

Package ‘limma’

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Contents

| | |
|-------------------------------|----|
| 01.Introduction | 6 |
| 02.Classes | 7 |
| 03.ReadingData | 8 |
| 04.Background | 9 |
| 05.Normalization | 10 |
| 06.LinearModels | 11 |
| 07.SingleChannel | 13 |
| 08.Tests | 14 |
| 09.Diagnostics | 15 |
| 10.GeneSetTests | 16 |
| 11.RNAseq | 17 |
| alias2Symbol | 18 |
| anova.MAList-method | 20 |
| arrayWeights | 21 |
| arrayWeightsQuick | 23 |
| as.data.frame | 24 |
| as.MAList | 25 |
| as.matrix | 26 |
| asMatrixWeights | 27 |
| auROC | 28 |
| avearrays | 29 |
| avedups | 30 |
| avereps | 31 |
| backgroundCorrect | 32 |
| barcodeplot | 34 |
| beadCountWeights | 38 |
| blockDiag | 40 |
| bwss | 41 |
| bwss.matrix | 42 |
| camera | 43 |
| cbind | 46 |
| changeLog | 47 |
| chooseLowessSpan | 48 |
| classifyTestsF | 49 |
| contrastAsCoef | 51 |
| contrasts.fit | 52 |
| controlStatus | 54 |
| coolmap | 55 |
| cumOverlap | 57 |
| decideTests | 58 |

| | |
|---------------------------------|-----|
| designI2M | 60 |
| detectionPValues | 61 |
| diffSplice | 63 |
| dim | 65 |
| dimnames | 66 |
| dupcor | 67 |
| eBayes | 69 |
| EList-class | 73 |
| exprs.MA | 75 |
| fitFDist | 75 |
| fitGammaIntercept | 78 |
| fitmixture | 79 |
| fitted.MArrayLM | 80 |
| genas | 81 |
| geneSetTest | 84 |
| getEAWP | 86 |
| getLayout | 87 |
| getSpacing | 89 |
| gls.series | 90 |
| goana | 91 |
| goanaTrend | 96 |
| gridr | 98 |
| head | 98 |
| heatdiagram | 99 |
| helpMethods | 101 |
| ids2indices | 102 |
| imageplot | 103 |
| imageplot3by2 | 104 |
| intraspotCorrelation | 105 |
| is.fullrank | 107 |
| isNumeric | 108 |
| kooperberg | 109 |
| LargeDataObject-class | 110 |
| limmaUsersGuide | 111 |
| lm.series | 112 |
| lmFit | 113 |
| lmscFit | 116 |
| loessFit | 117 |
| logcosh | 120 |
| logsumexp | 121 |
| ma3x3 | 122 |
| makeContrasts | 123 |
| makeUnique | 124 |
| MAList-class | 125 |
| MArrayLM-class | 126 |
| mdplot | 127 |
| merge | 128 |
| mergeScans | 129 |

| | |
|--------------------------------------|-----|
| modelMatrix | 131 |
| modifyWeights | 133 |
| mrlm | 134 |
| nec | 135 |
| normalizeBetweenArrays | 137 |
| normalizeCyclicLoess | 140 |
| normalizeForPrintorder | 141 |
| normalizeMedianAbsValues | 143 |
| normalizeQuantiles | 144 |
| normalizeRobustSpline | 145 |
| normalizeVSN | 147 |
| normalizeWithinArrays | 148 |
| normexp.fit | 150 |
| normexp.fit.control | 152 |
| normexp.fit.detection.p | 154 |
| normexp.signal | 156 |
| plotDensities | 157 |
| plotExonJunc | 158 |
| plotExons | 160 |
| plotFB | 161 |
| plotlines | 162 |
| plotMA | 163 |
| plotMA3by2 | 165 |
| plotMD | 167 |
| plotMDS | 169 |
| plotPrintTipLoess | 172 |
| plotRLDF | 173 |
| plotSA | 175 |
| plotSplice | 176 |
| plotWithHighlights | 177 |
| poolVar | 179 |
| predFCm | 181 |
| printHead | 182 |
| PrintLayout | 183 |
| printorder | 184 |
| printtipWeights | 185 |
| propexpr | 187 |
| propTrueNull | 189 |
| protectMetachar | 191 |
| qqt | 192 |
| QualityWeights | 193 |
| rankSumTestWithCorrelation | 194 |
| read.columns | 196 |
| read.idat | 197 |
| read.ilmn | 199 |
| read.ilmn.targets | 201 |
| read.maimages | 202 |
| readGAL | 206 |

| | |
|----------------------------------|-----|
| readHeader | 207 |
| readImaGeneHeader | 208 |
| readSpotTypes | 209 |
| readTargets | 210 |
| removeBatchEffect | 211 |
| removeExt | 213 |
| residuals.MArrayLM | 214 |
| RGList-class | 215 |
| roast | 216 |
| romer | 221 |
| sampleInfoFromGEO | 223 |
| selectModel | 224 |
| squeezeVar | 226 |
| strsplit2 | 228 |
| subsetting | 229 |
| summary | 230 |
| targetsA2C | 231 |
| TestResults-class | 233 |
| tmixture | 234 |
| topGO | 235 |
| topRomer | 236 |
| topSplice | 237 |
| topTable | 238 |
| tricubeMovingAverage | 241 |
| trigammaInverse | 243 |
| trimWhiteSpace | 244 |
| uniquegenelist | 244 |
| unwrapdups | 245 |
| venn | 246 |
| volcanoplot | 248 |
| voom | 249 |
| vooma | 252 |
| voomaLmFit | 255 |
| voomWithQualityWeights | 258 |
| weighted.median | 260 |
| weightedLowess | 261 |
| write.fit | 264 |
| wsva | 265 |
| zscore | 266 |
| zscoreT | 268 |

Description

LIMMA is a package for the analysis of gene expression microarray data, especially the use of linear models for analysing designed experiments and the assessment of differential expression. LIMMA provides the ability to analyse comparisons between many RNA targets simultaneously in arbitrary complicated designed experiments. Empirical Bayesian methods are used to provide stable results even when the number of arrays is small. The linear model and differential expression functions apply to all gene expression technologies, including microarrays, RNA-seq and quantitative PCR.

Details

There are three types of documentation available:

1. The *LIMMA User's Guide* can be reached through the "User Guides and Package Vignettes" links at the top of the LIMMA contents page. The function `limmaUsersGuide` gives the file location of the User's Guide.
2. An overview of limma functions grouped by purpose is contained in the numbered chapters at the foot of the LIMMA package index page, of which this page is the first.
3. The LIMMA contents page gives an alphabetical index of detailed help topics.

The function `changeLog` displays the record of changes to the package.

Author(s)

Gordon Smyth, with contributions from many colleagues

References

Law CW, Chen Y, Shi W, Smyth GK (2014). Voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biology* 15, R29. doi:10.1186/gb2014152r29. See also the Preprint Version at <https://gksmyth.github.io/pubs/VoomPreprint.pdf> incorporating some notational corrections.

