equal"),
sizefactrec = TRUE,
size_scale = c("sum", "first"),
sizescalebythreshold = FALSE,
covrob = FALSE,
precilcon = 1/25,
cutoff = 15,
confac = 1,
calhes = FALSE
)

## S4 method for signature 'matrix'
fitPoisthNorm(
  object,
  probenum = rep(1, NROW(object)),
  features_high,
  features_all,
  sizefact_start,
  sizefact_BG,
  threshold_mean,
  preci2 = 10000,
  iterations = 2,
  prior_type = c("contrast", "equal"),
sizefactrec = TRUE,
size_scale = c("sum", "first"),
sizescalebythreshold = FALSE,
covrob = FALSE,
precilcon = 1/25,
cutoff = 15,
confac = 1,
calhes = FALSE
)

Arguments

object          count matrix with features in rows and samples in columns
...
additional argument list that might be used
split           indicator variable on whether it is for multiple slides (Yes, TRUE; No, FALSE)
ROIs_high       ROIs with high expressions defined based on featfact and featfact
features_high   subset of features which are well above the background
features_all    full feature vector to apply the normalization on
sizefact_start  initial value for size factors
sizefact_BG     size factor for background
threshold_mean  average threshold level
preci2          precision for threshold, default=10000
fitPoisthNorm

iterations
iteration number, default=2, the first iteration using the features_high to construct the prior for parameters then refit the model on all features. precision matrix for threshold: preci2

prior_type
prior type for preci1, "equal" or "contrast", default=“contrast"

sizefactrec
XXXX, default = TRUE

size_scale
method to scale the sizefact, sum(sizefact)=1 when size_scale=“sum”, sizefact[1]=1 when size_scale=“first”

sizescalebythreshold
XXXX, default = FALSE

covrob
whether to use robust covariance in calculating the prior precision matrix 1, default = FALSE

preci1con
The user input constant term in specifying precision matrix 1, default=1/25

cutoff
term in calculating precision matrix 1, default=15

confac
The user input factor for contrast in precision matrix 1, default=1

calhes
The user input whether to calculate hessian: calhes, default=FALSE

probenum
a vector of numbers of probes in each gene

Value

if split is FALSE, a valid GeoMx S4 object including the following items

• para0_norm, matrix of estimated parameters for iter=1, features in columns and parameters(log2 expression, threshold) in rows, in featureData.
• para_norm, matrix of estimated parameters for iter=2, features in columns and parameters(log2 expression, threshold) in rows, in featureData.
• normmat0, matrix of log2 expression for iter=1, features in columns and log2 expression in rows, in assay slot.
• normmat, matrix of log2 expression for iter=2, features in columns and log2 expression in rows, in assay slot.
• sizefact_norm, estimated sizefact, in phenoData.
• sizefact0_norm, estimated sizefact in iter=1, in phenoData.
• preci1, precision matrix 1, in experimentData.
• conv0, vector of convergence for iter=1, 0 converged, 1 not converged, in featureData
• conv, vector of convergence for iter=2, 0 converged, 1 not converged, in featureData
• features_high, same as the input features_high, in featureData
• features_all, same as the input features_all, in featureData

if split is TRUE, a valid GeoMx S4 object with the following items appended.

• threshold0, matrix of estimated threshold for iter=1, features in columns and threshold for different slides in rows, in featureData.
• threshold, matrix of estimated threshold for iter=2, features in columns and threshold for different slides in rows, in featureData.
- normmat0_sp, matrix of log2 expression for iter=1, features in columns and log2 expression in rows, in assay slot.
- normmat_sp, matrix of log2 expression for iter=2, features in columns and log2 expression in rows, in assay slot.
- sizefact_norm_sp, estimated sizefact, in phenoData
- sizefact0_norm_sp, estimated sizefact in iter=1, in phenoData
- preci1, precision matrix 1, in experimentData
- conv0_sp_XX, vector of convergence for each unique slide value for iter=1, 0 converged, 1 not converged, in featureData for each unique slide.
- conv_sp_XX, vector of convergence for each unique slide value for iter=2, 0 converged, 1 not converged, in featureData for each unique slide.
- features_high_sp, same as the input features_high, in featureData.
- features_all_sp, same as the input features_all, in featureData.

