

Package ‘OutSeekR’

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Type Package

Title Statistical Approach to Outlier Detection in RNA-Seq and Related Data

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Description An approach to outlier detection in RNA-seq and related data based on five statistics. 'OutSeekR' implements an outlier test by comparing the distributions of these statistics in observed data with those of simulated null data.

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Author Jee Yun Han [aut],
John Sahrman [aut],
Jaron Arbet [ctb],
Paul Boutros [aut, cre, cph]

Maintainer Paul Boutros <pboutros@sbpdiscovery.org>

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Contents

calculate.p.values	2
calculate.residuals	4
detect.outliers	5
example.data.for.calculate.p.values	6
kmeans.fraction	6
outlier.detection.cosine	7
outliers	8
quantify.outliers	10
simulate.null	12
trim.sample	13
zrange	13
Index	15

calculate.p.values	<i>Calculate p-values</i>
--------------------	---------------------------

Description

Calculate p-values for each sample of a single transcript.

Usage

```
calculate.p.values(  
  x,  
  x.zrange.mean,  
  x.zrange.median,  
  x.zrange.trimmean,  
  x.fraction.kmeans,  
  x.cosine.similarity,  
  null.zrange.mean,  
  null.zrange.median,  
  null.zrange.trimmean,  
  null.fraction.kmeans,  
  null.cosine.similarity,  
  kmeans.nstart = 1  
)
```

Arguments

- | | |
|-----------------|---|
| x | A numeric vector of values for an observed transcript. |
| x.zrange.mean | A number, the range of the z-scores calculated using the mean and standard deviation of x. |
| x.zrange.median | A number, the range of the z-scores calculated using the median and median absolute deviation of x. |

`x.zrange.trimmean`
A number, the range of the z-scores calculated using the trimmed mean and trimmed standard deviation of `x`.

`x.fraction.kmeans`
A number, the k-means fraction of `x`.

`x.cosine.similarity`
A number, the cosine similarity of `x`.

`null.zrange.mean`
A numeric vector, the ranges of the z-scores calculated using the mean and standard deviation of each transcript in the null data.

`null.zrange.median`
A numeric vector, the ranges of the z-scores calculated using the median and median absolute deviation of each transcript in the null data.

`null.zrange.trimmean`
A numeric vector, the ranges of the z-scores calculated using the trimmed mean and trimmed standard deviation of each transcript in the null data.

`null.fraction.kmeans`
A numeric vector, the k-means fraction of each transcript in the null data.

`null.cosine.similarity`
A numeric vector, the cosine similarity of each transcript in the null data.

`kmeans.nstart` The number of random starts when computing k-means fraction; default is 1. See `?stats::kmeans` for further details.

Value

A list consisting of the following entries:

- `p.values`: a vector of p-values for the outlier test run on each sample (up until the p-value exceeds `p.value.threshold`); and
- `outlier.statistics.list`, a list of vectors containing the values of the outlier statistics calculated from the remaining samples. The list will be of length equal to one plus the total number of outliers (i.e., the number of samples with an outlier test p-value less than `p.value.threshold`) and will contain entries `outlier.statistics.N`, where `N` is between zero and the total number of outliers. `outlier.statistics.N` is the vector of outlier statistics after excluding the `N`th outlier sample, with `outlier.statistics.0` being for the complete transcript.

Examples

```
data(example.data.for.calculate.p.values);
i <- 1; # row index of transcript to test
calculate.p.values(
  x = example.data.for.calculate.p.values$data[i,],
  x.zrange.mean = example.data.for.calculate.p.values$x.zrange.mean[i],
  x.zrange.median = example.data.for.calculate.p.values$x.zrange.median[i],
  x.zrange.trimmean = example.data.for.calculate.p.values$x.zrange.trimmean[i],
  x.fraction.kmeans = example.data.for.calculate.p.values$x.fraction.kmeans[i],
  x.cosine.similarity = example.data.for.calculate.p.values$x.cosine.similarity[i],
```

```

null.zrange.mean = example.data.for.calculate.p.values$null.zrange.mean,
null.zrange.median = example.data.for.calculate.p.values$null.zrange.median,
null.zrange.trimmean = example.data.for.calculate.p.values$null.zrange.trimmean,
null.fraction.kmeans = example.data.for.calculate.p.values$null.fraction.kmeans,
null.cosine.similarity = example.data.for.calculate.p.values$null.cosine.similarity,
kmeans.nstart = example.data.for.calculate.p.values$kmeans.nstart
);

```

calculate.residuals	<i>Calculate residuals</i>
---------------------	----------------------------

Description

Calculate residuals between quantiles of the input and quantiles of a Gaussian mixture model fitted to the data.

