Package ‘missMethyl’

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Type Package

Title Analysing Illumina HumanMethylation BeadChip Data

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Depends R (>= 2.3.0)

Imports limma, minfi, methylumi, IlluminaHumanMethylation450kmanifest, statmod, ruv, stringr, IlluminaHumanMethylation450kanno.ilmn12.hg19, org.Hs.eg.db, AnnotationDbi, BiasedUrn, GO.db, IlluminaHumanMethylationEPICmanifest, IlluminaHumanMethylationEPICanno.ilm10b2.hg19

VignetteBuilder knitr

Suggests minfiData, BiocStyle, knitr, rmarkdown, edgeR, tweeDEseqCountData

Description Normalisation and testing for differential variability and differential methylation for data from Illumina’s Infinium HumanMethylation450 array. The normalisation procedure is subset-quantile within-array normalisation (SWAN), which allows Infinium I and II type probes on a single array to be normalised together. The test for differential variability is based on an empirical Bayes version of Levene’s test. Differential methylation testing is performed using RUV, which can adjust for systematic errors of unknown origin in high-dimensional data by using negative control probes. Gene ontology analysis is performed by taking into account the number of probes per gene on the array.

License GPL-2

biocViews Normalization, DNAMethylation, MethylationArray, GenomicVariation, GeneticVariability, DifferentialMethylation, GeneSetEnrichment

NeedsCompilation no
Introduction to the missMethyl package

Description

missMethyl is a library for the analysis of Illumina’s 450K human methylation BeadChip. Specifically, functions for SWAN normalisation and differential variability analysis are provided. SWAN normalisation uses probe specific information, and the differential variability procedure uses linear models which can handle any designed experiment.

Details

Package: missMethyl
Type: Package
Version: 0.99.0
Date: 2014-06-30
License: GPL-2

Normalisation of the 450K arrays can be performed using the function SWAN.

Differential variability analysis can be performed by calling varFit followed by topVar for a list of the top ranked differentially variable CpGs between conditions.

More detailed help documentation is provided in each function’s help page.

Author(s)

Belinda Phipson and Jovana Maksimovic

Maintainer: Belinda Phipson <belinda.phipson@mcri.edu.au>, Jovana Maksimovic <jovana.maksimovic@mcri.edu.au>
contrasts.varFit

References


contrasts.varFit

Compute contrasts for a varFit object.

Description

Compute estimated coefficients, standard errors and LogVarRatios for a given set of contrasts.

Usage

contrasts.varFit(fit, contrasts=NULL)

Arguments

fit list containing a linear model fit produced by varFit. The fit object should be of class MArrayLM.

contrasts numeric matrix with rows corresponding to coefficients in fit and columns containing contrasts.

Details

This function calls the contrasts.fit function in limma to compute coefficients and standard errors for the specified contrasts corresponding to a linear model fit obtained from the varFit function. LogVarRatios are also computed in terms of the contrasts. A contrasts matrix can be computed using the makeContrasts function.

Value

A list object of the same class as fit.

Author(s)

Belinda Phipson

See Also

varFit, contrasts.fit, makeContrasts
Examples

# Randomly generate data for a 3 group problem with 100 CpG sites and 4 arrays in each group.
library(limma)

y<-matrix(rnorm(1200),ncol=12)
group<-factor(rep(c(1,2,3),each=4))
design<-model.matrix(~0+group)
colnames(design)<-c("grp1","grp2","grp3")

# Fit linear model for differential variability
vfit<-varFit(y,design)

# Specify contrasts
contr<-makeContrasts(grp2-grp1,grp3-grp1,grp3-grp2,levels=colnames(design))

# Compute contrasts from fit object
vfit.contr<-contrasts.varFit(vfit,contrasts=contr)

summary(decideTests(vfit.contr))

# Look at top table of results for first contrast
topVar(vfit.contr,coef=1)

---

densityByProbeType

Plot the beta value distributions of the Infinium I and II probe types relative to the overall beta value distribution.

Description

Plot the overall density distribution of beta values and the density distributions of the Infinium I and II probe types.

Usage

densityByProbeType(data, legendPos = "top", colors = c("black", "red", "blue"), main = "", lwd = 3, cex.legend = 1)

Arguments

data A MethylSet or a matrix or a vector. We either use the getBeta function to get Beta values (in the first case) or we assume the matrix or vector contains Beta values.

legendPos 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 legend for details.

colors Colors to be used for the different beta value density distributions. Must be a vector of length 3.

main Plot title.

lwd The line width to be used for the different beta value density distributions.

cex.legend The character expansion factor for the legend text.
getINCs

Details

The density distribution of the beta values for a single sample is plotted. The density distributions of the Infinium I and II probes are then plotted individually, showing how they contribute to the overall distribution. This is useful for visualising how using SWAN affects the data.

Author(s)

Jovana Maksimovic <jovana.maksimovic@mcri.edu.au>.

References

No return value. Plot is produced as a side-effect.