A list of following items
- para0, matrix of estimated parameters for iter=1, features in columns and parameters (log2 expression, threshold) in rows.
- para, matrix of estimated parameters for iter=2, features in columns and parameters (log2 expression, threshold) in rows.
- normmat0, matrix of log2 expression for iter=1, features in columns and log2 expression in rows.
- normmat, matrix of log2 expression for iter=2, features in columns and log2 expression in rows.
- sizefact, estimated sizefact
- sizefact0, estimated sizefact in iter=1
- preci1, precision matrix 1
- Im0, Information matrix of parameters in iter=1
- Im, Information matrix of parameters in iter=2
- conv0, vector of convergence for iter=1, 0 converged, 1 not converged
- conv, vector of convergence for iter=2, 0 converged, 1 not converged
- features_high, same as the input features_high
- features_all, same as the input features_all

Examples

```r
library(Biobase)
library(dplyr)
data(demoData)
demoData <- fitPoisBG(demoData, size_scale = "sum")
demoData <- aggreprobe(demoData, use = "cor")
demoData <- BGScoreTest(demoData)
thmean <- 1 * mean(fData(demoData)$featfact, na.rm = TRUE)
demo_pos <- demoData[which(!fData(demoData)$CodeClass == "Negative"), ]
```
demo_neg <- demoData[which(fData(demoData)$CodeClass == "Negative"),]
scl_scores <- fData(demo_pos)[, "scores"]
names(scl_scores) <- fData(demo_pos)[, "TargetName"]
features_high <- ((scl_scores > quantile(scl_scores, probs = 0.4)) &
                   (scl_scores < quantile(scl_scores, probs = 0.95))) |>
                   which() |>
                   names()
set.seed(123)
features_high <- sample(features_high, 100)
demoData <- fitNBth(demoData,
                   features_high = features_high,
                   sizefact_BG = demo_neg$sizefact,
                   threshold_start = thmean,
                   iterations = 5,
                   start_para = c(200, 1),
                   lower_sizefact = 0,
                   lower_threshold = 100,
                   tol = 1e-8)
ROIs_high <- sampleNames(demoData)[which((quantile(fData(demoData)[["para"]], [1],
                                          probs = 0.90, na.rm = TRUE) -
                                          notes(demoData)[["threshold"]]) * demoData$sizefact_fitNBth > 2)]
features_all <- rownames(demo_pos)
thsmean <- mean(fData(demo_neg)[["featfact"]])
demoData <- fitPoisthNorm(
               object = demoData,
               split = FALSE,
               ROIs_high = ROIs_high,
               features_high = features_high,
               features_all = features_all,
               sizefact_start = demoData[, ROIs_high][["sizefact_fitNBth"]],
               sizefact_BG = demoData[, ROIs_high][["sizefact"]],
               threshold_mean = thmean,
               preci2 = 10000,
               prior_type = "contrast",
               covrob = FALSE,
               preci1con = 1 / 25)

---

**fitPoisthNorm_sp**  
Poisson threshold model based normalization-log2 transformation for multiple slides

**Description**

Poisson threshold model based normalization-log2 transformation for multiple slides

**Usage**

`fitPoisthNorm_sp(object, ...)`
## S4 method for signature 'matrix'
fitPoisthNorm_sp(
  object,
  probenum,
  features_high,
  features_all = colnames(object),
  sizefact_start,
  sizefact_BG,
  threshold_mean,
  preci2 = 10000,
  id,
  iterations = 2,
  prior_type = c("contrast", "equal"),
  sizefactrec = TRUE,
  size_scale = c("sum", "first"),
  sizescalebythreshold = FALSE,
  covrob = FALSE,
  preci1con = 1/25,
  cutoff = 15,
  confac = 1
)