Phipson B, Lee S, Majewski IJ, Alexander WS, and Smyth GK (2016). Robust hyperparameter estimation protects against hypervariable genes and improves power to detect differential expression. *Annals of Applied Statistics* 10, 946-963. doi:10.1214/16AOAS920

Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* 43, e47. doi:10.1093/nar/gkv007

Smyth GK (2004). Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Statistical Applications in Genetics and Molecular Biology* Volume 3, Issue 1, Article 3. doi:10.2202/15446115.1027. See also the Preprint Version <https://gksmyth.github.io/pubs/ebayes.pdf> incorporating corrections to 30 June 2009.

See Also

[02.Classes](#), [03.ReadingData](#), [04.Background](#), [05.Normalization](#), [06.LinearModels](#), [07.SingleChannel](#), [08.Tests](#), [09.Diagnostics](#), [10.GeneSetTests](#), [11.RNAseq](#)

02.Classes

Topic: Classes Defined by this Package

Description

This package defines the following data classes.

RGList A class used to store raw intensities as they are read in from an image analysis output file, usually by `read.maimages`.

MAList Intensities converted to M-values and A-values, i.e., to with-spot and whole-spot contrasts on the log-scale. Usually created from an **RGList** using `MA.RG` or `normalizeWithinArrays`. Objects of this class contain one row for each spot. There may be more than one spot and therefore more than one row for each probe.

EListRaw A class to store raw intensities for one-channel microarray data. May or may not be background corrected. Usually created by `read.maimages`.

EList A class to store normalized log₂ expression values for one-channel microarray data. Usually created by `normalizeBetweenArrays`.

MArrayLM Store the result of fitting gene-wise linear models to the normalized intensities or log-ratios. Usually created by `lmFit`. Objects of this class normally contain only one row for each unique probe.

TestResults Store the results of testing a set of contrasts equal to zero for each probe. Usually created by `decideTests`. Objects of this class normally contain one row for each unique probe.

All these data classes obey many analogies with matrices. In the case of **RGList**, **MAList**, **EListRaw** and **EList**, rows correspond to spots or probes and columns to arrays. In the case of **MArrayLM**, rows correspond to unique probes and the columns to parameters or contrasts. The functions `summary`, `dim`, `length`, `ncol`, `nrow`, `dimnames`, `rownames`, `colnames` have methods for these classes. Objects of any of these classes may be **subsetting**. Multiple data objects may be **combined** by rows (to add extra probes) or by columns (to add extra arrays).

Furthermore all of these classes may be coerced to actually be of class `matrix` using `as.matrix`, although this entails loss of information. Fitted model objects of class **MArrayLM** can be coerced to class `data.frame` using `as.data.frame`.

The first three classes belong to the virtual class **LargeDataObject**. A `show` method is defined for **LargeDataObjects** which uses the utility function `printHead`.

Author(s)

Gordon Smyth

See Also

[01.Introduction](#), [02.Classes](#), [03.ReadingData](#), [04.Background](#), [05.Normalization](#), [06.LinearModels](#), [07.SingleChannel](#), [08.Tests](#), [09.Diagnostics](#), [10.GeneSetTests](#), [11.RNAseq](#)

03.ReadingData

Topic: Reading Microarray Data from Files

Description

This help page gives an overview of LIMMA functions used to read data from files.

Reading Target Information

The function [readTargets](#) is designed to help with organizing information about which RNA sample is hybridized to each channel on each array and which files store information for each array.

Reading Intensity Data

The first step in a microarray data analysis is to read into R the intensity data for each array provided by an image analysis program. This is done using the function [read.maimages](#).

[read.maimages](#) optionally constructs quality weights for each spot using quality functions listed in [QualityWeights](#).

If the data is two-color, then [read.maimages](#) produces an `RGList` object. If the data is one-color (single channel) then an `EListRaw` object is produced. In either case, [read.maimages](#) stores only the information required from each image analysis output file. [read.maimages](#) uses utility functions [removeExt](#), [read.imagene](#) and [read.columns](#). There are also a series of utility functions which read the header information from image output files including [readGPRHeader](#), [readImaGeneHeader](#) and [readGenericHeader](#).

[read.ilmn](#) reads probe or gene summary profile files from Illumina BeadChips, and produces an `EListRaw` object.

[read.idat](#) reads Illumina files in IDAT format, and produces an `EListRaw` object. [detectionPValues](#) can be used to add detection p-values.

The function [as.MAList](#) can be used to convert a `marrayNorm` object to an `MAList` object if the data was read and normalized using the `marray` and `marrayNorm` packages.

Reading the Gene List

Most image analysis software programs provide gene IDs as part of the intensity output files, for example GenePix, Imagen and the Stanford Microarray Database do this. In other cases the probe ID and annotation information may be in a separate file. The most common format for the probe annotation file is the GenePix Array List (GAL) file format. The function [readGAL](#) reads information from a GAL file and produces a data frame with standard column names.

The function [getLayout](#) extracts from the GAL-file data frame the print layout information for a spotted array. The functions [gridr](#), [gridc](#), [spotr](#) and [spotc](#) use the extracted layout to compute grid positions and spot positions within each grid for each spot. The function [printorder](#) calculates

the printorder, plate number and plate row and column position for each spot given information about the printing process. The utility function `getSpacing` converts character strings specifying spacings of duplicate spots to numeric values.

The Australian Genome Research Facility in Australia often produces GAL files with composite probe IDs or names consisting of multiple strings separated by a delimiter. These can be separated into name and annotation information using `strsplit2`.

If each probe is printed more than once of the arrays in a regular pattern, then `uniquegenelist` will remove duplicate names from the gal-file or gene list.

Identifying Control Spots

The functions `readSpotTypes` and `controlStatus` assist with separating control spots from ordinary genes in the analysis and data exploration.

Manipulating Data Objects

`cbind`, `rbind`, `merge` allow different RGList or MAList objects to be combined. `cbind` combines data from different arrays assuming the layout of the arrays to be the same. `merge` can combine data even when the order of the probes on the arrays has changed. `merge` uses utility function `makeUnique`.

Author(s)

Gordon Smyth

See Also

[01.Introduction](#), [02.Classes](#), [03.ReadingData](#), [04.Background](#), [05.Normalization](#), [06.LinearModels](#), [07.SingleChannel](#), [08.Tests](#), [09.Diagnostics](#), [10.GeneSetTests](#), [11.RNAseq](#)

04. Background

Topic: Background Correction

Description

This page deals with background correction methods provided by the `backgroundCorrect`, `kooperberg` or `neqc` functions. Microarray data is typically background corrected by one of these functions before normalization and other downstream analysis.

`backgroundCorrect` works on matrices, `EListRaw` or `RGList` objects, and calls `backgroundCorrect.matrix`.

The `movingmin` method of `backgroundCorrect` uses utility functions `ma3x3.matrix` and `ma3x3.spottedarray`.

The `normexp` method of `backgroundCorrect` uses utility functions `normexp.fit` and `normexp.signal`.

`kooperberg` is a Bayesian background correction tool designed specifically for two-color GenePix data. It is computationally intensive and requires several additional columns from the GenePix data files. These can be read in using `read.maimages` and specifying the other `.columns` argument.

`neqc` is for single-color data. It performs `normexp` background correction and quantile normalization using control probes. It uses utility functions `normexp.fit.control` and `normexp.signal`. If `robust=TRUE`, then `normexp.fit.control` uses the function `huber` in the MASS package.

