

# Package ‘diffGeneAnalysis’

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**Title** Performs differential gene expression Analysis

**Version** 1.94.0

**Author** Choudary Jagarlamudi

**Description** Analyze microarray data

**Maintainer** Choudary Jagarlamudi <choudary.jagar@swosu.edu>

**License** GPL

**Collate** dataTrim.R curveFit.R normalize.R biasAdjust.R assocAnalysis.R  
refGroup.R

**Imports** graphics, grDevices, minpack.lm (>= 1.0-4), stats, utils

**biocViews** Microarray, DifferentialExpression

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 assocAnalysis

*Associative Analysis*


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### Description

assocAnalysis performs the Associative-T method of differential gene analysis.

### Usage

```
assocAnalysis(bAdjusted, numSlides, ctrl, exp, sdrefgrp,
pctrl, pexpm, ctrlavjs, expmavjs, ctrlstds, expstds, rgrplen)
```

### Arguments

|           |                                                                              |
|-----------|------------------------------------------------------------------------------|
| bAdjusted | bAdjusted is a matrix of bias adjusted normalized microarray data.           |
| numSlides | numSlides represents the total number of chips in the microarray experiment. |
| ctrl      | ctrl is the number of control chips in the microarray experiment.            |
| exp       | exp is the number of control chips in the microarray experiment.             |
| sdrefgrp  | standard deviation of the computed reference group.                          |
| pctrl     | p values of the control chips on performin ttests.                           |
| pexpm     | p values of the experiment chips on performin ttests.                        |
| ctrlavjs  | averages of genes across all control chips.                                  |
| expmavjs  | averages of genes across all experiment chips.                               |
| ctrlstds  | standard deviations of genes across all control chips.                       |
| expstds   | standard deviations of genes across all experiment chips.                    |
| rgrplen   | total number of genes of the computed reference group.                       |

### Details

AssociativeAnalysis performs the Associative-T method of differential gene analysis. The results are displayed in a 10 column matrix as follows

Column Representation.

1 Gene Bank Id

2 Average Signal of the Control Chips/Channels.

3 SD of Control Chips/Channels.

4 Probability that a given gene in the Control Chips/Channels belongs or does not belong to background.

5 Average Signal of the Experimental Chips/Channels.

6 SD of Experimental Chips/Channels.

7 Probability that a given gene in the Experimental Chips/Channels belongs or does not belong to background.

8 P-value from a Student T-test.

9 P-value from an Associative T-test.

10 Ratio of mean expression values (Control/Experimental).

11 Group Number.

Group Numbers are defined as follows:.

A1 Expressed above background in both sample types, but over-expressed on the Experimental Chips/Channels.

A2 Expressed above background in both sample types, but over-expressed on the Control Chips/Channels.

A3 Expressed above background only on the Experimental Chips/Channel.

A4 Expressed above background only in the Control Chips/Channel.

0 None of the above.

### Value

A matrix of 11 columns as described in the details and n rows where n stands for the number of genes in the microarray dataset.

### Author(s)

Choudary L Jagarlamudi

### References

Dozmorov I,Centola,M. An associative analysis of gene expression array data.Bioinformatics.2003 Jan22;19(2):204-11

Knowlton N,Dozmorov I, Centola M. Microarray data Analysis Tool box(MDAT): for normalization,adjustment and analysis of gene expression data.Bioinformatics. 2004 Dec 12;20(18):3687-90

### Examples

```
#see refGroup for more details
```

---

biasAdjust

*baisAdjust performs bias adjustment of normalized data*

---

### Description

biasAdjust takes a normalized dataset and applies a multiplicative scalar derived from the data to help account for expression biases. These expression biases can come from many sources including dye bias,hybridization efficacy,changes in personnel,etc.After bias adjustment the data is ready for differential gene analysis.

### Usage

```
biasAdjust(normalized, numSlides)
```

**Arguments**

|            |                                                                                                                                      |
|------------|--------------------------------------------------------------------------------------------------------------------------------------|
| normalized | normalized is a matrix of normalized microarray data. The first row consists of headers and the first column consists of gene names. |
| numSlides  | numSlides is the total number of chips of the microarray dataset.                                                                    |

**Value**

Returns a matrix of bias adjusted normalized data.

**Note**

biasAdjust takes 2-3 seconds to execute under optimal conditions of size of datasets and speed of the machine. Tested on a Pentium IV 1.6Ghz, 256Mb RAM with 22464 genes and 10 chips it took 12 seconds to execute.