See Also

densityPlot, densityBeanPlot, par, legend

Examples

```r
if (require(minfi) & require(minfiData)) {
  dat <- preprocessRaw(RGsetEx)
  datSwan <- SWAN(dat)
  par(mfrow=c(1,2))
  densityByProbeType(dat[,1], main="Raw")
  densityByProbeType(datSwan[,1], main="SWAN")
}
```

---

getINCs  
Extract intensity data for 613 Illumina negative controls found on 450k arrays.

Description

Extracts the intensity data for the 613 Illumina negative controls found on 450k arrays and returns a matrix of M-values (log2 ratio of the green to red intensities).

Usage

getINCs(rgSet)

Arguments

rgSet  
An object of class RGChannelSet.

Details

The getINCs function extracts the intensity data for the INCs from an RGChannelSet object. The function retrieves both the green and red channel intensity values and returns the data as the log2 ratio of the green to red intensities. Essentially, the INCs are being treated like 450k Type II probes for which the M-values are also given as the log2 ratio of the green to red intensities.
getLeveneResiduals

Obtain Levene residuals

Description

Obtain absolute or squared Levene residuals for each CpG given a series of methylation arrays

Usage

getLeveneResiduals(data, design = NULL, type = NULL)

Arguments

data object of class matrix of M values, with rows corresponding to features of interest such as CpG sites and columns corresponding to samples or arrays
design the design matrix of the experiment, with rows corresponding to arrays/samples and columns to coefficients to be estimated. Defaults to the unit vector.
type character string, "AD" for absolute residuals or "SQ" for squared residuals. Default is "AD".

Details

This function will return absolute or squared Levene residuals given a matrix of M values and a design matrix. This can be used for graphing purposes or for downstream analysis such as a gene set testing based on differential variability rather than differential methylation. If no design matrix is given, the residuals are determined by treating all samples as coming from one group.

Value

Returns a list with three components. data contains a matrix of absolute or squared residuals, AvgVar is a vector of sample variances and LogVarRatio corresponds to the columns of the design matrix and is usually the ratios of the log of the group variances.

Author(s)

Jovana Maksimovic <jovana.maksimovic@mcri.edu.au>

See Also

RGChannelSet

Examples

if (require(minfi) & require(minfiData)) {
  INCs <- getINCs(RGsetEx)
  head(INCs)
  dim(INCs)
}
getMappedEntrezIDs

Author(s)

Belinda Phipson

References


See Also

varFit

Examples

# Randomly generate data for a 2 group problem with 100 CpG sites and 5 arrays in each group
y <- matrix(rnorm(1000), ncol=10)

group <- factor(rep(c(1,2), each=5))
design <- model.matrix(~group)

# Get absolute Levene Residuals
resid <- getLeveneResiduals(y, design)

# Plot the first CpG
barplot(resid$data[1,], col=rep(c(2,4), each=5), ylab="Absolute Levene Residuals", names=group)

getMappedEntrezIDs

*Get mapped Entrez Gene IDs from CpG probe names*

**Description**

Given a set of CpG probe names and optionally all the CpG sites tested, this function outputs a list containing the mapped Entrez Gene IDs as well as the numbers of probes per gene, and a vector indicating significance.

**Usage**

getMappedEntrezIDs(sig.cpg, all.cpg = NULL, array.type)

**Arguments**

- `sig.cpg`: character vector of significant CpG sites used for testing gene set enrichment
- `all.cpg`: character vector of all CpG sites tested. Defaults to all CpG sites on the array.
- `array.type`: the Illumina methylation array used. Options are "450K" or "EPIC".

**Details**

This function is used by the gene set testing functions gometh and gsameth. It maps the significant CpG probe names to Entrez Gene IDs, as well as all the CpG sites tested. It also calculated the numbers of probes for gene.

Genes associated with each CpG site are obtained from the annotation package `IlluminaHumanMethylation450kanno.ilmn12.hg19` if the array type is "450K". For the EPIC array, the annotation package `IlluminaHumanMethylationEPICanno.ilm10b2.hg19` is used.
Value
A list with the following elements

- `sig.eg`: mapped Entrez Gene IDs for the significant probes
- `universe`: mapped Entrez Gene IDs for all probes on the array, or for all the CpG probes tested.
- `freq`: table output with numbers of probes associated with each gene
- `de`: a vector of ones and zeroes of the same length of universe indicating which genes in the universe are significantly differentially methylated.

Author(s)
Belinda Phipson

See Also
`gometh, gsameth`

Examples

```r
library(IlluminaHumanMethylation450kanno.ilmn12.hg19)
library(org.Hs.eg.db)
library(limma)
ann <- getAnnotation(IlluminaHumanMethylation450kanno.ilmn12.hg19)

# Randomly select 1000 CpGs to be significantly differentially methylated
sigcpgs <- sample(rownames(ann), 1000, replace=FALSE)

# All CpG sites tested
allcpgs <- rownames(ann)

mappedEz <- getMappedEntrezIDs(sigcpgs, allcpgs, array.type="450K")
mappedEz$sig.eg[1:10]
mappedEz$universe[1:10]
mappedEz$freq[1:10]
mappedEz$de[1:10]
```

Description
Tests gene ontology enrichment for significant CpGs from Illumina's Infinium HumanMethylation450 or MethylationEPIC array, taking into account the differing number of probes per gene present on the array.