Author(s)

Gordon Smyth

See Also

[01.Introduction](#), [02.Classes](#), [03.ReadingData](#), [04.Background](#), [05.Normalization](#), [06.LinearModels](#), [07.SingleChannel](#), [08.Tests](#), [09.Diagnostics](#), [10.GeneSetTests](#), [11.RNAseq](#)

05.Normalization

Topic: Normalization of Microarray Data

Description

This page gives an overview of the LIMMA functions available to normalize data from single-channel or two-colour microarrays. Smyth and Speed (2003) give an overview of the normalization techniques implemented in the functions for two-colour arrays.

Usually data from spotted microarrays will be normalized using [normalizeWithinArrays](#). A minority of data will also be normalized using [normalizeBetweenArrays](#) if diagnostic plots suggest a difference in scale between the arrays.

In rare circumstances, data might be normalized using [normalizeForPrintorder](#) before using [normalizeWithinArrays](#).

All the normalization routines take account of spot quality weights which might be set in the data objects. The weights can be temporarily modified using [modifyWeights](#) to, for example, remove ratio control spots from the normalization process.

If one is planning analysis of single-channel information from the microarrays rather than analysis of differential expression based on log-ratios, then the data should be normalized using a single channel-normalization technique. Single channel normalization uses further options of the [normalizeBetweenArrays](#) function. For more details see the [LIMMA User's Guide](#) which includes a section on single-channel normalization.

[normalizeWithinArrays](#) uses utility functions [MA.RG](#), [loessFit](#) and [normalizeRobustSpline](#).

[normalizeBetweenArrays](#) is the main normalization function for one-channel arrays, as well as an optional function for two-colour arrays. [normalizeBetweenArrays](#) uses utility functions [normalizeMedianValues](#), [normalizeMedianAbsValues](#), [normalizeQuantiles](#) and [normalizeCyclicLoess](#), none of which need to be called directly by users.

[neqc](#) is a between array normalization function customized for Illumina BeadChips.

The function [normalizeVSN](#) is also provided as an interface to the vsn package. It performs variance stabilizing normalization, an algorithm which includes background correction, within and between normalization together, and therefore doesn't fit into the paradigm of the other methods.

[removeBatchEffect](#) can be used to remove a batch effect, associated with hybridization time or some other technical variable, prior to unsupervised analysis.

Author(s)

Gordon Smyth

References

Smyth, G. K., and Speed, T. P. (2003). Normalization of cDNA microarray data. *Methods* 31, 265-273. <https://gksmyth.github.io/pubs/normalize.pdf>

See Also

[01.Introduction](#), [02.Classes](#), [03.ReadingData](#), [04.Background](#), [05.Normalization](#), [06.LinearModels](#), [07.SingleChannel](#), [08.Tests](#), [09.Diagnostics](#), [10.GeneSetTests](#), [11.RNAseq](#)

06.LinearModels

Topic: Linear Models for Microarrays

Description

This page gives an overview of the LIMMA functions available to fit linear models and to interpret the results. This page covers models for two color arrays in terms of log-ratios or for single-channel arrays in terms of log-intensities. If you wish to fit models to the individual channel log-intensities from two colour arrays, see [07.SingleChannel](#).

The core of this package is the fitting of gene-wise linear models to microarray data. The basic idea is to estimate log-ratios between two or more target RNA samples simultaneously. See the LIMMA User's Guide for several case studies.

Fitting Models

The main function for model fitting is `lmFit`. This is recommended interface for most users. `lmFit` produces a fitted model object of class `MArrayLM` containing coefficients, standard errors and residual standard errors for each gene. `lmFit` calls one of the following three functions to do the actual computations:

`lm.series` Straightforward least squares fitting of a linear model for each gene.

`mrlm` An alternative to `lm.series` using robust regression as implemented by the `r1m` function in the MASS package.

`gls.series` Generalized least squares taking into account correlations between duplicate spots (i.e., replicate spots on the same array) or related arrays. The function `duplicateCorrelation` is used to estimate the inter-duplicate or inter-block correlation before using `gls.series`.

All the functions which fit linear models use `link{getEAW}` to extract data from microarray data objects, and `unwrappedups` which provides an unified method for handling duplicate spots.

Forming the Design Matrix

`lmFit` has two main arguments, the expression data and the design matrix. The design matrix is essentially an indicator matrix which specifies which target RNA samples were applied to each channel on each array. There is considerable freedom in choosing the design matrix - there is always more than one choice which is correct provided it is interpreted correctly.

Design matrices for Affymetrix or single-color arrays can be created using the function `model.matrix` which is part of the R base package. The function `modelMatrix` is provided to assist with creation of an appropriate design matrix for two-color microarray experiments. For direct two-color designs, without a common reference, the design matrix often needs to be created by hand.

Making Comparisons of Interest

Once a linear model has been fit using an appropriate design matrix, the command `makeContrasts` may be used to form a contrast matrix to make comparisons of interest. The fit and the contrast matrix are used by `contrasts.fit` to compute fold changes and t-statistics for the contrasts of interest. This is a way to compute all possible pairwise comparisons between treatments for example in an experiment which compares many treatments to a common reference.

Assessing Differential Expression

After fitting a linear model, the standard errors are moderated using a simple empirical Bayes model using `eBayes` or `treat`. A moderated t-statistic and a log-odds of differential expression is computed for each contrast for each gene. `treat` tests whether log-fold-changes are greater than a threshold rather than merely different to zero.

`eBayes` and `treat` use internal functions `squeezeVar`, `fitFDist`, `fitFDistRobustly`, `fitFDistUnequalDF1`, `tmixture.matrix` and `tmixture.vector`.

Summarizing Model Fits

After the above steps the results may be displayed or further processed using:

`topTable` Presents a list of the genes most likely to be differentially expressed for a given contrast or set of contrasts.

`topTableF` Presents a list of the genes most likely to be differentially expressed for a given set of contrasts. Equivalent to `topTable` with `coef` set to all the coefficients, `coef=1:ncol(fit)`.

`volcanoplot` Volcano plot of fold change versus the B-statistic for any fitted coefficient.

`plotlines` Plots fitted coefficients or log-intensity values for time-course data.

`genas` Estimates and plots biological correlation between two coefficients.

`write.fit` Writes an `MarrayLM` object to a file. Note that if `fit` is an `MarrayLM` object, either `write.fit` or `write.table` can be used to write the results to a delimited text file.

For multiple testing functions which operate on linear model fits, see [08.Tests](#).

Model Selection

`selectModel` provides a means to choose between alternative linear models using AIC or BIC information criteria.

Author(s)

Gordon Smyth

References

- Phipson, B, Lee, S, Majewski, IJ, Alexander, WS, and Smyth, GK (2016). Robust hyperparameter estimation protects against hypervariable genes and improves power to detect differential expression. *Annals of Applied Statistics* 10, 946-963. <http://projecteuclid.org/euclid.aos/1469199900>
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See Also

[01.Introduction](#), [02.Classes](#), [03.ReadingData](#), [04.Background](#), [05.Normalization](#), [06.LinearModels](#), [07.SingleChannel](#), [08.Tests](#), [09.Diagnostics](#), [10.GeneSetTests](#), [11.RNAseq](#)

07.SingleChannel

Topic: Individual Channel Analysis of Two-Color Microarrays

Description

This page gives an overview of the LIMMA functions fit linear models to two-color microarray data in terms of the log-intensities rather than log-ratios.