**Author(s)**

Choudary L Jagarlamudi

**References**

Dozmorov I, Centola M. An associative analysis of gene expression array data. *Bioinformatics*. 2003 Jan 22;19(2):204-11

Knowlton N, Dozmorov I, Centola M. Microarray data Analysis Tool box (MDAT): for normalization, adjustment and analysis of gene expression data. *Bioinformatics*. 2004 Dec 12;20(18):3687-90

**Examples**

```
#biasAdjust(normalized,7)
```

---

curveFit

*CurveFit data to a Gaussian distribution*

---

**Description**

CurveFit takes a vector of chipdata from microarray slides and fits the data to a Gaussian distribution through a non-linear least-squares optimization algorithm. The results are graphically depicted in a series of histograms. Each histogram represents a different initial seed (left to right: 2 bins, 3 bins, 4 bins, 4.5 bins, 5 bins, and 5.5 bins) that is passed to the curve fitting algorithm. The resulting fit for each histogram is superimposed with a solid blue line. The user is then able to visually select the 'best' fit.

**Usage**

```
curveFit(chipdata, plot)
```

**Arguments**

chipdata      a vector of chipdata from microarray slides.

plot            plot can take values of 1 or 0. If plot is 1 then the histogram with the curve fit will be shown graphically.

**Value**

an object res which is a list containing the following components. res[1]: mean of the computed background. res[2]: standard deviation of the computed background.

**Author(s)**

Choudary L Jagarlamudi

**References**

Dozmorov I,Centola,M. An associative analysis of gene expression array data. Bioinformatics.2003 Jan22;19(2):204-11

Knowlton N,Dozmorov I, Centola M. Microarray data Analysis Tool box(MDAT): for normalization,adjustment and analysis of gene expression data. Bioinformatics.2004 Dec 12;20(18):3687-90

**Examples**

```
#see normalize for details.
```

---

dataTrim

*DataTrim using pseudo winsorization algorithm*


---

**Description**

DataTrim cleans the data through a pseudo winsorization algorithm. First, the mean and SD are calculated. Then, any values above 2SD are trimmed.The mean is recalculated and any values less than 2 SD are trimmed.This process of cutting 2 SD above the mean and then 2 SD below the mean is repeated until no further cuts are possible.

**Usage**

```
dataTrim(chipdata)
```

**Arguments**

chipdata      chipdata is a vector of chipdata from microarray chips.

**Value**

Returns a vector of trimmed chipdata representing background based on pseudo winsorization algorithm.

Author(s)

Choudary L Jagarlamudi

References

Dozmorov I,Centola,M. An associative analysis of gene expression array data. Bioinformatics.2003 Jan22;19(2):204-11

Knowlton N,Dozmorov I, Centola M. Microarray data Analysis Tool box(MDAT): for normalization,adjustment and analysis of gene expression data. Bioinformatics.2004 Dec 12;20(18):3687-90

Examples

#see normalize for details

---

|           |                                         |
|-----------|-----------------------------------------|
| normalize | <i>Normalization of microarray data</i> |
|-----------|-----------------------------------------|

---

Description

Normalization of data utilizing information obtained from background fluorescence. Background fluorescence intensity values are used to determine a Gaussian distribution of lowly expressed genes, yielding the background estimates (mean and standard deviation).

Usage

normalize(rawdata, numSlides,ctrl,expm,ctrlbg,expmbg)

Arguments

- |           |                                                                                                                                                                                                             |
|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| rawdata   | rawdata is matrix of microarray data. The first column consists of gene Names and the first row consists of headers.                                                                                        |
| numSlides | numSlides represents the total number of chips/slides in the microarray dataset including control and experiment. Control slides are always followed by experiment slides from left to right in the matrix. |
| ctrl      | ctrl represents the total number of control chips in the microarray dataset.                                                                                                                                |
| expm      | expm represents the total number of experiment chips in the microarray dataset.                                                                                                                             |
| ctrlbg    | ctrlbg represents the percent of data to pick for background computation of the control chips. 30 percent is the default.                                                                                   |
| expmbg    | expmbg represents the percent of data to pick for background computation of the experiment chips. 30 percent is the default.                                                                                |

**Details**

The normalization algorithm trims the data based on initial empirical estimates of the mean and standard deviation. All data beyond  $\pm 2SD$  of the mean are cut iteratively. This procedure is repeated until no more cuts can be made. The trimmed data is then subjected to a non linear curve fitting procedure. The user is presented with six different pictures obtained using bars 2,3,4,4.5,5, and 5.5 as mean. The user is given the freedom to select the best visual estimate of background. The user selected parameters are used to perform a z-Transformation on the data. The percent of data selected to compute background depends on the data obtained. The default is 30 percent. A normal distributed histogram should confirm that, else the user is allowed to pick a percent and make changes until the user sees a normal distributed histogram. Upon running normalize the user is presented with a set of 6 histograms. If the user is not happy with the default 30 percent, the user should go ahead and select a mean and confirm curvefit, then select 'no' to confirm histogram distribution. The user will be presented with a new set of 6 histograms. This process is repeated until the user selects the best Histogram distribution. This process is repeated for each individual chip.

