Package ‘nudge’

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Title Normal Uniform Differential Gene Expression detection

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Description Package for normalizing microarray data in single and multiple
replicate experiments and fitting a normal-uniform mixture to detect
differentially expressed genes in the cases where the two samples are being
compared directly or indirectly (via a common reference sample)

biocViews Microarray, TwoChannel, DifferentialExpression

Imports stats

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NeedsCompilation no

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**hiv**  
*Microarray intensity expression levels for HIV type 1 infection of CD4+ T-Cell lines*

**Description**

The “hiv” section of this dataset consists of cDNA from CD4+ T cell lines at 1 hour after infection with HIV-1BRU. There are 4608 genes’ expression levels. There are four replicates with balanced dye sways i.e. two of the replicates have sample 1 labeled with red and sample 2 labeled with green and the other two have the opposite labeling scheme. It is useful in testing the specificity and sensitivity of methods identifying differentially expressed genes, since there are 13 genes known to be differentially expressed (HIV-1 genes), called positive controls, identified in the vector of logical values “pos.contr” and 29 genes known not to be differentially expressed (non-human genes), called negative controls, identified in the vector of logical values “neg.contr”.

**Usage**

data(hiv)

**Format**

A matrix containing 4608 observations on eight variables

**Source**

Bumgarner Lab

**References**

[http://www.biomedcentral.com/1471-2105/6/173](http://www.biomedcentral.com/1471-2105/6/173)


**See Also**

pos.contr,neg.contr

---

**like**  
*Microarray intensity expression levels for like-like data*

**Description**

This data set gives the expression levels for the same sample tested against itself, i.e. one sample split into two and labeled with both red and green dyes. Useful for testing false positive rate of methods for detecting differential expression since there are, theoretically no differentially expressed genes. There are 7680 different genes’ expression levels measured.
Usage
data(like)

Format
A matrix containing 7680 observations on two variables

Source
Bumgarner Lab

References
http://www.biomedcentral.com/1471-2105/6/173

Description
The “hiv” section of this dataset consists of cDNA from CD4+ T cell lines at 1 hour after infection with HIV-1BRU. There are 4608 genes’ expression levels. There are four replicates with balanced dye sways i.e. two of the replicates have sample 1 labeled with red and sample 2 labeled with green and the other two have the opposite labeling scheme. It is useful in testing the specificity of methods identifying differentially expressed genes, since there are 29 genes known not to be differentially expressed (non-human genes), called negative controls, identified in the vector of logical values “neg.contr”.

Usage
data(hiv)

Format
A logical vector containing 4608 indicators of known non differential expression

Source
Bumgarner Lab

References
http://www.biomedcentral.com/1471-2105/6/173
**See Also**

pos.contr.hiv

---

**norm1a**  
*Function for normalizing the mean of single replicate log ratios*

**Description**

This is essentially the same as the lowess normalization suggested in the paper "Statistical methods for identifying differentially expressed genes in replicated cDNA microarrays" by Dudoit et al (2002), except the loess function is used instead of lowess and the recommended span is between 0.6 and 0.8. The normalization is done for each gene by subtracting from its log ratio the loess estimated mean for the log ratio based on the regression of log ratios on log intensities.

**Usage**

```r
norm1a(logratio, logintensity, span = 0.6)
```

**Arguments**

- `logratio`: A vector or single-column matrix of log (base 2) ratios of gene expressions in two samples.
- `logintensity`: A vector or single-column matrix of log (base 2) total intensities (defined as the product) of gene expressions in the two samples.
- `span`: Proportion of data used to fit the loess regression of the log ratios on the log intensities.

**Value**

A vector or single-column matrix of mean normalized log ratios.

**Author(s)**

N. Dean and A. E. Raftery

**References**

[http://www.biomedcentral.com/1471-2105/6/173](http://www.biomedcentral.com/1471-2105/6/173)


**See Also**

norm1b,norm1c,norm1d,norm2c,norm2d
Examples

```r
data(like)
1R<-log(like[,1],2)-log(like[,2],2)
1I<-log(like[,1],2)+log(like[,2],2)
1Rnorm<-norm1a(1R,1I)
```

---

**norm1b**  
*Function for normalizing the mean and variance (or just the variance) of single replicate log ratios*

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**Description**

This performs a robust loess normalization of the variance of the log ratios in a single replicate experiment by regressing the absolute (mean normalized) log ratios on the log intensities and using the fitted values to scale the (mean normalized) log ratio for each gene.