The function `intraspotCorrelation` estimates the intra-spot correlation between the two channels. The regression function `lmScFit` takes the correlation as an argument and fits linear models to the two-color data in terms of the individual log-intensities. The output of `lmScFit` is an `MArrayLM` object just the same as from `lmFit`, so inference proceeds in the same way as for log-ratios once the linear model is fitted. See [06.LinearModels](#).

The function `targetsA2C` converts two-color format target data frames to single channel format, i.e., converts from array-per-line to channel-per-line, to facilitate the formulation of the design matrix.

Author(s)

Gordon Smyth

See Also

[01.Introduction](#), [02.Classes](#), [03.ReadingData](#), [04.Background](#), [05.Normalization](#), [06.LinearModels](#), [07.SingleChannel](#), [08.Tests](#), [09.Diagnostics](#), [10.GeneSetTests](#), [11.RNAseq](#)

Description

LIMMA provides a number of functions for multiple testing across both contrasts and genes. The starting point is an `MArrayLM` object, called `fit` say, resulting from fitting a linear model and running `eBayes` and, optionally, `contrasts.fit`. See [06.LinearModels](#) or [07.SingleChannel](#) for details.

Multiple testing across genes and contrasts

The key function is `decideTests`. This function writes an object of class `TestResults`, which is basically a matrix of -1 , 0 or 1 elements, of the same dimension as `fit$coefficients`, indicating whether each coefficient is significantly different from zero. A number of different multiple testing strategies are provided. `decideTests` calls `classifyTestsF` to implement the nested F-test strat.

`selectModel` chooses between linear models for each probe using AIC or BIC criteria. This is an alternative to hypothesis testing and can choose between non-nested models.

A number of other functions are provided to display the results of `decideTests`. The functions `heatDiagram` (or the older version `heatdiagram`) displays the results in a heat-map style display. This allows visual comparison of the results across many different conditions in the linear model.

The functions `vennCounts` and `vennDiagram` provide Venn diagram style summaries of the results. `Summary` and `show` method exists for objects of class `TestResults`.

The results from `decideTests` can also be included when the results of a linear model fit are written to a file using `write.fit`.

Gene Set Tests

Competitive gene set testing for an individual gene set is provided by `wilcoxGST` or `geneSetTest`, which permute genes. The gene set can be displayed using `barcodeplot`.

Self-contained gene set testing for an individual set is provided by `roast`, which uses rotation technology, analogous to permuting arrays.

Gene set enrichment analysis for a large database of gene sets is provided by `romer`. `topRomer` is used to rank results from `romer`.

The functions `alias2Symbol`, `alias2SymbolTable` and `alias2SymbolUsingNCBI` are provided to help match gene sets with microarray probes by way of official gene symbols.

Global Tests

The function `genas` can test for associations between two contrasts in a linear model.

Given a set of p-values, the function `propTrueNull` can be used to estimate the proportion of true null hypotheses.

When evaluating test procedures with simulated or known results, the utility function `auROC` can be used to compute the area under the Receiver Operating Curve for the test results for a given probe.

Author(s)

Gordon Smyth

See Also

[01.Introduction](#), [02.Classes](#), [03.ReadingData](#), [04.Background](#), [05.Normalization](#), [06.LinearModels](#), [07.SingleChannel](#), [08.Tests](#), [09.Diagnostics](#), [10.GeneSetTests](#), [11.RNAseq](#)

09.Diagnostics

Topic: Diagnostics and Quality Assessment

Description

This page gives an overview of the LIMMA functions available for microarray quality assessment and diagnostic plots.

This package provides an [anova](#) method which is designed for assessing the quality of an array series or of a normalization method. It is not designed to assess differential expression of individual genes. [anova](#) uses utility functions [bwss](#) and [bwss.matrix](#).

The function [arrayWeights](#) estimates the empirical reliability of each array following a linear model fit.

Diagnostic plots can be produced by

[imageplot](#) Produces a spatial picture of any spot-specific measure from an array image. If the log-ratios are plotted, then this produces an in-silico representation of the well known false-color TIFF image of an array. [imageplot3by2](#) will write imageplots to files, six plots to a page.

[plotFB](#) Plots foreground versus background log-intensities.

[plotMD](#) Mean-difference plots. Very versatile plot. For two color arrays, this plots the M-values vs A-values. For single channel technologies, this plots one column of log-expression values vs the average of the other columns. For fitted model objects, this plots a log-fold-change versus average log-expression. [mdplot](#) can also be useful for comparing two one-channel microarrays.

[plotMA](#) MA-plots, essentially the same as mean-difference plots. [plotMA3by2](#) will write MA-plots to files, six plots to a page.

[plotWithHighlights](#) Scatterplots with highlights. This is the underlying engine for [plotMD](#) and [plotMA](#).

[plotPrintTipLoess](#) Produces a grid of MA-plots, one for each print-tip group on an array, together with the corresponding lowess curve. Intended to help visualize print-tip loess normalization.

[plotPrintorder](#) For an array, produces a scatter plot of log-ratios or log-intensities by print order.

[plotDensities](#) Individual channel densities for one or more arrays. An essential plot to accompany between array normalization, especially quantile normalization.

[plotMDS](#) Multidimensional scaling plot for a set of arrays. Useful for visualizing the relationship between the set of samples.

[plotSA](#) Sigma vs A plot. After a linear model is fitted, this checks constancy of the variance with respect to intensity level.

[plotPrintTipLoess](#) uses utility functions [gridr](#) and [gridc](#). [plotDensities](#) uses utility function [RG.MA](#).

Author(s)

Gordon Smyth

See Also

[01.Introduction](#), [02.Classes](#), [03.ReadingData](#), [04.Background](#), [05.Normalization](#), [06.LinearModels](#), [07.SingleChannel](#), [08.Tests](#), [09.Diagnostics](#), [10.GeneSetTests](#), [11.RNAseq](#)

10.GeneSetTests

Topic: Gene Set Tests

Description

This page gives an overview of the LIMMA functions for gene set testing and pathway analysis.

[roast](#) Self-contained gene set testing for one set. Uses [zscoreT](#) to normalize t-statistics.

[mroast](#) Self-contained gene set testing for many sets. Uses [zscoreT](#) to normalize t-statistics.

[fry](#) Fast approximation to [mroast](#), especially useful when heteroscedasticity of genes can be ignored.

[camera](#) Competitive gene set testing.

[cameraPR](#) Competitive gene set testing with a pre-ranked gene set.

[romer](#) **and** [topRomer](#) Gene set enrichment analysis.

[ids2indices](#) Convert gene sets consisting of vectors of gene identifiers into a list of indices suitable for use in the above functions.

[alias2Symbol](#), [alias2SymbolTable](#) **and** [alias2SymbolUsingNCBI](#) Convert gene symbols or aliases to current official symbols.

[geneSetTest](#) **or** [wilcoxGST](#) Simple gene set testing based on gene or probe permutation.

[barcodeplot](#) Enrichment plot of a gene set.

[goana](#) **and** [topGO](#) Gene ontology over-representation analysis of gene lists using Entrez Gene IDs. [goana](#) can work directly on a fitted model object or on one or more lists of genes.

[kegga](#) **and** [topKEGG](#) KEGG pathway over-representation analysis of gene lists using Entrez Gene IDs. [kegga](#) can work directly on a fitted model object or on one or more lists of genes.