Usage

```r
gometh(sig.cpg, all.cpg = NULL, collection = c("GO","KEGG"), array.type = c("450K","EPIC"), plot.bias = FALSE, prior.prob = TRUE)
```
Arguments

sig.cpg character vector of significant CpG sites to test for GO term enrichment
all.cpg character vector of all CpG sites tested. Defaults to all CpG sites on the array.
collection the collection of pathways to test. Options are "GO" and "KEGG". Defaults to "GO".
array.type the Illumina methylation array used. Options are "450K" or "EPIC". Defaults to "450K".
plot.bias logical, if true a plot showing the bias due to the differing numbers of probes per gene will be displayed
prior.prob logical, if true will take into account the probability of significant differentially methylation due to numbers of probes per gene. If false, a hypergeometric test is performed ignoring any bias in the data.

Details

This function takes a character vector of significant CpG sites, maps the CpG sites to Entrez Gene IDs, and tests for GO term or KEGG pathway enrichment using a hypergeometric test, taking into account the number of CpG sites per gene on the 450K/EPIC array.

Geleheer et al. (2013) showed that a severe bias exists when performing gene set analysis for genome-wide methylation data that occurs due to the differing numbers of CpG sites profiled for each gene. gometh is based on the goseq method (Young et al., 2010) and calls the goana function for GO testing, or the kegga function for KEGG testing, both of which are from the limma package (Ritchie et al. 2015). If prior.prob is set to FALSE, then prior probabilities are not used and it is assumed that each gene is equally likely to have a significant CpG site associated with it.

Genes associated with each CpG site are obtained from the annotation package IlluminaHumanMethylation450kanno.ilmn12.hg19 if the array type is "450K". For the EPIC array, the annotation package IlluminaHumanMethylationEPICanno.ilm10b2.hg19 is used. In order to get a list which contains the mapped Entrez gene IDs, please use the getMappedEntrezIDs function.

gometh tests all GO or KEGG terms, and false discovery rates are calculated using the method of Benjamini and Hochberg (1995).

The limma functions topGO and topKEGG can be used to display the top 20 most enriched pathways.

For more generalised gene set testing where the user can specify the gene set/s of interest to be tested, please use the gsameth function.

Value

A data frame with a row for each GO or KEGG term and the following columns:

<table>
<thead>
<tr>
<th>Term</th>
<th>GO term if testing GO pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ont</td>
<td>ontology that the GO term belongs to if testing GO pathways. &quot;BP&quot; - biological process, &quot;CC&quot; - cellular component, &quot;MF&quot; - molecular function.</td>
</tr>
<tr>
<td>Pathway</td>
<td>the KEGG pathway being tested if testing KEGG terms.</td>
</tr>
<tr>
<td>N</td>
<td>number of genes in the GO or KEGG term</td>
</tr>
<tr>
<td>DE</td>
<td>number of genes that are differentially methylated</td>
</tr>
<tr>
<td>P.DE</td>
<td>p-value for over-representation of the GO or KEGG term</td>
</tr>
<tr>
<td>FDR</td>
<td>False discovery rate</td>
</tr>
</tbody>
</table>
References


See Also
goana, kegga, gsameth

Examples

```
library(IlluminaHumanMethylation450kanno.ilmn12.hg19)
library(limma)
ann <- getAnnotation(IlluminaHumanMethylation450kanno.ilmn12.hg19)

# Randomly select 1000 Cpgs to be significantly differentially methylated
sigcpgs <- sample(rownames(ann),1000,replace=FALSE)

# All CpG sites tested
allcpgs <- rownames(ann)

# GO testing with prior probabilities taken into account
# Plot of bias due to differing numbers of CpG sites per gene
gst <- gometh(sig.cpg = sigcpgs, all.cpg = allcpgs, collection = "GO", plot.bias = TRUE, prior.prob = TRUE)

# Total number of GO categories significant at 5% FDR
table(gst$FDR<0.05)

# Table of top GO results
topGO(gst)

# GO testing ignoring bias
gst.bias <- gometh(sig.cpg = sigcpgs, all.cpg = allcpgs, collection = "GO", prior.prob=FALSE)

# Total number of GO categories significant at 5% FDR ignoring bias
table(gst.bias$FDR<0.05)

# Table of top GO results ignoring bias
topGO(gst.bias)
```
gsameth

# KEGG testing
kegg <- gometh(sig.cpg = sigcpgs, all.cpg = allcpgs, collection = "KEGG", prior.prob=TRUE)

# Table of top KEGG results
topKEGG(kegg)

---

gsameth

Generalised gene set testing for Illumina’s methylation array data

Description

Given a user specified list of gene sets to test, gsameth tests whether significantly differentially methylated CpG sites are enriched in these gene sets.