**Usage**

```r
norm1b(logratio, logintensity, span1 = 0.6, span2 = 0.2, mean.norm=TRUE)
```

**Arguments**

- **logratio**: A vector or single-column matrix of log (base 2) ratios of gene expressions in two samples, if mean.norm is FALSE the log ratios should be already mean normalized.
- **logintensity**: A vector or single-column matrix of log (base 2) total intensities (defined as the product) of gene expressions in the two samples.
- **span1**: Proportion of data used to fit the loess regression of the log ratios on the log intensities for the mean normalization.
- **span2**: Proportion of data used to fit the loess regression of the absolute (mean normalized) log ratios on the log intensities for the variance normalization.
- **mean.norm**: A logical value indicating whether or not a mean normalization should be performed prior to the variance normalization.

**Value**

A vector or single-column matrix of mean and variance normalized log (base 2) ratios of gene expressions in two samples.

**Author(s)**

N. Dean and A. E. Raftery

**References**


[http://www.biomedcentral.com/1471-2105/6/173](http://www.biomedcentral.com/1471-2105/6/173)
norm1c

Function for normalizing the mean of average-across-replicates log ratios

Description

This is essentially the same as the lowess normalization suggested in the paper "Statistical methods for identifying differentially expressed genes in replicated cDNA microarrays" by Dudoit et al (2002), except the loess function and average-across-replicates log ratios are used and the recommended span is between 0.6 and 0.8. The normalization is done for each gene by subtracting from its average-across-replicates log ratio the loess estimated mean for average-across-replicates log ratio based on the loess regression of average-across-replicates log ratios on average-across-replicates log total intensities.

Usage

norm1c(logratio, logintensity, span = 0.6)

Arguments

logratio A multiple-column matrix of replicates of log (base 2) ratios of gene expressions in two samples.

logintensity A multiple-column matrix of replicates of log (base 2) total intensities (defined as the product) of gene expressions in two samples.

span Proportion of data used to fit the loess regression of the average-across-replicates log ratios on the average-across-replicates log total intensities

Value

A vector of mean normalized average-across-replicates log ratios.

Author(s)

N. Dean and A. E. Raftery

Examples

data(like)
1R<-log(like[,1],2)-log(like[,2],2)
1I<-log(like[,1],2)+log(like[,2],2)

1Rnorm<-norm1b(1R,1I,mean.norm=TRUE)
norm1d

References


http://www.biomedcentral.com/1471-2105/6/173


See Also

norm1d,norm1a,norm1b,norm2c,norm2d

Examples

data(hiv)
lR<-log(hiv[,1:4],2)-log(hiv[,5:8],2)
lI<-log(hiv[,1:4],2)+log(hiv[,5:8],2)
lRnorm<-norm1c(lR,lI)

norm1d

Function for normalizing the mean and variance of average-across-replicates log ratios

Description

This performs a robust normalization of the variance of the (mean normalized) average-across-replicates log ratios by scaling the (mean normalized) average-across-replicates log ratio for each gene either by the standard deviation of the log ratios for that gene across replicates (if bigger than its absolute (mean normalized) average-across-replicates log ratio) or scaling by a constant (a quantile of the distribution of standard deviations of log ratios across replicates for all genes whose standard deviation was bigger than their absolute (mean normalized) average-across-replicates log ratio.

Usage

norm1d(logratio, logintensity, span = 0.6, quant = 0.99, dye.swap = FALSE)

Arguments

logratio A multiple-column matrix of replicates of log (base 2) ratios of gene expressions in two samples.

logintensity A multiple-column matrix of replicates of log (base 2) total intensities (defined as the product) of gene expressions in two samples.

span Proportion of data used to fit the loess regression of the average-across-replicates log ratios on the average-across-replicates log total intensities.

quant Quantile to be used from the distribution of standard deviations of log ratios across replicates for all genes whose standard deviation was bigger than their absolute (mean normalized) average-across-replicates log ratio.

dye.swap A logical value indicating whether or not the data consists of a balanced dye swap (if FALSE a mean normalization will be performed prior to the variance normalization rather than a simple averaging across replicates).
Details

A balanced dye swap is a data set where the data are split into two sets of replicates (same number of replicates in each set) where one set has the reverse dye assignment of the other set.

Value

A vector of mean and variance normalized average-across-replicates log ratios.

