Package 'spkTools'

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Title Methods for Spike-in Arrays

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Depends R (>= 2.7.0), Biobase (>= 2.5.5)

Suggests xtable

Imports Biobase (>= 2.5.5), graphics, grDevices, gtools, methods,RColorBrewer, stats, utils

Description The package contains functions that can be used to compare expression measures on different array platforms.

biocViews Software, AssayTechnologies, Microarray

License GPL (>= 2)

URL http://bioconductor.org

R topics documented:

`affy` *SpikeInExpressionSet of Affymetrix Spike-in Experiment Data*

Description

This is a SpikeInExpressionSet object containing the data from the Affymetrix HGU133A Spike-in Experiment.

Usage

```
data(affy)
```

Format

It contains a matrix of expression values and a matrix of nominal concentrations.

Source

For more information see Irizarry, R.A., et al. NAR (2003) http://www.biostat.jhsph.edu/~ririzarr/papers/index.html

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`plotSpkBox` *Boxplots of Fold Changes Calculated by spkBox*

Description

Plots boxplots of the data resulting from a call to spkBox.

Usage

```
plotSpkBox(boxs, fc=2, box.names=NULL, ...)
```

Arguments

`boxs`	the output of a call to spkBox
`fc`	expected fold change
`box.names`	names to be printed below each boxplot
`...`	parameters passed to boxplot

Value

Boxplots for spike-in and non-spike-in comparisons stratified by ALE strata are produced.

Author(s)

Matthew N. McCall

Examples

```
data(affy)
affySlope <- spkSlope(affy)
affyBox <- spkBox(affy, affySlope)
plotSpkBox(affyBox)
```

SpikeInExpressionSet-class

Class to Contain and Describe High-Throughput Expression Level Assays with Spike-in Data

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Description

This is a class representation for spike-in expression data. `SpikeInExpressionSet` class is derived from `ExpressionSet`, and requires a matrix names `exprs` and a matrix named `spikeIn`.

Extends

Extends class `ExpressionSet`.

Creating Objects

`createSpikeInExpressionSet(exprs, spikeIn, ...)`

`new("SpikeInExpressionSet", phenoData = new("AnnotatedDataFrame"), featureData = new("AnnotatedDa`

This creates a `SpikeInExpressionSet` with `assayData` implicitly created to contain `exprs` and `spikeIn`. Additional named matrix arguments with the same dimensions as `exprs` are added to `assayData`; the row and column names of these additional matrices should match those of `exprs` and `spikeIn`.

`new("SpikeInExpressionSet", assayData = assayDataNew(exprs=new("matrix"),spikeIn=new("matrix")),`

This creates a `SpikeInExpressionSet` with `assayData` provided explicitly. In this form, the only required named argument is `assayData`.

Slots

Inherited from ExpressionSet:

assayData: Contains matrices with equal dimensions, and with column number equal to nrow(phenoData). assayData must contain a matrix exprs and a matrix spikeIn with rows representing features and columns representing samples.

phenoData: See eSet

annotation See eSet

featureData See eSet

experimentData: See eSet

Methods

Class-specific methods:

spikeIn(SpikeInExpressionSet), spikeIn(SpikeInExpressionSet)<- Access and set elements named spikeIn in the AssayData-class slot.

spkSplit(SpikeInExpressionSet) creates two SpikeInExpressionSet objects – one with the spike-in probes and one with the non-spike-in probes.

For derived methods (see ExpressionSet).

See Also

eSet-class, ExpressionSet-class.

Examples

```
# create an instance of SpikeInExpressionSet
new("SpikeInExpressionSet")

new("SpikeInExpressionSet", exprs=matrix(runif(1000), nrow=100), spikeIn=matrix(rep(1:10,100), nrow=100))

# class specific methods
data(affy)
affySpikes <- spikeIn(affy)
affySplit <- spkSplit(affy)
```

---

spkAccSD *Accuracy Standard Deviation*

Description

Estimates the standard deviation for spike-ins at the lowest possible fold change in each bin.