Usage

```
gsameth(sig.cpg, all.cpg = NULL, collection, array.type=c("450K","EPIC"),plot.bias = FALSE, prior.prob = TRUE)
```

Arguments

- `sig.cpg` character vector of significant CpG sites to test for gene set enrichment
- `all.cpg` character vector of all CpG sites tested. Defaults to all CpG sites on the array.
- `collection` a list of user specified gene sets to test. Can also be a single character vector gene set. Gene identifiers must be Entrez Gene IDs.
- `array.type` the Illumina methylation array used. Options are "450K" or "EPIC". Defaults to "450K".
- `plot.bias` logical, if true a plot showing the bias due to the differing numbers of probes per gene will be displayed
- `prior.prob` logical, if true will take into account the probability of significant differentially methylation due to numbers of probes per gene. If false, a hypergeometric test is performed ignoring any bias in the data.

Details

This function extends gometh, which only tests GO and KEGG pathways. gsameth can take a list of user specified gene sets and test whether the significant CpG sites are enriched in these pathways. gsameth maps the CpG sites to Entrez Gene IDs and tests for pathway enrichment using a hypergeometric test, taking into account the number of CpG sites per gene on the 450K/EPIC arrays. Please note the gene ids for the collection of gene sets must be Entrez Gene IDs.

Geeleher et al. (2013) showed that a severe bias exists when performing gene set analysis for genome-wide methylation data that occurs due to the differing numbers of CpG sites profiled for each gene. gsameth and gometh is based on the goseq method (Young et al., 2010). If prior.prob is set to FALSE, then prior probabilities are not used and it is assumed that each gene is equally likely to have a significant CpG site associated with it.

Genes associated with each CpG site are obtained from the annotation package IlluminaHumanMethylation450kanno.ilmn12.hg19 if the array type is "450K". For the EPIC array, the annotation package IlluminaHumanMethylationEPICanno.ilm10b2.hg19 is used. In order to get a list which contains the mapped Entrez gene IDS, please use the getMappedEntrezIDs function.
**Value**

A data frame with a row for each gene set and the following columns:

- **N**: number of genes in the gene set
- **DE**: number of genes that are differentially methylated
- **P.DE**: p-value for over-representation of the gene set
- **FDR**: False discovery rate, calculated using the method of Benjamini and Hochberg (1995).

**Author(s)**

Belinda Phipson

**References**


**See Also**

gometh, getMappedEntrezIDs

**Examples**

```r
library(IlluminaHumanMethylation450kanno.ilmn12.hg19)
library(org.Hs.eg.db)
library(limma)
ann <- getAnnotation(IlluminaHumanMethylation450kanno.ilmn12.hg19)

# Randomly select 1000 CpGs to be significantly differentially methylated
sigcpgs <- sample(rownames(ann),1000,replace=FALSE)

# All CpG sites tested
allcpgs <- rownames(ann)

# Use org.Hs.eg.db to extract a GO term
GOTOID <- toTable(org.Hs.egGO2EG)
setname1 <- GOTOID$go_id[1]
setname1
keep.set1 <- GOTOID$go_id %in% setname1
set1 <- GOTOID$gene_id[keep.set1]
```
```
setname2 <- GOtoID$go_id[2]
setname2
keep.set2 <- GOtoID$go_id %in% setname2
set2 <- GOtoID$gene_id[keep.set2]

# Make the gene sets into a list
sets <- list(set1, set2)
names(sets) <- c(setname1, setname2)

# Testing with prior probabilities taken into account
# Plot of bias due to differing numbers of CpG sites per gene
gst <- gsameth(sig.cpg = sigcpgs, all.cpg = allcpgs, collection = sets, plot.bias = TRUE, prior.prob = TRUE)
topGSA(gst)

# Testing ignoring bias
gst.bias <- gsameth(sig.cpg = sigcpgs, all.cpg = allcpgs, collection = sets, prior.prob = FALSE)
topGSA(gst.bias)
```

---

**RUVadj**

*Adjust estimated variances*

**Description**

Calculate rescaled variances, empirical variances, etc. For use with RUV model fits produced using RUVfit.

**Usage**

```r
RUVadj(fit, ebayes = TRUE, evar = FALSE, rsvar = FALSE, ...)
```

**Arguments**

- `fit`:
  - An object of class `MArrayLM` as returned by RUVfit.
- `ebayes`:
  - A logical variable. Should empirical bayes estimates be calculated?
- `evar`:
  - A logical variable. Should empirical variance estimates be calculated?
- `rsvar`:
  - A logical variable. Should rescaled variance estimates be calculated?
- `...`:
  - Additional arguments that can be passed to `variance_adjust`. See linked function documentation for details.