### Arguments

- **object**: count matrix with features in rows and samples in columns
- **...**: additional argument list that might be used
- **probenum**: a vector of numbers of probes in each gene
- **features_high**: subset of features which are well above the background
- **features_all**: full feature vector to apply the normalization on
- **sizefact_start**: initial value for size factors
- **sizefact_BG**: size factor for background
- **threshold_mean**: average threshold level
- **preci2**: precision for threshold, default=10000
- **id**: character vector of slide name of each sample
- **iterations**: iteration number, default=2, the first iteration using the features_high to construct the prior for parameters then refit the model on all features. precision matrix for threshold: preci2
- **prior_type**: prior type for preci1, "equal" or "contrast", default="contrast"
- **sizefactrec**: XXXX, default = TRUE
- **size_scale**: method to scale the sizefact, sum(sizefact)=1 when size_scale="sum", sizefact[1]=1 when size_scale="first"
- **sizescalebythreshold**: XXXX, default = FALSE
covrob whether to use robust covariance in calculating the prior precision matrix 1, default = FALSE
preci1con The user input constant term in specifying precision matrix 1, default=1/25
cutoff term in calculating precision matrix 1, default=15
confac The user input factor for contrast in precision matrix 1, default=1

Value

a list of following items

- threshold0, matrix of estimated threshold for iter=1, features in columns and threshold for different slides in rows.
- threshold, matrix of estimated threshold for iter=2, features in columns and threshold for different slides in rows.
- normmat0, matrix of log2 expression for iter=1, features in columns and log2 expression in rows.
- normmat, matrix of log2 expression for iter=2, features in columns and log2 expression in rows.
- sizefact, estimated sizefact
- sizefact0, estimated sizefact in iter=1
- preci1, precision matrix 1
- Im0, Information matrix in iter=1
- Im, Information matrix in iter=2
- conv0, vector of convergence for iter=1, 0 converged, 1 not converged
- conv, vector of convergence for iter=2, 0 converged, 1 not converged
- features_high, same as the input features_high
- features_all, same as the input features_all

kidney

A demo dataset for GeoMx Human Whole Transcriptome Atlas (WTA) panel

Description

A demo dataset contains 276 ROIs and 18642 features

Usage
data(kidney)

Format

A NanoStringGeoMxSet S4 object with 18642 features and 276 samples
Examples

data(kidney)

NBthDEmod2

A demo example output list returned by function fitNBthDE

Description

A list used to demonstrate the function coefNBth

Usage

data(NBthDEmod2)

Format

A list

Examples

data(NBthDEmod2)

NBthmDEmod2

A demo example output list returned by function fitNBthmDE

Description

A list used to demonstrate the function coefNBth

Usage

data(NBthmDEmod2)

Format

A list

Examples

data(NBthmDEmod2)
NBthmDEmod2slope

A demo example output list returned by function fitNBthmDE

Description
A list used to demonstrate the function coefNBth

Usage
data(NBthmDEmod2slope)

Format
A list

Examples
data(NBthmDEmod2slope)

QuanRange
Compute Quantile Range

Description
Compute Quantile Range, a metric representing signal strength for QC purpose

Usage
QuanRange(object, ...)

## S4 method for signature 'NanoStringGeoMxSet'
QuanRange(object, split = FALSE, probs, removeoutlier = FALSE, ...)

## S4 method for signature 'matrix'
QuanRange(object, probenum, BGmod, probs, removeoutlier = FALSE)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>object</td>
<td>count matrix with features in rows and samples in columns</td>
</tr>
<tr>
<td>...</td>
<td>additional argument list that might be used</td>
</tr>
<tr>
<td>split</td>
<td>indicator variable on whether it is for multiple slides</td>
</tr>
<tr>
<td>probs</td>
<td>numeric vector of probabilities with values in [0,1] passed to quantile</td>
</tr>
<tr>
<td>removeoutlier</td>
<td>indicator on whether to remove outliers, default: FALSE</td>
</tr>
<tr>
<td>probenum</td>
<td>a vector of numbers of probes in each gene</td>
</tr>
<tr>
<td>BGmod</td>
<td>a list of sizefact, sizefact, countmat, and id (if it is for multiple slides)</td>
</tr>
</tbody>
</table>
QuanRange