Usage

```
spkAccSD(object, spkSlopeOut, tol=3)
```

Arguments

object	a SpikeInExpressionSet object
spkSlopeOut	the output from the spkSlope function
tol	number of digits after decimal point

Value

returns the median absolute deviation (MAD) for each bin.

Author(s)

Matthew N. McCall

Examples

```
data(affy)
affySlope <- spkSlope(affy)
spkAccSD <- spkAccSD(affy, affySlope)
```

---

spkAll *Spike-in Functions Wrapper*

Description

A wrapper for the functions contained in the spkTools package, which calls each function.

Usage

```
spkAll(object, label, model=expr~spike+probe+array, fc=NULL, tol=3,
xrngs=NULL, yrngs=NULL, cuts=c(.6,.99), potQuantile=.995,
gnn=c(25,100,10000), pch=".", output="eps")
```

Arguments

object	a SpikeInExpressionSet object
label	a character string to insert into the graphs and tables produced
model	model to be passed to spkAnova
fc	the fold change for which fold change plots will be produced
tol	the number of digits after the decimal point in fc
xrngs	ranges for the x-axis of each plot. d=density, s=slope, v=box, m=M vs A
yrngs	ranges for the y-axis of each plot. d=density, s=slope, v=box, m=M vs A
cuts	quantiles used to make the low, medium, and high bins
potQuantile	the desired quantile to compute the probability of being above
gnn	a vector of 3 numbers passed to spkGNN: the desired number of true positives, the number of truly expressed genes, and the number of truly unexpressed genes
pch	plotting point to be used in spkSlope
output	the format in which to save the plots produced. Options are "pdf" and "eps"

Value

The full complement of plots and tables described in the vignette are created and saved in the current working directory.

Author(s)

Matthew N. McCall

Examples

```
data(affy)
spkAll(affy, label="affy", fc=2)
```

---

spkAnova *Anova Model for Microarray Spike-in Data*

Description

Computes the mean squared errors of a microarray spike-in design due to concentration, probe, array, and error.

Usage

```
spkAnova(object, model=expr~spike+probe+array)
```

Arguments

object	a SpikeInExpressionSet object
model	the anova model

Value

A vector of the mean squared errors from the anova model.

Author(s)

Matthew N. McCall

Examples

```
data(affy)
spkAnova(affy)
```

spkBal *Quantify Microarry Spike-in Design Imbalance*

Description

Computes the imbalance of a microarray spike-in design due to probes and arrays.

Usage

```
spkBal(object)
```

Arguments

object	a SpikeInExpressionSet object

Value

The probe and array imbalances.

Author(s)

Matthew N. McCall

References

Wu, Chien-Fu, Iterative Construction of Nearly Balanced Assignments I: Categorical Covariates. Technometrics, Vol. 23, No. 1. (Feb, 1981), pp. 37-44.

Examples

```
data(affy)
spkBal(affy)
```

spkBox *Fold Change Calculations*

Description

A function to calculate the log-ratios stratified by which ALE groups yield the comparison. They are stratified by which bins are being compared to produce the given fold change.

Usage

```
spkBox(object, spkSlopeOut, fc = 2, tol = 3, reduce=TRUE)
```

Arguments

object	a SpikeInExpressionSet object
spkSlopeOut	the output of the spkSlope function
fc	the fold change of interest
tol	the precision (number of digits after decimal point) in fc
reduce	if TRUE the number of points plotted in the null bins is reduced

Details

This function requires the output of spkSlope.

Value

A list with the log-ratios separated by ALE strata comparison.

Author(s)

Matthew N. McCall

Examples

```
data(affy)
affySlope <- spkSlope(affy)
spkBox(affy,affySlope)
```

---

spkDensity *Spike-in Density Plot*

Description

A density plot of the non-spike-in expression with a rug of the average expression at each spike-in level.