**Details**

Adjust variance. By default only the empirical bayes method of Smyth (2004) is performed.

**Value**

An object of class `MArrayLM` containing:

- `coefficients`:
  - The estimated coefficients of the factor(s) of interest.
- `sigma2`:
  - Estimates of the features’ variances.
- `t`:
  - t statistics for the factor(s) of interest.
- `p`:
  - P-values for the factor(s) of interest.
multiplier The constant by which \( \sigma^2 \) must be multiplied in order to get an estimate of the variance of coefficients.

df The number of residual degrees of freedom.

\( W \) The estimated unwanted factors.

\( \alpha \) The estimated coefficients of \( W \).

\( b_{yx} \) The coefficients in a regression of \( Y \) on \( X \) (after both \( Y \) and \( X \) have been "adjusted" for \( Z \)). Useful for projection plots.

\( b_{wx} \) The coefficients in a regression of \( W \) on \( X \) (after \( X \) has been "adjusted" for \( Z \)). Useful for projection plots.

\( X \) \( X \) Included for reference.

\( k \) \( k \) Included for reference.

\( c_{tl} \) \( c_{tl} \) Included for reference.

\( Z \) \( Z \) Included for reference.

\( \text{fullW0} \) Can be used to speed up future calls of \( \text{RUVfit} \).

The following items may or may not be present depending on the options selected when \( \text{RUVadj} \) was run:

\( p_{.rsvar} \) P-values, after applying the method of rescaled variances.

\( p_{.evar} \) P-values, after applying the method of empirical variances.

\( p_{.ebayes} \) P-values, after applying the empirical bayes method of Smyth (2004).

\( p_{.rsvar.ebayes} \) P-values, after applying the empirical bayes method of Smyth (2004) and the method of rescaled variances.

\( p_{.BH} \) P-values adjusted for false discovery rate (FDR) using the method of Benjamini and Hochberg (1995).

\( p_{.rsvar.BH} \) FDR-adjusted p-values, after applying the method of rescaled variances.

\( p_{.evar.BH} \) FDR-adjusted p-values, after applying the method of empirical variances.

\( p_{.ebayes.BH} \) FDR-adjusted p-values, after applying the empirical bayes method of Smyth (2004).

\( p_{.rsvar.ebayes.BH} \) FDR-adjusted p-values, after applying the empirical bayes method of Smyth (2004) and the method of rescaled variances.

**Author(s)**

Jovana Maksimovic <jovana.maksimovic@mcri.edu.au>

**References**


RUVfit

Remove unwanted variation when testing for differential methylation

Description

Provides an interface similar to lmFit from limma to the RUV2, RUV4, RUVinv and RUVrinv functions from the ruv package, which facilitates the removal of unwanted variation in a differential methylation analysis. A set of negative control variables, as described in the references, must be specified.

Usage

RUVfit(data, design, coef, ctl, method=c("inv", "rinv", "ruv4", "ruv2"),
       k = NULL, ...)

Arguments

data numeric matrix with rows corresponding to the features of interest such as CpG sites and columns corresponding to samples or arrays.
**design**
the design matrix of the experiment, with rows corresponding to arrays/samples and columns to coefficients to be estimated.

**coef**
integer, column of the design matrix containing the comparison to test for differential methylation. Default is the last column of the design matrix.

**ctl**
logical vector, length == nrow(data). Features that are to be used as negative control variables are indicated as TRUE, all other features are FALSE.

**method**
character string, indicates which RUV method should be used. Default method is RUVinv.

**k**
integer, required if method is "ruv2" or "ruv4". Indicates the number of unwanted factors to use. Can be 0.

**...**
additional arguments that can be passed to RUV2, RUV4, RUVinv and RUVrinv. See linked function documentation for details.

**Details**
This function depends on the ruv and limma packages and is used to estimate and adjust for unwanted variation in a differential methylation analysis. Briefly, the unwanted factors $W$ are estimated using negative control variables. $Y$ is then regressed on the variables $X$, $Z$, and $W$. For methylation data, the analysis is performed on the M-values, defined as the log base 2 ratio of the methylated signal to the unmethylated signal.

**Value**
An object of class MArrayLM (see MArrayLM-class) containing:

- **coefficients** The estimated coefficients of the factor(s) of interest.
- **sigma2** Estimates of the features’ variances.
- **t** t statistics for the factor(s) of interest.
- **p** P-values for the factor(s) of interest.
- **multiplier** The constant by which $sigma2$ must be multiplied in order to get an estimate of the variance of coefficients
- **df** The number of residual degrees of freedom.
- **W** The estimated unwanted factors.
- **alpha** The estimated coefficients of $W$.
- **byx** The coefficients in a regression of $Y$ on $X$ (after both $Y$ and $X$ have been "adjusted" for $Z$). Useful for projection plots.
- **bwx** The coefficients in a regression of $W$ on $X$ (after $X$ has been "adjusted" for $Z$). Useful for projection plots.
- **X** $X$. Included for reference.
- **k** $k$. Included for reference.
- **ctl** $ctl$. Included for reference.
- **Z** $Z$. Included for reference.
- **fullW0** Can be used to speed up future calls of RUVfit.