Usage

```
calculate.residuals(x)
```

Arguments

x	A numeric vector.
---	-------------------

Value

A numeric vector of the same length as x. Names are not retained.

Examples

```

# Generate fake data.
set.seed(1234);
x <- rgamma(
  n = 20,
  shape = 2,
  scale = 2
);
names(x) <- paste(
  'Sample',
  seq_along(x),
  sep = '.'
);
calculate.residuals(
  x = x
);

```

detect.outliers	<i>Detect outliers</i>
-----------------	------------------------

Description

Detect outliers in normalized RNA-seq data.

Usage

```
detect.outliers(
  data,
  num.null = 1000,
  initial.screen.method = c("fdr", "p.value"),
  p.value.threshold = 0.05,
  fdr.threshold = 0.01,
  kmeans.nstart = 1
)
```

Arguments

<code>data</code>	A matrix or data frame of normalized RNA-seq data, organized with transcripts on rows and samples on columns. Transcript identifiers should be stored as <code>rownames(data)</code> .
<code>num.null</code>	The number of transcripts to generate when simulating from null distributions; default is 1000. We recommend using at least 10,000 iterations for publication-level results, with 100,000 or even one million iterations providing more robust estimates.
<code>initial.screen.method</code>	The statistical criterion for initial gene selection; valid options are 'FDR' and 'p-value'.
<code>p.value.threshold</code>	The p-value threshold for the outlier test; default is 0.05. Once the p-value for a sample exceeds <code>p.value.threshold</code> , testing for that transcript ceases, and all remaining samples will have p-values equal to NA.
<code>fdr.threshold</code>	The false discovery rate (FDR)-adjusted p-value threshold for determining the final count of outliers; default is 0.01.
<code>kmeans.nstart</code>	The number of random starts when computing k-means fraction; default is 1. See <code>?stats::kmeans</code> for further details.

Value

A list consisting of the following entries:

- `fdr`: a matrix of FDR-adjusted p-values for the outlier test run on each transcript in data.
- `p.values`: a matrix of unadjusted p-values for the outlier test run on each transcript in data.

- `num.outliers`: a vector giving the number of outliers detected for each transcript based on the threshold.
- `outlier.test.results.list`: a list of length `max(num.outliers) + 1` containing entries `roundN`, where `N` is between one and `max(num.outliers) + 1`. `roundN` is the data frame of results for the outlier test after excluding the `(N-1)`th outlier sample, with `round1` being for the original data set (i.e., before excluding any outlier samples).
- `initial.screen.method`: Specifies the statistical criterion for initial feature selection. Valid options are 'FDR' and 'p-value' (FDR used by default).

Examples

```
data(outliers);
outliers.subset <- outliers[1:10,];
results <- detect.outliers(
  data = outliers.subset,
  num.null = 10
);
```

```
example.data.for.calculate.p.values
```

example.data.for.calculate.p.values

Description

Example data (list object) for testing `calculate.p.values()`.

Usage

```
example.data.for.calculate.p.values
```

Format

An object of class `list` of length 12.

```
kmeans.fraction
```

k-means fraction

Description

Given a vector of cluster assignments from `quantify.outliers()` run with `method = 'kmeans'`, compute the fraction of observations belonging to the smaller of the two clusters.

Usage

```
kmeans.fraction(x)
```

Arguments

x A numeric vector.

Details

This function only considers clusters 1 and 2 even if `quantify.outliers()` was run with `exclude.zero = TRUE`. In that case, zeros are effectively excluded from the counts used to define the k-means fraction. See examples.

Value

A number.

