

# 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>

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affy	<i>SpikeInExpressionSet of Affymetrix Spike-in Experiment Data</i>
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---

### 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>

---

plotSpkBox	<i>Boxplots of Fold Changes Calculated by spkBox</i>
------------	--

---

### 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*

---

**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("Anno
```

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**

<code>object</code>	a <code>SpikeInExpressionSet</code> object
<code>spkSlopeOut</code>	the output from the <code>spkSlope</code> function
<code>tol</code>	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**

<code>object</code>	a <code>SpikeInExpressionSet</code> object
<code>model</code>	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 Microarray Spike-in Design Imbalance*

---

**Description**

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

**Usage**

```
spkBal(object)
```

**Arguments**

<code>object</code>	a <code>SpikeInExpressionSet</code> 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**

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



**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**

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

---

**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	<i>Signal Detect Slope Plot</i>
----------	---------------------------------

---

**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(boxes)
```

**Arguments**

`boxes`            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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