**Author(s)**
Jovana Maksimovic <jovana.maksimovic@mcri.edu.au>
References


See Also

RUV2, RUV4, RUVinv, RUVrinv, topRUV

Examples

```r
if(require(minfi) & require(minfiData) & require(limma)) {
  # Get methylation data for a 2 group comparison
  meth <- getMeth(MsetEx)
  unmeth <- getUnmeth(MsetEx)
  Mval <- log2((meth + 100)/(unmeth + 100))
  group<-factor(pData(MsetEx)$Sample_Group)
  design<model.matrix(~group)

  # Perform initial analysis to empirically identify negative control features
  # when not known a priori
  lFit = lmFit(Mval,design)
  lFit2 = eBayes(lFit)
  lTop = topTable(lFit2,coef=2,num=Inf)

  # The negative control features should *not* be associated with factor of interest
  # but *should* be affected by unwanted variation
  ctl = rownames(Mval) %in% rownames(lTop[lTop$adj.P.Val > 0.5,])

  # Perform RUV adjustment and fit
  fit = RUVfit(data=Mval, design=design, coef=2, ctl=ctl)
  fit2 = RUVadj(fit)

  # Look at table of top results
  top = topRUV(fit2)
}
```

---

**SWAN**: Subset-quantile Within Array Normalisation for Illumina Infinium HumanMethylation450 BeadChips

**Description**

Subset-quantile Within Array Normalisation (SWAN) is a within array normalisation method for the Illumina Infinium HumanMethylation450 platform. It allows Infinium I and II type probes on a single array to be normalized together.
Usage

SWAN(data, verbose = FALSE)

Arguments

data An object of class either MethylSet, RGChannelSet or MethyLumiSet.
verbose Should the function be verbose?

Details

The SWAN method has two parts. First, an average quantile distribution is created using a subset of probes defined to be biologically similar based on the number of CpGs underlying the probe body. This is achieved by randomly selecting N Infinium I and II probes that have 1, 2 and 3 underlying CpGs, where N is the minimum number of probes in the 6 sets of Infinium I and II probes with 1, 2 or 3 probe body CpGs. If no probes have previously been filtered out e.g. sex chromosome probes, etc. N=11,303. This results in a pool of 3N Infinium I and 3N Infinium II probes. The subset for each probe type is then sorted by increasing intensity. The value of each of the 3N pairs of observations is subsequently assigned to be the mean intensity of the two probe types for that row or 'quantile’. This is the standard quantile procedure. The intensities of the remaining probes are then separately adjusted for each probe type using linear interpolation between the subset probes.

Value

An object of class MethylSet

Note

SWAN uses a random subset of probes to perform the within-array normalization. In order to achieve reproducible results, the seed needs to be set using set.seed.

Author(s)

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References


See Also

RGChannelSet and MethylSet as well as MethyLumiSet and IlluminaMethylationManifest.

Examples

if (require(minfi) & require(minfiData)) {
  set.seed(100)
  datSwan1 <- SWAN(RGsetEx)
  dat <- preprocessRaw(RGsetEx)
  set.seed(100)
  datSwan2 <- SWAN(dat)
After using gsameth, calling topGSA will output the top 20 most significantly enriched pathways.

Usage

```r
topGSA(gsa, number = 20, sort = TRUE)
```

Arguments

- `gsa` matrix, from output of gsameth
- `number` scalar, number of pathway results to output. Default is 20
- `sort` logical, should the table be ordered by p-value. Default is TRUE.

Details

This function will output the top 20 most significant pathways from a pathway analysis using the gsameth function. The output is ordered by p-value.

Value

A matrix ordered by P.DE, with a row for each gene set and the following columns:

- `N` number of genes in the gene set
- `DE` number of genes that are differentially methylated
- `P.DE` p-value for over-representation of the gene set

Author(s)

Belinda Phipson

See Also

- gsameth
Examples

```r
library(IlluminaHumanMethylation450kanno.ilmn12.hg19)
library(org.Hs.eg.db)
library(limma)
ann <- getAnnotation(IlluminaHumanMethylation450kanno.ilmn12.hg19)

# Randomly select 1000 CpGs to be significantly differentially methylated
sigcpgs <- sample(rownames(ann),1000,replace=FALSE)

# All CpG sites tested
allcpgs <- rownames(ann)

# Use org.Hs.eg.db to extract a GO term
GOtoID <- toTable(org.Hs.egGO2EG)
setname1 <- GOtoID$go_id[1]
setname1
keep.set1 <- GOtoID$go_id %in% setname1
set1 <- GOtoID$gene_id[keep.set1]
setname2 <- GOtoID$go_id[2]
setname2
keep.set2 <- GOtoID$go_id %in% setname2
set2 <- GOtoID$gene_id[keep.set2]

# Make the gene sets into a list
sets <- list(set1, set2)
names(sets) <- c(setname1,setname2)

# Testing with prior probabilities taken into account
# Plot of bias due to differing numbers of CpG sites per gene
gst <- gsameth(sig.cpg = sigcpgs, all.cpg = allcpgs, collection = sets, plot.bias = TRUE, prior.prob = TRUE)
topGSA(gst)

# Testing ignoring bias
gst.bias <- gsameth(sig.cpg = sigcpgs, all.cpg = allcpgs, collection = sets, prior.prob = FALSE)
topGSA(gst.bias)
```