Examples

```
x <- c(1, 1, 2, 2, 2, 2, 2, 2, 2, 2);
names(x) <- letters[1:length(x)];
kmeans.fraction(x);
```

outlier.detection.cosine

Cosine similarity

Description

Compute cosine similarity for detection of outliers. Generate theoretical quantiles based on the Gaussian mixture model of the data, and compute cosine similarity between a point made up of the largest observed quantile and the largest theoretical quantile and a point on the line $y = x$.

Usage

```
outlier.detection.cosine(x)
```

Arguments

x A numeric vector.

Value

A number.

Examples

```
# Generate fake data.
set.seed(1234);
x <- rgamma(
  n = 20,
  shape = 2,
  scale = 2
);
outlier.detection.cosine(
  x = x
);
```

outliers

Example data set for outlier testing

Description

Example data set for outlier testing

Usage

```
outliers
```

Format

A data frame with 500 rows and 50 columns:

- S01** simulated fragments per kilobase of transcript per million fragments mapped (FPKM) values for sample 1
- S02** simulated FPKM values for sample 2
- S03** simulated FPKM values for sample 3
- S04** simulated FPKM values for sample 4
- S05** simulated FPKM values for sample 5
- S06** simulated FPKM values for sample 6
- S07** simulated FPKM values for sample 7
- S08** simulated FPKM values for sample 8
- S09** simulated FPKM values for sample 9
- S10** simulated FPKM values for sample 10
- S11** simulated FPKM values for sample 11
- S12** simulated FPKM values for sample 12
- S13** simulated FPKM values for sample 13
- S14** simulated FPKM values for sample 14
- S15** simulated FPKM values for sample 15

S16 simulated FPKM values for sample 16
S17 simulated FPKM values for sample 17
S18 simulated FPKM values for sample 18
S19 simulated FPKM values for sample 19
S20 simulated FPKM values for sample 20
S21 simulated FPKM values for sample 21
S22 simulated FPKM values for sample 22
S23 simulated FPKM values for sample 23
S24 simulated FPKM values for sample 24
S25 simulated FPKM values for sample 25
S26 simulated FPKM values for sample 26
S27 simulated FPKM values for sample 27
S28 simulated FPKM values for sample 28
S29 simulated FPKM values for sample 29
S30 simulated FPKM values for sample 30
S31 simulated FPKM values for sample 31
S32 simulated FPKM values for sample 32
S33 simulated FPKM values for sample 33
S34 simulated FPKM values for sample 34
S35 simulated FPKM values for sample 35
S36 simulated FPKM values for sample 36
S37 simulated FPKM values for sample 37
S38 simulated FPKM values for sample 38
S39 simulated FPKM values for sample 39
S40 simulated FPKM values for sample 40
S41 simulated FPKM values for sample 41
S42 simulated FPKM values for sample 42
S43 simulated FPKM values for sample 43
S44 simulated FPKM values for sample 44
S45 simulated FPKM values for sample 45
S46 simulated FPKM values for sample 46
S47 simulated FPKM values for sample 47
S48 simulated FPKM values for sample 48
S49 simulated FPKM values for sample 49
S50 simulated FPKM values for sample 50

quantify.outliers	<i>Compute quantities for outlier detection</i>
-------------------	---

Description

Compute quantities for use in the detection of outliers. Specifically, compute z-scores based on the mean / standard deviation, the trimmed mean / trimmed standard deviation, or the median / median absolute deviation, or the cluster assignment from k-means with two clusters.

Usage

```
quantify.outliers(
  x,
  method = "mean",
  trim = 0,
  nstart = 1,
  exclude.zero = FALSE
)
```

Arguments

<code>x</code>	A numeric vector.
<code>method</code>	A string indicating the quantities to be computed. Possible values are • 'mean' : z-scores based on mean and standard deviation or trimmed mean and trimmed standard deviation if <code>trim > 0</code>, • 'median' : z-scores based on median and median absolute deviation, or • 'kmeans' : cluster assignment from k-means with two clusters. The default is z-scores based on the mean and standard deviation.
<code>trim</code>	A number, the fraction of observations to be trimmed from each end of <code>x</code> . Default is no trimming.
<code>nstart</code>	A number, for k-means clustering, the number of random initial centers for the clusters. Default is 1. See stats::kmeans() for further information.
<code>exclude.zero</code>	A logical, whether zeros should be excluded (TRUE) or not excluded (FALSE, the default) from computations. For <code>method = 'mean'</code> and <code>method = 'median'</code> , this means zeros will not be included in computing the summary statistics; for <code>method = 'kmeans'</code> , this means zeros will be placed in their own cluster, coded 0.