Usage

```
spkDensity(object, spkSlopeOut, cuts=TRUE, label = NULL, ...)
```

Arguments

object	a SpikeInExpressionSet object
spkSlopeOut	the output from the spkSlope function
cuts	if TRUE vertical lines are drawn at the expression values separating low vs medium and medium vs high ALE strata
label	a character string to insert into the plot title
...	arguments passed to the plot function

Details

This function requires the output of spkSlope.

Value

Density plot is produced.

Author(s)

Matthew N. McCall

Examples

```
data(affy)
affySlope <- spkSlope(affy)
spkDensity(affy,affySlope)
```

---

spkGNN *Genes Needed to Detect N True Positives*

Description

Computes the number of genes one would need to consider to obtain a given number of truly positive genes if one considered genes in order of decreasing observed fold change.

Usage

```
spkGNN(n, n.expr, n.unexpr, AccuracySlope, AccuracySD, nullfc)
```

Arguments

n	the desired number of true positives
n.expr	the actual number of truly expressed genes
n.unexpr	the actual number of truly unexpressed genes
AccuracySlope	the signal detect slope from the spkSlope function
AccuracySD	the standard deviation of the signal detect slope from the spkAccSD function
nullfc	a vector of null fold changes from the spkBox function

Value

This function returns the expected number of genes one would have to consider to obtain N true positives under the given conditions.

Author(s)

Matthew N. McCall

Examples

```
data(affy)
spkSlopeOut <- spkSlope(affy)
spkBoxOut <- spkBox(affy, spkSlopeOut, fc=2)
AccuracySlope <- round(spkSlopeOut$slope[-1], digits=2)
AccuracySD <- round(spkAccSD(affy, spkSlopeOut), digits=2)
spkGNN(n=25, n.expr=100, n.unexpr=10000, AccuracySlope[2],
AccuracySD[2], spkBoxOut[[2]])
```

---

spkMA *MA Plots*

Description

Plots log-ratios (M) vs. average log expression (A) for a SpikeInExpressionSet object.

Usage

```
spkMA(object, spkSlopeOut, fc=2, tol=3, label=NULL, ylim=NULL,
outlier=1, reduce=TRUE, plot.legend=TRUE)
```

Arguments

object	a SpikeInExpressionSet object
spkSlopeOut	the output from the spkSlope function
fc	the fold change of interest
tol	the precision (number of digits after decimal point) in fc
label	a character string to insert into the plot title
ylim	limits of y-axis
outlier	log fold change cut-off for outliers
reduce	if TRUE some points are removed from the background to speed plotting
plot.legend	if TRUE a legend is plotted

Value

The MA plot is produced.

Author(s)

Matthew N. McCall

Examples

```
data(affy)
affySlope <- spkSlope(affy)
spkMA(affy, affySlope)
```

spkPair *Pairwise Comparisons for Spike-in Genes*

Description

Compute log-ratios among spike-in genes.

Usage

```
spkPair(object)
```

Arguments

object	a SpikeInExpressionSet object

Value

An array containing either log-ratios (M), average log expression (A), and nominal concentrations (N1 & N2). Dimension one is genes, dimension two is array pairings, dimension three is M, A, N1, and N2.

Author(s)

Matthew N. McCall

Examples

```
data(affy)
affyPair <- spkPair(affy)
```

spkPairNS *Pairwise Comparisons for Non-Spike-in Genes*

Description

Compute log-ratios among non-spike-in genes.

Usage

```
spkPairNS(object, output="M")
```

Arguments

object	a SpikeInExpressionSet object
output	what to return; either "M" for log-ratios or "A" for average log expression.

Value

A matrix containing either log-ratios (M) or average log expression (A). Rows are genes and columns are array pairings.

Author(s)

Matthew N. McCall

Examples

```
data(affy)
affyPairNS <- spkPairNS(affy)
```

---

spkPot *Probability of being in the Top*

Description

Compute the probability that a spike-in with a nominal fold change of 2 appears in the the top 0.5% (default) of log-ratios.