### `topRUV`

**Table of top-ranked differentially methylated CpGs obtained from a differential methylation analysis using RUV**

#### Description

Extract a table of the top-ranked CpGs from a linear model fit after performing a differential methylation analysis using RUVfit.

#### Usage

```r
topRUV(fit, number=10, p.value.cut = 1,
```
Arguments

fit  An object containing a linear model fit produced by RUVfit, followed by RUVadj. The fit object should be of class MArrayLM.

number  integer, maximum number of genes to list. Default is 10.

p.value.cut  numeric, cutoff value for adjusted p-values. Only features with lower p-values are listed. Must be between 0 and 1. Default is 1.

cut.on  numeric, the type of adjusted p-value that the cutoff should be applied to. Default is p.ebayes.BH. Other options are: p.BH, p.rsvar.BH, p.evar.BH or p.rsvar.ebayes.BH.

sort.by  character string, the type of adjusted p-value that should be used for sorting. Default is p.ebayes.BH. Other options are: p.BH, p.rsvar.BH, p.evar.BH or p.rsvar.ebayes.BH.

Details

This function summarises the results of a differential methylation analysis performed using RUVfit, followed by RUVadj. The top ranked CpGs are selected by first ranking the adjusted p-values (Default: p.ebayes.BH), then ranking the raw p-values (Default: p.ebayes).

Value

Produces a dataframe with rows corresponding to the top number CpGs and the following columns:

coefficients  The estimated coefficients of the factor(s) of interest.

sigma2  Estimates of the features' variances.

t  t statistics for the factor(s) of interest.

p  P-values for the factor(s) of interest.

multiplier  The constant by which sigma2 must be multiplied in order to get an estimate of the variance of coefficients.

df  The number of residual degrees of freedom.

W  The estimated unwanted factors.

alpha  The estimated coefficients of W.

byx  The coefficients in a regression of Y on X (after both Y and X have been "adjusted" for Z). Useful for projection plots.

bwx  The coefficients in a regression of W on X (after X has been "adjusted" for Z). Useful for projection plots.

X  X. Included for reference.

k  k. Included for reference.

ctl  ctl. Included for reference.

Z  Z. Included for reference.

fullW0  Can be used to speed up future calls of RUVfit.

The following columns may or may not be present depending on the options selected when RUVadj was run:

p.rsvar  P-values, after applying the method of rescaled variances.

p.evar  P-values, after applying the method of empirical variances.


p.rsvar.BH FDR-adjusted p-values, after applying the method of rescaled variances.

p.evar.BH FDR-adjusted p-values, after applying the method of empirical variances.


Author(s)

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References


See Also

`RUVfit`, `RUVadj`, `MArrayLM`

Examples

```
if(require(minfi) & require(minfiData) & require(limma)){

  # Get methylation data for a 2 group comparison
  meth <- getMeth(MsetEx)
  unmeth <- getUnmeth(MsetEx)
  Mval <- log2((meth + 100)/(unmeth + 100))

  group<-factor(pData(MsetEx)$Sample_Group)
  design<-model.matrix(~group)

  # Perform initial analysis to empirically identify negative control features
  # when *not* known a priori
  lFit = lmFit(Mval,design)
  lFit2 = eBayes(lFit)
  lTop = topTable(lFit2,coef=2,num=Inf)

  # The negative control features should *not* be associated with factor of interest
  # but *should* be affected by unwanted variation
  ctl = rownames(Mval) %in% rownames(lTop[lTop$adj.P.Val > 0.5,])

  # Perform RUV adjustment and fit
  fit = RUVfit(data=Mval, design=design, coef=2, ctl=ctl)
```

```r
fit2 = RUVadj(fit)

# Look at table of top results
top = topRUV(fit2)
```

### Description

Extract a table of the top-ranked CpGs from a linear model fit after a differential variability analysis.

### Usage

```r
topVar(fit, coef = NULL, number = 10, sort = TRUE)
```

### Arguments

- **fit**: list containing a linear model fit produced by `varFit`. The fit object should be of class `MArrayLM`.
- **coef**: column number or column name specifying which coefficient of the linear model fit is of interest. It should be the same coefficient that the differential variability testing was performed on. Default is last column of fit object.
- **number**: maximum number of genes to list. Default is 10.
- **sort**: logical, default is TRUE. Sorts output according the P-value. FALSE will return results in same order as fit object.