Author(s)

Gordon Smyth

See Also

[01.Introduction](#), [02.Classes](#), [03.ReadingData](#), [04.Background](#), [05.Normalization](#), [06.LinearModels](#), [07.SingleChannel](#), [08.Tests](#), [09.Diagnostics](#), [10.GeneSetTests](#), [11.RNAseq](#)

11.RNAseq

Topic: Analysis of RNA-seq Data

Description

This page gives an overview of LIMMA functions to analyze RNA-seq data.

[voom](#) Transform RNA-seq or ChIP-seq counts to log counts per million (log-cpm) with associated precision weights. After this transformation, RNA-seq or ChIP-seq data can be analyzed using the same functions as would be used for microarray data.

[voomWithQualityWeights](#) Combines the functionality of `voom` and `arrayWeights`.

[diffSplice](#) Test for differential exon usage between experimental conditions.

[topSplice](#) Show a data.frame of top results from `diffSplice`.

[plotSplice](#) Plot results from `diffSplice`.

[plotExons](#) Plot logFC for individual exons for a given gene.

References

Law, CW, Chen, Y, Shi, W, Smyth, GK (2014). Voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biology* 15, R29. doi:10.1186/gb2014152r29

Ritchie, ME, Phipson, B, Wu, D, Hu, Y, Law, CW, Shi, W, and Smyth, GK (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* 43, e47. doi:10.1093/nar/gkv007

See Also

See also the `edgeR` package for normalization and data summaries of RNA-seq data, as well as for alternative differential expression methods based on the negative binomial distribution. `voom` accepts `DGEList` objects and normalization factors from `edgeR`.

The `edgeR` function `voomLmFit` is a drop-in replacement for either `voom` or `voomWithQualityWeights`.

[01.Introduction](#), [02.Classes](#), [03.ReadingData](#), [04.Background](#), [05.Normalization](#), [06.LinearModels](#), [07.SingleChannel](#), [08.Tests](#), [09.Diagnostics](#), [10.GeneSetTests](#), [11.RNAseq](#)

alias2Symbol

*Convert Gene Aliases to Official Gene Symbols***Description**

Maps gene alias names to official gene symbols.

Usage

```
alias2Symbol(alias, species = "Hs", expand.symbols = FALSE)
alias2SymbolTable(alias, species = "Hs")
alias2SymbolUsingNCBI(alias, gene.info.file,
                      required.columns = c("GeneID", "Symbol", "description"))
```

Arguments

| | |
|------------------|---|
| alias | character vector of gene aliases |
| species | character string specifying the species. Possible values include "Hs" (human), "Mm" (mouse), "Rn" (rat), "Dm" (fly) or "Pt" (chimpanzee), but other values are possible if the corresponding organism package is available. |
| expand.symbols | logical. This affects those elements of alias that are the official gene symbol for one gene and also an alias for another gene. If FALSE, then these elements will just return themselves. If TRUE, then all the genes for which they are aliases will also be returned. |
| gene.info.file | either the name of a gene information file downloaded from the NCBI or a data.frame resulting from reading such a file. |
| required.columns | character vector of columns from the gene information file that are required in the output. |

Details

Aliases are mapped via NCBI Entrez Gene identity numbers using Bioconductor organism packages.

alias2Symbol maps a set of aliases to a set of symbols, without necessarily preserving order. The output vector may be longer or shorter than the original vector, because some aliases might not be found and some aliases may map to more than one symbol.

alias2SymbolTable returns of vector of the same length as the vector of aliases. If an alias maps to more than one symbol, then the one with the lowest Entrez ID number is returned. If an alias can't be mapped, then NA is returned.

species can be any character string XX for which an organism package org.XX.eg.db exists and is installed. The only requirement of the organism package is that it contains objects org.XX.egALIAS2EG and org.XX.egSYMBOL linking the aliases and symbols to Entrez Gene Ids. At the time of writing, the following organism packages are available from Bioconductor 3.16:

| Package | Species |
|-------------------|---------------------|
| org.Ag.eg.db | Anopheles |
| org.Bt.eg.db | Bovine |
| org.Ce.eg.db | Worm |
| org.Cf.eg.db | Canine |
| org.Dm.eg.db | Fly |
| org.Dr.eg.db | Zebrafish |
| org.EcK12.eg.db | E coli strain K12 |
| org.EcSakai.eg.db | E coli strain Sakai |
| org.Gg.eg.db | Chicken |
| org.Hs.eg.db | Human |
| org.Mm.eg.db | Mouse |
| org.Mmu.eg.db | Rhesus |
| org.Pt.eg.db | Chimp |
| org.Rn.eg.db | Rat |
| org.Ss.eg.db | Pig |
| org.Xl.eg.db | Xenopus |

alias2SymbolUsingNCBI is analogous to alias2SymbolTable but uses a gene-info file from NCBI instead of a Bioconductor organism package. It also gives the option of returning multiple columns from the gene-info file. NCBI gene-info files can be downloaded from https://ftp.ncbi.nlm.nih.gov/gene/DATA/GENE_INFO/. For example, the human file is https://ftp.ncbi.nlm.nih.gov/gene/DATA/GENE_INFO/Mammalia/Homo_sapiens.gene_info.gz and the mouse file is ftp://ftp.ncbi.nlm.nih.gov/gene/DATA/GENE_INFO/Mammalia/Mus_musculus.gene_info.gz.

Value

alias2Symbol and alias2SymbolTable produce a character vector of gene symbols. alias2SymbolTable returns a vector of the same length and order as alias, including NA values where no gene symbol was found. alias2Symbol returns an unordered vector that may be longer or shorter than alias.

alias2SymbolUsingNCBI returns a data.frame with rows corresponding to the entries of alias and columns as specified by required.columns.

Author(s)

Gordon Smyth and Yifang Hu

See Also

This function is often used to assist gene set testing, see [10.GeneSetTests](#).

Examples

```
alias2Symbol(c("PUMA", "NOXA", "BIM"), species="Hs")
alias2Symbol("RS1", expand=TRUE)
```

anova.MAList-method *ANOVA Table - method*

Description

Analysis of variance method for objects of class `MAList`. Produces an ANOVA table useful for quality assessment by decomposing between and within gene sums of squares for a series of replicate arrays. This method produces a single ANOVA Table rather than one for each gene and is not used to identify differentially expressed genes.

Usage

```
anova(object, design=NULL, ndups=2, ...)
```

Arguments

`object` object of class `MAList`. Missing values in the M-values are not allowed.

`design` numeric vector or single-column matrix containing the design matrix for linear model. The length of the vector or the number of rows of the matrix should agree with the number of columns of M.

`ndups` number of duplicate spots. Each gene is printed `ndups` times in adjacent spots on each array.
... other arguments are not used

Details

This function aids in quality assessment of microarray data and in the comparison of normalization methodologies. It applies only to replicated two-color experiments in which all the arrays are hybridized with the same RNA targets, possibly with dye-swaps, so the design matrix should have only one column. The function has not been heavily used and is somewhat experimental.

Value

An object of class `anova` containing rows for between genes, between arrays, gene x array interaction, and between duplicate with array sums of squares. Variance components are estimated for each source of variation.

Note

This function does not give valid results in the presence of missing M-values.