Author(s)

N. Dean and A. E. Raftery

References


http://www.biomedcentral.com/1471-2105/6/173

See Also

norm1c,norm1a,norm1b,norm2c,norm2d

Examples

data(hiv)
lR<-log(hiv[,1:4],2)-log(hiv[,5:8],2)
lI<-log(hiv[,1:4],2)+log(hiv[,5:8],2)

lRnorm<-norm1d(lR,lI,dye.swap=TRUE)

---

**norm2c**

*Function for normalizing the mean of average-across-replicates log ratio differences*

Description

This normalization is used when the two samples (control and treatment, say) are not being directly compared on the slides but instead are being compared to a common reference sample. The quantity of interest for each gene is thus the average difference between control and treatment log ratios. The normalization is done for each gene by subtracting from its average-across-replicates log ratio difference the loess estimated mean for average-across-replicates log ratio difference based on the loess regression of average-across-replicates log ratio differences on average-across-replicates log total intensities.

Usage

```
norm2c(control.logratio, txt.logratio, control.logintensity, txt.logintensity, span = 0.6)
```
Arguments

control.logratio
A multiple-column matrix of replicates of log (base 2) ratios of gene expressions for the control versus reference slides.

txt.logratio
A multiple-column matrix of replicates of log (base 2) ratios of gene expressions for the treatment versus reference slides.

control.logintensity
A multiple-column matrix of replicates of log (base 2) total intensities (defined as the product) of gene expressions for the control versus reference slides.

txt.logintensity
A multiple-column matrix of replicates of log (base 2) total intensities (defined as the product) of gene expressions for the treatment versus reference slides.

span
Proportion of data used to fit the loess regression of the average-across-replicates log ratio differences on the average-across-replicates log total intensities.

Value

A vector of mean normalized average-across-replicates log ratio differences.

Author(s)

N. Dean and A. E. Raftery

References


http://www.biomedcentral.com/1471-2105/6/173


See Also

norm2d,norm1a,norm1b,norm1c,norm1d

Examples

apo<-read.csv("http://www.stat.berkeley.edu/users/terry/zarray/Data/ApoA1/rg_a1ko_morph.txt", header=TRUE)
rownames(apo)<-apo[,1]
apo<-apo[,1]
apo<-apo+1

lRctl<-log(apo[,c(seq(2,16,2))],2)-log(apo[,c(seq(1,15,2))],2)
lRtxt<-log(apo[,c(seq(18,32,2))],2)-log(apo[,c(seq(17,31,2))],2)
lIctl<-log(apo[,c(seq(2,16,2))],2)+log(apo[,c(seq(1,15,2))],2)
lItxt<-log(apo[,c(seq(18,32,2))],2)+log(apo[,c(seq(17,31,2))],2)
lRnorm<-norm2c(lRctl,lRtxt,lIctl,lItxt)
norm2d

Function for normalizing the mean and variance of average-across-replicates log ratio differences

Description

This normalization is used when the two samples (control and treatment, say) are not being directly compared on the slides but instead are being compared to a common reference sample. The quantity of interest for each gene is thus the average difference between control and treatment log ratios. This function performs a robust normalization of the variance of the (mean normalized) average-across-replicates log ratio differences by scaling the (mean normalized) average-across-replicates log ratio difference for each gene either by the standard deviation of the log ratio differences for that gene across replicates (if bigger than the absolute (mean normalized) average-across-replicates log ratio difference) or scaling by a constant (a quantile of the distribution of standard deviations of (mean normalized) average-across-replicates log ratio differences for all genes whose standard deviation was bigger than their absolute (mean normalized) average-across-replicates log ratio difference.

Usage

```r
norm2d(control.logratio, txt.logratio, control.logintensity, txt.logintensity, span = 0.6, quant = 0.99)
```

Arguments

- `control.logratio`: A multiple-column matrix of replicates of log (base 2) ratios of gene expressions for the control versus reference slides.
- `txt.logratio`: A multiple-column matrix of replicates of log (base 2) ratios of gene expressions for the treatment versus reference slides.
- `control.logintensity`: A multiple-column matrix of replicates of log (base 2) total intensities (defined as the product) of gene expressions for the control versus reference slides.
- `txt.logintensity`: A multiple-column matrix of replicates of log (base 2) total intensities (defined as the product) of gene expressions for the treatment versus reference slides.
- `span`: Proportion of data used to fit the loess regression of the average-across-replicates log ratio differences on the average-across-replicates log intensities.
- `quant`: Quantile to be used from the distribution of standard deviations of log ratio differences across replicates for all genes whose standard deviation was smaller than their absolute (mean normalized) average-across-replicates log ratio difference.