**Value**

A matrix of normalized values of rawdata

**Author(s)**

Choudary L Jagarlamudi

**References**

Dozmorov I, Centola M. An associative analysis of gene expression array data. *Bioinformatics*. 2003 Jan 22; 19(2):204-11

Knowlton N, Dozmorov I, Centola M. Microarray data Analysis Tool box (MDAT): for normalization, adjustment and analysis of gene expression data. *Bioinformatics*. 2004 Dec 12; 20(18):3687-90

**Examples**

```
#rawdata is loaded in the package. Run example as follows:  
#Read the description file for best results.  
#data(rawdata)  
#normalize(rawdata, 7, 3, 4, 0.15, 0.60)
```

---

rawdata

*Micro array dataset*

---

**Description**

Microarray dataset consisting of 7 chips. 3 control and 4 experiment with 2382 genes. The first column consists of gene names, hence the dataset has 8 columns in total.

Usage

data(rawdata)

Format

A data frame with 2382 observations on the following 11 variables.

- GeneID** Gene IDS or Gene names
- c1** a numeric vector of gene intensities for control chip 1
- c2** a numeric vector of gene intensities for control chip 2
- c3** a numeric vector of gene intensities for control chip 3
- e1** a numeric vector of gene intensities for experiment chip 1
- e2** a numeric vector of gene intensities for experiment chip 2
- e3** a numeric vector of gene intensities for experiment chip 3
- e4** a numeric vector of gene intensities for experiment chip 4

Source

Dozmorov I,Centola,M. An associative analysis of gene expression array data. Bioinformatics.2003 Jan22;19(2):204-11

Knowlton N,Dozmorov I, Centola M. Microarray data Analysis Tool box(MDAT): for normalization,adjustment and analysis of gene expression data. Bioinformatics.2004 Dec 12;20(18):3687-90

Examples

data(rawdata)

---

|          |                                                                                               |
|----------|-----------------------------------------------------------------------------------------------|
| refGroup | <i>Reference Group computes a robust estimate of inter-assay variability (Standard Error)</i> |
|----------|-----------------------------------------------------------------------------------------------|

---

Description

refGroup takes a normalized, bias adjusted dataset and computes a robust estimate of inter-assay variability (Standard Error).This value is used here to perform T-tests. It can also be used in sample size calculations.The associative analysis method is then applied to the computed reference group.

Usage

refGroup(biasAdjusted, numSlides, ctrl, exp, pval)

### Arguments

|              |                                                                                                                                                              |
|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| biasAdjusted | biasAdjusted is a matrix of bias adjusted normalized microarray data. The first row consists of headers and the first column must consist of Gene Bank Id's. |
| numSlides    | numSlides represents the total number of chips in a microarray dataset including control and experiment.                                                     |
| ctrl         | ctrl represents the total number of ctrl chips in the microarray.                                                                                            |
| exp          | exp represents the total number of experiment chips in the microarray.                                                                                       |
| pval         | pval is the stringency value for computing the reference group. 0.05 is the normally suggested value.                                                        |

### Details

AssociativeAnalysis performs the Associative-T method of differential gene analysis. The user is asked to enter values for E and R. E stand for the increase in fold over background and R stands for the ratio of experimental chips average over control chips average. The higher these values the higher will be the stringency. Example dataset used here was run with an E values of 1 and R value of 1.5.

The results are displayed in a 10 column matrix as follows

Column Representation.

1 Gene Bank ID

2 Average Signal of the Control Chips/Channels.

3 SD of Control Chips/Channels.

4 Probability that a given gene in the Control Chips/Channels belongs or does not belong to background.

5 Average Signal of the Experimental Chips/Channels.

6 SD of Experimental Chips/Channels.

7 Probability that a given gene in the Experimental Chips/Channels belongs or does not belong to background.

8 P-value from a Student T-test.

9 P-value from an Associative T-test.

10 Ratio of mean expression values (Control/Experimental).

11 Group Number.

Group Numbers are defined as follows:

A1 Expressed above background in both sample types, but over-expressed on the Experimental Chips/Channels.

A2 Expressed above background in both sample types, but over-expressed on the Control Chips/Channels.

A3 Expressed above background only on the Experimental Chips/Channel.

A4 Expressed above background only in the Control Chips/Channel.

0 None of the above.

**Value**

A matrix of 11 columns as described in the details and n rows where n stands for the number of genes in the microarray dataset.

**Note**

refGroup takes 4-5 seconds to execute under optimal conditions of size of datasets and speed of the machine. Tested on Pentium IV 1.6Ghz, 256 MB RAM with 22464 genes and 10 chips it took 25 seconds to execute.

**Author(s)**

Choudary L Jagarlamudi

**References**

Dozmorov I, Centola M. An associative analysis of gene expression array data. *Bioinformatics*. 2003 Jan 22;19(2):204-11

Knowlton N, Dozmorov I, Centola M. Microarray data Analysis Tool box (MDAT): for normalization, adjustment and analysis of gene expression data. *Bioinformatics*. 2004 Dec 12;20(18):3687-90

**Examples**

```
#refGroup(bAdjusted, 7, 3, 4, 0.05)
```

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