Author(s)

Gordon Smyth

See Also

[MAList-class](#), [bwss.matrix](#), [anova](#).

An overview of quality assessment and diagnostic functions in LIMMA is given by [09.Diagnostics](#).

| | |
|--------------|------------------------------|
| arrayWeights | <i>Array Quality Weights</i> |
|--------------|------------------------------|

Description

Estimate relative quality weights for each array or group in a multi-array experiment.

Usage

```
arrayWeights(object, design = NULL, weights = NULL,
             var.design = NULL, var.group = NULL, prior.n = 10,
             method = "auto", maxiter = 50, tol = 1e-5, trace = FALSE)
```

Arguments

| | |
|------------|---|
| object | any matrix-like object containing log-expression values or log-ratio expression values, for example an EList or ExpressionSet object. See help("getEAWP") for a list of possible classes. |
| design | the design matrix of the microarray experiment, with rows corresponding to arrays and columns to coefficients to be estimated. Defaults to the unit vector meaning that the arrays are treated as replicates. |
| weights | numeric matrix containing prior weights for each expression value. |
| var.design | design matrix for the variance model. Defaults to the sample-specific model whereby each sample has a distinct variance. |
| var.group | vector or factor indicating groups to have different array weights. This is another way to specify var.design for groupwise variance models. |
| prior.n | prior number of genes. Larger values squeeze the array weights more strongly towards equality. |
| method | character string specifying the estimating algorithm to be used. Choices are "genebygene", "reml" or "auto". |
| maxiter | maximum number of iterations allowed when method="reml". |
| tol | convergence tolerance when method="reml". |
| trace | logical. If TRUE then progress information is printed at each iteration of the "reml" algorithm or at every 1000th gene for the "genebygene" algorithm. |

Details

The relative reliability of each array is estimated by measuring how well the expression values for that array follow the linear model. Arrays that tend to have larger residuals are assigned lower weights.

The basic method is described by Ritchie et al (2006) and the extension to custom variance models by Liu et al (2015). A weighted linear model is fitted to the expression values for each gene. The variance model is fitted to the squared residuals from the linear model fit and is updated either by full REML scoring iterations (method="reml") or using an efficient gene-by-gene update algorithm

(method="genebygene"). The final estimates of these array variances are converted to weights. The gene-by-gene algorithm is described by Ritchie et al (2006) while the REML algorithm is an adaption of the algorithm of Smyth (2002).

For stability, the array weights are squeezed slightly towards equality. This is done by adding a prior likelihood corresponding to unit array weights equivalent to `prior.n` genes. The gene-by-gene algorithm is started from the prior genes while the REML algorithm adds the prior to the log-likelihood derivatives.

By default, `arrayWeights` chooses between the REML and gene-by-gene algorithms automatically (method="auto"). REML is chosen if there are no prior weights or missing values and otherwise the gene-by-gene algorithm is used.

The input object is interpreted as for `lmFit` and `getEAWP`. In particular, the arguments `design` and `weights` will be extracted from the data object if available and do not normally need to be set explicitly in the call; if any of these are set in the call then they will over-ride the slots or components in the data object.

Value

A numeric vector of array weights, which multiply to 1.

Author(s)

Matthew Ritchie and Gordon Smyth

References

Liu, R., Holik, A. Z., Su, S., Jansz, N., Chen, K., Leong, H. S., Blewitt, M. E., Asselin-Labat, M.-L., Smyth, G. K., Ritchie, M. E. (2015). Why weight? Combining voom with estimates of sample quality improves power in RNA-seq analyses. *Nucleic Acids Research* 43, e97. doi:10.1093/nar/gkv412

Ritchie, M. E., Diyagama, D., Neilson, van Laar, R., J., Dobrovic, A., Holloway, A., and Smyth, G. K. (2006). Empirical array quality weights in the analysis of microarray data. *BMC Bioinformatics* 7, 261. doi:10.1186/147121057261

Smyth, G. K. (2002). An efficient algorithm for REML in heteroscedastic regression. *Journal of Computational and Graphical Statistics* 11, 836-847. <https://gksmyth.github.io/pubs/remlalgo.pdf>

See Also

[arrayWeightsQuick](#), [voomWithQualityWeights](#)

An overview of linear model functions in `limma` is given by [06.LinearModels](#).

Examples

```
ngenes <- 1000
narrays <- 6
y <- matrix(rnorm(ngenes*narrays), ngenes, narrays)
var.group <- c(1,1,1,2,2,2)
y[,var.group==1] <- 2*y[,var.group==1]
arrayWeights(y, var.group=var.group)
```

| | |
|-------------------|------------------------------|
| arrayWeightsQuick | <i>Array Quality Weights</i> |
|-------------------|------------------------------|

Description

Estimates relative quality weights for each array in a multi-array experiment with replication.

Usage

```
arrayWeightsQuick(y, fit)
```

Arguments

| | |
|-----|--|
| y | the data object used to estimate fit. Can be of any class which can be coerced to matrix, including matrix, MAlist, marrayNorm or ExpressionSet. |
| fit | MArrayLM fitted model object |

Details

Estimates the relative reliability of each array by measuring how well the expression values for that array follow the linear model.

This is a quick and dirty version of [arrayWeights](#).

Value

Numeric vector of weights of length `ncol(fit)`.

Author(s)

Gordon Smyth

References

Ritchie, M. E., Diyagama, D., Neilson, van Laar, R., J., Dobrovic, A., Holloway, A., and Smyth, G. K. (2006). Empirical array quality weights in the analysis of microarray data. BMC Bioinformatics 7, 261. doi:10.1186/147121057261

See Also

See [arrayWeights](#). An overview of LIMMA functions for reading data is given in [03.ReadingData](#).

Examples

```
## Not run:  
fit <- lmFit(y, design)  
arrayWeightsQuick(y, fit)  
  
## End(Not run)
```

`as.data.frame`*Turn a Microarray Linear Model Object into a Dataframe*

Description

Turn a MArrayLM object into a data.frame.

Usage

```
## S3 method for class 'MArrayLM'  
as.data.frame(x, row.names = NULL, optional = FALSE, ...)
```

Arguments

| | |
|------------------------|---|
| <code>x</code> | an object of class MArrayLM |
| <code>row.names</code> | NULL or a character vector giving the row names for the data frame. Missing values are not allowed. |
| <code>optional</code> | logical. If TRUE, setting row names and converting column names (to syntactic names) is optional. |
| <code>...</code> | additional arguments to be passed to or from methods. |

Details

This method combines all the components of `x` which have a row for each probe on the array into a data.frame.

Value

A data.frame.

Author(s)

Gordon Smyth

See Also

[as.data.frame](#) in the base package.

[02.Classes](#) gives an overview of data classes used in LIMMA. [06.LinearModels](#) gives an overview of linear model functions in LIMMA.

| | |
|-----------|--|
| as.MAList | <i>Convert marrayNorm Object to an MAList Object</i> |
|-----------|--|

Description

Convert marrayNorm Object to an MAList Object

Usage

```
as.MAList(object)
```

Arguments

object an marrayNorm object

Details

The marrayNorm class is defined in the marray package. This function converts a normalized two color microarray data object created by the marray package into the corresponding limma data object.

Note that such conversion is not necessary to access the limma linear modelling functions, because lmFit will operate on a marrayNorm data object directly.