Usage

```
spkPot(object, spkSlopeOut, sig, SD, precisionQuantile)
```

Arguments

object	a SpikeInExpressionSet object
spkSlopeOut	the output from the spkSlope function
sig	the signal detect slopes from a call to spkSlope
SD	the standard deviation from spkAccSD
precisionQuantile	the desired quantile to compute the probability of being above

Value

A vector of probabilities for each ALE strata.

Author(s)

Matthew N. McCall

Examples

```
data(affy)
affySlope <- spkSlope(affy)
affyAccSD <- spkAccSD(affy, affySlope)
spkPot(affy, affySlope, affySlope$slopes, affyAccSD, .995)
```

spkQuantile *Empirical Quantiles*

Description

An internal function called by spkSlope.

Usage

```
spkQuantile(amt, avgE, ens, p)
```

Arguments

amt	a vector of nominal concentrations
avgE	the observed average expression corresponding to each nominal concentration
ens	the average expression across arrays of unexpressed genes
p	the quantiles to make the bins

Author(s)

Matthew N. McCall

Examples

```
data(affy)
affySlope <- spkSlope(affy)
```

spkSlope *Signal Detect Slope Plot*

Description

Plots observed expression vs. nominal concentration. The overall regression slope, as well as, regression slopes for low, medium, and high bins are computed and the regression lines plotted.

Usage

```
spkSlope(object, label = NULL, cuts=c(.6,.99), ...)
```

Arguments

object	a SpikeInExpressionSet object
label	a character string to insert into the plot title
cuts	quantiles used to make the low, medium, and high bins
...	arguments passed to the plot function

Details

The bins are created by computing the proportion of non-spike-in genes with expression values less than or equal to the average expression value at each nominal concentration. Using the default value of cuts, the high bin contains nominal concentrations with 99 percent or more of the non-spike-in expression values lower than it. The medium bin contains nominal concentrations with between 60 and 99 percent of the non-spike-in expression values lower than it. The low bin contains nominal concentrations with less than 60 percent of the non-spike-in expression values lower than it.

Value

`avgExp`	average expression at each nominal concentration
`slopes`	the regression slopes - overall and for each bin
`breaks`	which spike-in levels fall in each bin
`brkpts`	the expression value of the cut points between bins
`prop`	the proportion of non-spike-in probes with expression less than the average expression at each nominal concentration

Author(s)

Matthew N. McCall

Examples

```
data(affy)
spkSlope(affy)
```

`spkTools` *Tools for Spike-in Data Analysis and Visualization*

Description

A collection of functions to examine microarray datasets that include spike-ins. In particular, it allows one to explore the distribution of spike-ins within the range of possible expression values, the relationship between nominal concentration and expression, and the relationship between expected and observed fold change for different levels of comparison.

Details

Package:	spkTools
Type:	Package
Version:	0.0.1
Date:	2007-10-9
License:	GPL version 2 or newer

Author(s)

Matthew N. McCall

Maintainer: Matthew N. McCall <mmccall@jhsph.edu>

Examples

```
## The Three Plots
data(affy)
par(mfrow=c(2,2))
affySlope <- spkSlope(affy)
spkDensity(affy, affySlope)
spkBox(affy, affySlope)

## The Full Wrapper
data(affy)
spkAll(affy, label="Affymetrix", fc=2)
```

---

spkVar *Spike-in Variance*

Description

Compute an estimate of the standard deviation in expression at each nominal concentration.

Usage

```
spkVar(object)
```

Arguments

object	a SpikeInExpressionSet object

Value

a matrix containing spike-in levels and corresponding MADs.

Author(s)

Matthew N. McCall

Examples

```
data(affy)
spkVar(affy)
```

summarySpkBox *Summary of Fold Changes Calculated by spkBox*

Description

Prints a summary table of the data resulting from a call to spkBox.

Usage

```
summarySpkBox(boxs)
```

Arguments

boxs	the output of a call to spkBox

Value

A dataframe with 2 columns: the mean fold change and the median average distance of the fold changes.

Author(s)

Matthew N. McCall

Examples

```
data(affy)
affySlope <- spkSlope(affy)
affyBox <- spkBox(affy, affySlope)
plotSpkBox(affyBox)
```

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