Value

- a valid S4 object with probabilities in phenoData
- a matrix of quantile range in rows and probs in columns

Examples

```r
data(demoData)
demoData <- fitPoisBG(demoData, size_scale = "sum")
demoData <- diagPoisBG(demoData)
demoData <- aggreprobe(demoData, use = "cor")
Biobase::notes(demoData)$disper
demoData <- QuanRange(demoData, split = FALSE, probs = c(0.75, 0.8, 0.9, 0.95))

data(demoData)
demoData <- fitPoisBG(demoData, groupvar = "slide name")
demoData <- diagPoisBG(demoData, split = TRUE)
demoData <- aggreprobe(demoData, use = "cor")
Biobase::notes(demoData)$disper_sp
demoData <- QuanRange(demoData, split = TRUE, probs = c(0.75, 0.8, 0.9, 0.95))
```
# Index

* datasets
  - demoData, 8
  - kidney, 29
  - NBthDEmod2, 30
  - NBthmDEmod2, 30
  - NBthmDEmod2slope, 31

  - aggreprobe, 2
  - aggreprobe, matrix-method (aggreprobe), 2
  - aggreprobe, NanoStringGeoMxSet-method (aggreprobe), 2

  - BGScoreTest, 4
  - BGScoreTest, matrix-method (BGScoreTest), 4
  - BGScoreTest, NanoStringGeoMxSet-method (BGScoreTest), 4
  - BGScoreTest_sp, 5
  - BGScoreTest_sp, matrix-method (BGScoreTest_sp), 5

  - coefNBth, 6
  - coefNBth, list-method (coefNBth), 6
  - contrastNBth, 7
  - contrastNBth, list-method (contrastNBth), 7

  - demoData, 8
  - DENBth, 8
  - DENBth, list-method (DENBth), 8
  - diagPoisBG, 9
  - diagPoisBG, list-method (diagPoisBG), 9
  - diagPoisBG, NanoStringGeoMxSet-method (diagPoisBG), 9

  - fitNBth, 10
  - fitNBth, matrix-method (fitNBth), 10
  - fitNBth, NanoStringGeoMxSet-method (fitNBth), 10
  - fitNBthDE, 13
  - fitNBthDE, matrix-method (fitNBthDE), 13
  - fitNBthDE, NanoStringGeoMxSet-method (fitNBthDE), 13
  - fitNBthDE, matrix-method (fitNBthDE), 13
  - fitNBthmDE, 17
  - fitNBthmDE, matrix-method (fitNBthmDE), 17
  - fitNBthmDE, NanoStringGeoMxSet-method (fitNBthmDE), 17
  - fitPoisBG, 21
  - fitPoisBG, matrix-method (fitPoisBG), 21
  - fitPoisBG, NanoStringGeoMxSet-method (fitPoisBG), 21
  - fitPoisBG_sp, 22
  - fitPoisBG_sp, matrix-method (fitPoisBG_sp), 22
  - fitPoisthNorm, 23
  - fitPoisthNorm, matrix-method (fitPoisthNorm), 23
  - fitPoisthNorm, NanoStringGeoMxSet-method (fitPoisthNorm), 23
  - fitPoisthNorm_sp, 27
  - fitPoisthNorm_sp, matrix-method (fitPoisthNorm_sp), 27

  - kidney, 29

  - NBthDEmod2, 30
  - NBthmDEmod2, 30
  - NBthmDEmod2slope, 31

  - QuanRange, 31
  - QuanRange, matrix-method (QuanRange), 31
  - QuanRange, NanoStringGeoMxSet-method (QuanRange), 31