Value

Object of class [MAList](#)

Author(s)

Gordon Smyth

See Also

[02.Classes](#) gives an overview of all the classes defined by this package.

The marrayNorm class is defined in the marray package.

`as.matrix`*Turn a Microarray Data Object into a Matrix*

Description

Turn a microarray data object into a numeric matrix by extracting the expression values.

Usage

```
## S3 method for class 'MAList'  
as.matrix(x, ...)
```

Arguments

`x` an object of class `RGList`, `MAList`, `EList`, `MArrayLM`, `marrayNorm`, `PLMset`, `ExpressionSet`, `LumiBatch` or `vsn`.

`...` additional arguments, not used for these methods.

Details

These methods extract the matrix of log-ratios, for `MAList` or `marrayNorm` objects, or the matrix of expression values for other expression objects such as `EList` or `ExpressionSet`. For `MArrayLM` objects, the matrix of fitted coefficients is extracted.

These methods involve loss of information, so the original data object is not recoverable.

Value

A numeric matrix.

Author(s)

Gordon Smyth

See Also

[as.matrix](#) in the base package or [exprs](#) in the Biobase package.

[02.Classes](#) gives an overview of data classes used in LIMMA.

| | |
|-----------------|------------------------|
| asMatrixWeights | <i>asMatrixWeights</i> |
|-----------------|------------------------|

Description

Convert probe-weights or array-weights to a matrix of weights.

Usage

```
asMatrixWeights(weights, dim)
```

Arguments

| | |
|---------|--|
| weights | numeric matrix of weights, rows corresponding to probes and columns to arrays. Or vector of probe weights. Or vector of array weights. |
| dim | numeric dimension vector of length 2, i.e., the number of probes and the number of arrays. |

Details

This function converts a vector or probe-weights or a vector of array-weights to a matrix of the correct size. Probe-weights are repeated across rows while array-weights are repeated down the columns. If `weights` has length equal to the number of probes, it is assumed to contain probe-weights. If it has length equal to the number of arrays, it is assumed to contain array-weights. If the number of probes is equal to the number of arrays, then `weights` is assumed to contain array-weights if it is a row-vector of the correct size, i.e., if it is a matrix with one row.

This function is used internally by the linear model fitting functions in `limma`.

Value

Numeric matrix of dimension `dim`.

Author(s)

Gordon Smyth

See Also

[modifyWeights](#).

An overview of functions in LIMMA used for fitting linear models is given in [06.LinearModels](#).

Examples

```
asMatrixWeights(1:3,c(4,3))
asMatrixWeights(1:4,c(4,3))
```

`auROC`*Area Under Receiver Operating Curve*

Description

Compute exact area under the ROC for empirical data.

Usage

```
auROC(truth, stat=NULL)
```

Arguments

| | |
|--------------------|--|
| <code>truth</code> | logical vector, or numeric vector of 0s and 1s, indicating whether each case is a true positive. |
| <code>stat</code> | numeric vector containing test statistics used to rank cases, from largest to smallest. If <code>NULL</code> , then <code>truth</code> is assumed to be already sorted in decreasing test statistic order. |

Details

A receiver operating curve (ROC) is a plot of sensitivity (true positive rate) versus 1-specificity (false positive rate) for a statistical test or binary classifier. The area under the ROC is a well accepted measure of test performance. It is equivalent to the probability that a randomly chosen pair of cases is correctly ranked.

Here we consider a test statistic `stat`, with larger values being more significant, and a vector `truth` indicating whether the alternative hypothesis is in fact true. `truth==TRUE` or `truth==1` indicates a true discovery and `truth=FALSE` or `truth=0` indicates a false discovery. Correct ranking here means that `truth[i]` is greater than or equal to `truth[j]` when `stat[i]` is greater than `stat[j]`. The function computes the exact area under the empirical ROC curve defined by `truth` when ordered by `stat`.

If `stat` contains ties, then `auROC` returns the average area under the ROC for all possible orderings of `truth` for tied `stat` values.

The area under the curve is undefined if `truth` is all `TRUE` or all `FALSE` or if `truth` or `stat` contain missing values.

Value

Numeric value between 0 and 1 giving area under the curve, 1 being perfect and 0 being the minimum.

Author(s)

Gordon Smyth

Examples

```

auROC(c(1,1,0,0,0))
truth <- rbinom(30,size=1,prob=0.2)
stat <- rchisq(30,df=2)
auROC(truth,stat)

```

avearrays

*Average Over Replicate Arrays***Description**

Condense a microarray data object so that technical replicate arrays are replaced with (weighted) averages.

Usage

```

## Default S3 method:
avearrays(x, ID=colnames(x), weights=NULL)
## S3 method for class 'MList'
avearrays(x, ID=colnames(x), weights=x$weights)
## S3 method for class 'EList'
avearrays(x, ID=colnames(x), weights=x$weights)

```

Arguments

| | |
|---------|--|
| x | a matrix-like object, usually a matrix, MList or EList object. |
| ID | sample identifier. |
| weights | numeric matrix of non-negative weights |

Details

A new data object is computed in which technical replicate arrays are replaced by their (weighted) averages.

For an MList object, the components M and A are both averaged in this way, as are weights and any matrices found in object\$other.

EList objects are similar, except that the E component is averaged instead of M and A.

If x is of mode "character", then the replicate values are assumed to be equal and the first is taken as the average.

Value

A data object of the same class as x with a column for each unique value of ID.

Author(s)

Gordon Smyth

See Also

[avereps](#).

[02.Classes](#) gives an overview of data classes used in LIMMA.

Examples

```
x <- matrix(rnorm(8*3),8,3)
colnames(x) <- c("a","a","b")
avearrays(x)
```

avedups

Average Over Duplicate Spots

Description

Condense a microarray data object so that values for within-array replicate spots are replaced with their average.

Usage

```
## Default S3 method:
avedups(x, ndups=2, spacing=1, weights=NULL)
## S3 method for class 'MList'
avedups(x, ndups=x$printer$ndups, spacing=x$printer$spacing, weights=x$weights)
## S3 method for class 'EList'
avedups(x, ndups=x$printer$ndups, spacing=x$printer$spacing, weights=x$weights)
```

Arguments

| | |
|---------|--|
| x | a matrix-like object, usually a matrix, MList or EList object. |
| ndups | number of within-array replicates for each probe. |
| spacing | number of spots to step from a probe to its duplicate. |
| weights | numeric matrix of spot weights. |

Details

A new data object is computed in which each probe is represented by the (weighted) average of its duplicate spots. For an MList object, the components M and A are both averaged in this way. For an EList object, the component E is averaged in this way.

If x is of mode "character", then the duplicate values are assumed to be equal and the first is taken as the average.

Value

A data object of the same class as x with 1/ndups as many rows.

Author(s)

Gordon Smyth

See Also[avereps.](#)[02.Classes](#) gives an overview of data classes used in LIMMA.

avereps*Average Over Irregular Replicate Probes*

Description

Condense a microarray data object so that values for within-array replicate probes are replaced with their average.

Usage

```
## Default S3 method:
avereps(x, ID=rownames(x), ...)
## S3 method for class 'MAList'
avereps(x, ID=NULL, ...)
## S3 method for class 'EList'
avereps(x, ID=NULL, ...)
```

Arguments

| | |
|-----|---|
| x | a matrix-like object, usually a matrix, MAList or EList object. |
| ID | probe identifier. |
| ... | other arguments are not currently used. |

Details

A new data object is computed in which each probe ID is represented by the average of its replicate spots or features.