Value

A vector of mean and variance normalized average-across-replicates log ratio differences.

Author(s)

N. Dean and A. E. Raftery
References

http://www.biomedcentral.com/1471-2105/6/173


See Also

norm2c,norm1a,norm1b,norm1c,norm1d

Examples

rownames(apo)<-apo[,1]
apo<-apo[,1]
apo<-apo+1
lRctl<-log(apo[,c(seq(2,16,2))],2)-log(apo[,c(seq(1,15,2))],2)
lRtxt<-log(apo[,c(seq(18,32,2))],2)-log(apo[,c(seq(17,31,2))],2)
lIctl<-log(apo[,c(seq(2,16,2))],2)+log(apo[,c(seq(1,15,2))],2)
lItxt<-log(apo[,c(seq(18,32,2))],2)+log(apo[,c(seq(17,31,2))],2)
lRnorm<-norm2d(lRctl,lRtxt,lIctl,lItxt)

Description

After a mean and variance normalization, a two component mixture model is fitted to the data. The normal component represents the genes that are not differentially expressed and the uniform component represents the genes that are differentially expressed. Posterior probabilities for differential expression are computed from the fitted model.

Usage

nudge1(logratio, logintensity, dye.swap = FALSE, span1 = 0.6, span2 = 0.2, quant = 0.99, z = NULL, tol = 0.00001, iterlim=500)

Arguments

logratio A matrix or vector of log (base 2) ratios of intensity expressions in 2 samples, with rows indexing genes and columns (if necessary) indexing replicates.

logintensity A matrix or vector of total log (base 2) total intensities (defined as the product) of intensity expressions in 2 samples, with rows indexing genes and columns (if necessary) indexing replicates.
**dye.swap**  
A logical value indicating whether or not the data is from a balanced dye-swap. Only used for multiple replicate experiments.

**span1**  
Proportion of data used to fit the loess regression of the (average-across-replicates) log ratios on the (average-across-replicates) log total intensities for the mean normalization.

**span2**  
Proportion of data used to fit the loess regression of the absolute (mean normalized) log ratios on the log total intensities for the variance normalization. Only used for single replicate experiments.

**quant**  
Quantile to be used from the distribution of standard deviations of log ratios across replicates for all genes whose standard deviation was smaller than their absolute (mean normalized) average-across-replicates log ratio. Only used for multiple replicate experiments.

**z**  
An optional 2-column matrix with each row giving a starting estimate for the probability of the gene (in the corresponding row of the log ratio matrix/vector) not being differentially expressed and a starting estimate for the probability of the gene being differentially expressed. Each row should add up to 1.

**tol**  
A scalar tolerance for relative convergence of the loglikelihood.

**iterlim**  
The maximum number of iterations the EM is run for.

**Details**

A balanced dye swap is where a certain number of replicates have a particular dye to sample assignment and the same number of other replicates have the reversed assignment. Note in this case log ratios should be taken with numerators being the same sample and denominators the other sample, i.e. ratios should always be sample i/sample j rather than red dye/green dye for all replicates.

**Value**

A list including the following components

- **pdiff**  
A vector with the estimated posterior probabilities of being in the group of differentially expressed genes.

- **lRnorm**  
A vector with the normalized (average-across-replicates) log ratios.

- **mu**  
The estimated mean of the group of genes that are not differentially expressed.

- **sigma**  
The estimated variance of the group of genes that are not differentially expressed.

- **mixprob**  
The prior/mixing probability of a gene being in the group of genes that are not differentially expressed.

- **a**  
The minimum value of the normalized data.

- **b**  
The maximum value of the normalized data.

- **loglike**  
The log likelihood for the fitted mixture model.

- **iter**  
The number of iterations run by the EM algorithm until either convergence or iteration limit was reached.

**Author(s)**

N. Dean and A. E. Raftery
nudge2

References


http://www.biomedcentral.com/1471-2105/6/173


See Also

nudge2, norm1a, norm1b, norm1c, norm1d, norm2c, norm2d

Examples

data(like)
lR<-log(like[,1],2)-log(like[,2],2)
lI<-log(like[,1],2)+log(like[,2],2)

result<-nudge1(lR,lI)

data(hiv)
lR<-log(hiv[,1:4],2)-log(hiv[,5:8],2)
lI<-log(hiv[,1:4],2)+log(hiv[,5:8],2)

result<-nudge1(lR,lI,dye.swap=TRUE)

---

nudge2

Function for normalizing data, fitting a normal-uniform mixture and estimating probabilities of differential expression in the case where the two samples are being compared indirectly through a common reference sample

Description

After a mean and variance normalization a two component mixture model is fitted to the data. The normal component represents the genes that are not differentially expressed and the uniform component represents the genes that are differentially expressed. Posterior probabilities for differential expression are computed from the fitted model.