Value

A numeric vector the same size as `x` whose values are the requested quantities computed on the corresponding elements of `x`.

Examples

```
# Generate fake data.
set.seed(1234);
x <- rgamma(
  n = 20,
  shape = 2,
  scale = 2
);
# Add missing values and zeros for demonstration. Missing values are
# ignored, and zeros can be ignored with `exclude.zeros = TRUE`.
x[1:5] <- NA;
x[6:10] <- 0;

# Compute z-scores based on mean and standard deviation.
quantify.outliers(
  x = x,
  method = 'mean',
  trim = 0
);
# Exclude zeros from the calculation of the mean and standard
# deviation.
quantify.outliers(
  x = x,
  method = 'mean',
  trim = 0,
  exclude.zero = TRUE
);

# Compute z-scores based on the 5% trimmed mean and 5% trimmed
# standard deviation.
quantify.outliers(
  x = x,
  method = 'mean',
  trim = 0.05
);

# Compute z-scores based on the median and median absolute deviation.
quantify.outliers(
  x = x,
  method = 'median'
);

# Compute cluster assignments using k-means with k = 2.
quantify.outliers(
  x = x,
  method = 'kmeans'
);
# Try different initial cluster assignments.
quantify.outliers(
  x = x,
  method = 'kmeans',
  nstart = 10
);
```

```

    );
# Assign zeros to their own cluster.
quantify.outliers(
  x = x,
  method = 'kmeans',
  exclude.zero = TRUE
);

```

simulate.null	<i>Simulate from a null distribution</i>
---------------	--

Description

Simulate transcripts from a Gaussian mixture model fitted to the observed data.

Usage

```

## S3 method for class 'null'
simulate(x, r)

```

Arguments

x	A numeric vector of transcripts.
r	A numeric vector of residuals calculated for this transcript.

Value

A numeric vector of the same length as x. Names are not retained.

Examples

```

# Prepare fake data.
set.seed(1234);
x <- rgamma(
  n = 20,
  shape = 2,
  scale = 2
);
names(x) <- paste('Sample', seq_along(x), sep = '.');
r <- calculate.residuals(
  x = x
);
r.trimmed <- trim.sample(
  x = r
);
null <- simulate.null(
  x = x,
  r = r.trimmed
);

```

trim.sample	<i>Trim a vector of numbers</i>
-------------	---------------------------------

Description

Symmetrically trim a vector of numbers after sorting it.

Usage

```
trim.sample(x, trim = 0.01)
```

Arguments

x	A numeric vector.
trim	A number, the fraction of observations to be trimmed from each end of x.

Details

If `length(x) <= 10`, the function returns `x[2:(length(x) - 1)]`.

Value

A sorted, trimmed copy of x.

Examples

```
trim.sample(  
  x = 1:20,  
  trim = 0.01  
);
```

zrange	<i>Range of z-scores</i>
--------	--------------------------

Description

Compute the range of a vector of z-scores.

Usage

```
zrange(x)
```

Arguments

x	A numeric vector
---	------------------

Value

A number.

Examples

```
set.seed(1234);  
x <- rnorm(  
  n = 10  
);  
zrange(  
  x = x  
);
```

Index

* **datasets**

- example.data.for.calculate.p.values,
6
- outliers, 8

- calculate.p.values, 2
- calculate.residuals, 4

- detect.outliers, 5

- example.data.for.calculate.p.values, 6

- kmeans.fraction, 6

- outlier.detection.cosine, 7
- outliers, 8

- quantify.outliers, 10

- simulate.null, 12
- stats::kmeans(), 10

- trim.sample, 13

- zrange, 13