### Details

This function summarises the results of a differential variability analysis performed with `varFit`. The p-values from the comparison of interest are adjusted using Benjamini and Hochberg’s false discovery rate with the function `p.adjust`. The top ranked CpGs are selected by first ranking the adjusted p-values, then ranking the raw p-values. At this time no other sorting option is catered for.

### Value

Produces a dataframe with rows corresponding to the top CpGs and the following columns:

- **genelist**: one or more columns of annotation for each CpG, if the gene information is available in `fit`
- **AvgVar**: average of the absolute or squared Levene residuals across all samples
- **DiffVar**: estimate of the difference in the Levene residuals corresponding to the comparison of interest
- **t**: moderated t-statistic
- **P.Value**: raw p-value
- **Adj.P.Value**: adjusted p-value

### Author(s)

Belinda Phipson
References

data shows CpG islands are highly variably methylated in cancers. *Genome Biology*, **15**:465.
Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and pow-

See Also

varFit, p.adjust

Examples

# Randomly generate data for a 2 group problem with 100 CpG sites and 5 arrays in each group.
y<-matrix(rnorm(1000),ncol=10)
group<-factor(rep(c(1,2),each=5))
design<-model.matrix(~group)

# Fit linear model for differential variability
vfit<-varFit(y,design)

# Look at top table of results
topVar(vfit,coef=2)

---

**varFit**

*Testing for differential variability*

**Description**

Fit linear model on mean absolute or squared deviations for each CpG given a series of methylation
arrays

**Usage**

`varFit(data, design = NULL, coef = NULL, type = NULL, trend = TRUE, robust = TRUE, weights = NULL)`

**Arguments**

data  
object of class `MethylSet` or matrix with rows corresponding to the features of interest such as CpG sites and columns corresponding to samples or arrays

design  
the design matrix of the experiment, with rows corresponding to arrays/samples and columns to coefficients to be estimated. Defaults to the unit vector.

coeff  
The columns of the design matrix containing the comparisons to test for differential variability.

type  
character string, "AD" for absolute residuals or "SQ" for squared residuals. Default is absolute.

trend  
logical, if true fits a mean variance trend on the absolute or squared deviations
robust logical, if true performs robust empirical Bayes shrinkage of the variances for the moderated t statistics

weights non-negative observation weights. Can be a numeric matrix of individual weights, of same size as the object matrix, or a numeric vector of array weights, or a numeric vector of gene/feature weights.

Details

This function depends on the limma package and is used to rank features such as CpG sites or genes in order of evidence of differential variability between different comparisons corresponding to the columns of the design matrix. A measure of variability is calculated for each CpG in each sample by subtracting out the group mean and taking the absolute or squared deviation. A linear model is then fitted to the absolute or squared deviations. The residuals of the linear model fit are subjected to empirical Bayes shrinkage and moderated t statistics (Smyth, 2004) calculated. False discovery rates are calculated using the method of Benjamini and Hochberg (1995).

If coef is not specified, then group means are estimated based on all the columns of the design matrix and subtracted out before testing for differential variability. If the design matrix contains nuisance parameters, then subsetting the design matrix columns by coef should remove these columns from the design matrix. If the design matrix includes an intercept term, this should be included in coef. The nuisance parameters are included in the linear model fit to the absolute or squared deviations, but should not be considered when subtracting group means to obtain the deviations. Note that design matrices without an intercept term are permitted, and specific contrasts tested using the function contrasts.varFit.

For methylation data, the analysis is performed on the M-values, defined as the log base 2 ratio of the methylated signal to the unmethylated signal. If a MethylSet object is supplied, M-values are extracted with an offset of 100 added to the numerator and denominator.

For testing differential variability on RNA-Seq data, a DGEList object can be supplied directly to the function. A voom transformation is applied before testing for differential variability. The weights calculated in voom are used in the linear model fit.

Since the output is of class MArrayLM, any functions that can be applied to fit objects from lmFit and eBayes can be applied, for example, topTable and decideTests.

Value

produces an object of class MArrayLM (see MArrayLM-class) containing everything found in a fitted model object produced by lmFit and eBayes as well as a vector containing the sample CpG-wise variances and a matrix of LogVarRatios corresponding to the differential variability analysis.

Author(s)

Belinda Phipson

References


See Also

`contrasts.varFit`, `topVar`, `getLeveneResiduals`, `lmFit`, `eBayes`, `topTable`, `decideTests`, `voom`

Examples

# Randomly generate data for a 2 group problem with 100 CpG sites and 5 arrays in each group.

```r
y<-matrix(rnorm(1000),ncol=10)
group<-factor(rep(c(1,2),each=5))
design<-model.matrix(~group)

# Fit linear model for differential variability
vfit<-varFit(y,design,coef=c(1,2))

# Look at top table of results

topVar(vfit,coef=2)
```
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