Usage

nudge2(control.logratio, txt.logratio, control.logintensity, txt.logintensity, span1 = 0.2, quant = 0.99, z = NULL, tol = 0.00001, iterlim=500)

Arguments

control.logratio

A multiple-column matrix of replicates of log (base 2) ratios of gene expressions for the control versus reference slides.

txt.logratio

A multiple-column matrix of replicates of log (base 2) ratios of gene expressions for the treatment versus reference slides.
control.logintensity
A multiple-column matrix of replicates of log (base 2) total intensities (defined as the product) of gene expressions for the control versus reference slides.

txt.logintensity
A multiple-column matrix of replicates of log (base 2) total intensities (defined as the product) of gene expressions for the treatment versus reference slides.

span1
Proportion of data used to fit the loess regression of the (average-across-replicates) log ratio differences on the (average-across-replicates) log intensities for the mean normalization.

quant
Quantile to be used from the distribution of standard deviations of log ratio differences across replicates for all genes whose standard deviation was smaller than their absolute (mean normalized) average-across-replicates log ratio difference.

z
An optional 2-column matrix with each row giving a starting estimate for the probability of the gene (in the corresponding row of the log ratio matrix/vector) not being differentially expressed and a starting estimate for the probability of the gene being differentially expressed. Each row should add up to 1.

tol
A scalar tolerance for relative convergence of the loglikelihood.

iterlim
The maximum number of iterations the EM is run for.

Value
A list including the following components

pdiff
A vector with the estimated posterior probabilities of being in the group of differentially expressed genes.

lRnorm
A vector with the normalized (average-across-replicates) log ratio differences.

mu
The estimated mean of the group of genes that are not differentially expressed.

sigma
The estimated variance of the group of genes that are not differentially expressed.

mixprob
The prior/mixing probability of a gene being in the group of genes that are not differentially expressed.

a
The minimum value of the normalized data.

b
The maximum value of the normalized data.

loglike
The log likelihood for the fitted mixture model.

iter
The number of iterations run by the EM algorithm until either convergence or iteration limit was reached.

Author(s)
N. Dean and A. E. Raftery

References
http://www.biomedcentral.com/1471-2105/6/173

**pos.contr**

**See Also**

nudge1, norm2c, norm2d, norm1a, norm1b, norm1c, norm1d

**Examples**

```r
apo <- read.csv("http://www.stat.berkeley.edu/users/terry/zarray/Data/ApoA1/rg_a1ko_morph.txt", header=TRUE)
rownames(apo) <- apo[,1]
apo <- apo[, -1]
apo <- apo + 1

lRctl <- log(apo[, c(seq(2, 16, 2))], 2) - log(apo[, c(seq(1, 15, 2))], 2)
lRtxt <- log(apo[, c(seq(18, 32, 2))], 2) - log(apo[, c(seq(17, 31, 2))], 2)
lIctl <- log(apo[, c(seq(2, 16, 2))], 2) + log(apo[, c(seq(1, 15, 2))], 2)
lItxt <- log(apo[, c(seq(18, 32, 2))], 2) + log(apo[, c(seq(17, 31, 2))], 2)

result <- nudge2(lRctl, lRtxt, lIctl, lItxt)
```

**pos.contr**  
Indicator of known truly differentially expressed genes in the hiv dataset

**Description**

The “hiv” section of this dataset consists of cDNA from CD4+ T cell lines at 1 hour after infection with HIV-1BRU. There are 4608 genes’ expression levels. There are four replicates with balanced dye sways i.e. two of the replicates have sample 1 labeled with red and sample 2 labeled with green and the other two have the opposite labeling scheme. It is useful in testing the sensitivity of methods identifying differentially expressed genes, since there are 13 genes known to be differentially expressed (HIV-1 genes), called positive controls, identified in the vector of logical values “pos.contr”

**Usage**

data(hiv)

**Format**

A logical vector containing 4608 indicators of known differential expression

**Source**

Bumgarner Lab

**References**

http://www.biomedcentral.com/1471-2105/6/173

See Also

hiv.neg.contr
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