For an MAList object, the components M and A are both averaged in this way, as are weights and any matrices found in object\$other. For an MAList object, ID defaults to MA\$genes\$ID if that exists, otherwise to rownames(MA\$M).

EList objects are similar, except that the E component is averaged instead of M and A.

If x is of mode "character", then the replicate values are assumed to be equal and the first is taken as the average.

Value

A data object of the same class as x with a row for each unique value of ID.

Note

This function should only be applied to normalized log-expression values, and not to raw unlogged expression values. It will generate an error message if applied to `RGList` or `EListRaw` objects.

Author(s)

Gordon Smyth

See Also

[avedups](#), [avearrays](#). Also [rowsum](#) in the base package.
[02.Classes](#) gives an overview of data classes used in LIMMA.

Examples

```
x <- matrix(rnorm(8*3),8,3)
colnames(x) <- c("S1","S2","S3")
rownames(x) <- c("b","a","a","c","c","b","b","b")
avereps(x)
```

backgroundCorrect

Correct Intensities for Background

Description

Background correct microarray expression intensities.

Usage

```
backgroundCorrect(RG, method="auto", offset=0, printer=RG$printer,
                  normexp.method="saddle", verbose=TRUE)
backgroundCorrect.matrix(E, Eb=NULL, method="auto", offset=0, printer=NULL,
                         normexp.method="saddle", verbose=TRUE)
```

Arguments

| | |
|--------|--|
| RG | a numeric matrix, EListRaw or RGList object. |
| E | numeric matrix containing foreground intensities. |
| Eb | numeric matrix containing background intensities. |
| method | character string specifying correction method. Possible values are "auto", "none", "subtract", "half", "minimum", "movingmin", "edwards" or "normexp". If RG is a matrix, possible values are restricted to "none" or "normexp". The default "auto" is interpreted as "subtract" if background intensities are available or "normexp" if they are not. |
| offset | numeric value to add to intensities |

| | |
|----------------|--|
| printer | a list containing printer layout information, see PrintLayout-class . Ignored if RG is a matrix. |
| normexp.method | character string specifying parameter estimation strategy used by normexp, ignored for other methods. Possible values are "saddle", "mle", "rma" or "rma75". |
| verbose | logical. If TRUE, progress messages are sent to standard output |

Details

This function implements the background correction methods reviewed or developed in Ritchie et al (2007) and Silver et al (2009). Ritchie et al (2007) recommend `method="normexp"` whenever RG contains local background estimates. Silver et al (2009) shows that either `normexp.method="mle"` or `normexp.method="saddle"` are excellent options for normexp. If RG contains morphological background estimates instead (available from SPOT or GenePix image analysis software), then `method="subtract"` performs well.

If `method="none"` then no correction is done, i.e., the background intensities are treated as zero. If `method="subtract"` then the background intensities are subtracted from the foreground intensities. This is the traditional background correction method, but is not necessarily recommended. If `method="movingmin"` then the background estimates are replaced with the minimums of the backgrounds of the spot and its eight neighbors, i.e., the background is replaced by a moving minimum of 3x3 grids of spots.

The remaining methods are all designed to produce positive corrected intensities. If `method="half"` then any intensity which is less than 0.5 after background subtraction is reset to be equal to 0.5. If `method="minimum"` then any intensity which is zero or negative after background subtraction is set equal to half the minimum of the positive corrected intensities for that array. If `method="edwards"` a log-linear interpolation method is used to adjust lower intensities as in Edwards (2003). If `method="normexp"` a convolution of normal and exponential distributions is fitted to the foreground intensities using the background intensities as a covariate, and the expected signal given the observed foreground becomes the corrected intensity. This results in a smooth monotonic transformation of the background subtracted intensities such that all the corrected intensities are positive.

The normexp method is available in a number of variants depending on how the model parameters are estimated, and these are selected by `normexp.method`. Here "saddle" gives the saddle-point approximation to maximum likelihood from Ritchie et al (2007) and improved by Silver et al (2009), "mle" gives exact maximum likelihood from Silver et al (2009), "rma" gives the background correction algorithm from the RMA-algorithm for Affymetrix microarray data as implemented in the affy package, and "rma75" gives the RMA-75 method from McGee and Chen (2006). In practice "mle" performs well and is nearly as fast as "saddle", but "saddle" is the default for backward compatibility. See [normexp.fit](#) for more details.

The `offset` can be used to add a constant to the intensities before log-transforming, so that the log-ratios are shrunk towards zero at the lower intensities. This may eliminate or reverse the usual 'fanning' of log-ratios at low intensities associated with local background subtraction.

Background correction (background subtraction) is also performed by the [normalizeWithinArrays](#) method for RGList objects, so it is not necessary to call backgroundCorrect directly unless one wants to use a method other than simple subtraction. Calling backgroundCorrect before [normalizeWithinArrays](#) will over-ride the default background correction.

Value

A matrix, EListRaw or RGList object in which foreground intensities have been background corrected and any components containing background intensities have been removed.

Author(s)

Gordon Smyth

References

Edwards, D. E. (2003). Non-linear normalization and background correction in one-channel cDNA microarray studies *Bioinformatics* 19, 825-833.

McGee, M., and Chen, Z. (2006). Parameter estimation for the exponential-normal convolution model for background correction of Affymetrix GeneChip data. *Stat Appl Genet Mol Biol*, Volume 5, Article 24.

Ritchie, M. E., Silver, J., Oshlack, A., Silver, J., Holmes, M., Diyagama, D., Holloway, A., and Smyth, G. K. (2007). A comparison of background correction methods for two-colour microarrays. *Bioinformatics* 23, 2700-2707. <http://bioinformatics.oxfordjournals.org/content/23/20/2700>

Silver, J., Ritchie, M. E., and Smyth, G. K. (2009). Microarray background correction: maximum likelihood estimation for the normal-exponential convolution model. *Biostatistics* 10, 352-363. <http://biostatistics.oxfordjournals.org/content/10/2/352>

See Also

[kooperberg](#), [neqc](#).

An overview of background correction functions is given in [04.Background](#).

Examples

```
RG <- new("RGList", list(R=c(1,2,3,4),G=c(1,2,3,4),Rb=c(2,2,2,2),Gb=c(2,2,2,2)))
backgroundCorrect(RG)
backgroundCorrect(RG, method="half")
backgroundCorrect(RG, method="minimum")
backgroundCorrect(RG, offset=5)
```

barcodeplot

Barcode Enrichment Plot

Description

Display the enrichment of one or two gene sets in a ranked gene list.

Usage

```
barcodeplot(statistics, index = NULL, index2 = NULL, gene.weights = NULL,
            weights.label = "Weight", labels = c("Down", "Up"),
            quantiles = c(-1,1)*sqrt(2), col.bars = NULL, alpha = 0.4,
            worm = TRUE, span.worm = 0.45, xlab = "Statistic", ...)
```

Arguments

| | |
|----------------------------|--|
| <code>statistics</code> | numeric vector giving the values of statistics to rank genes by. |
| <code>index</code> | index vector for the gene set. This can be a vector of indices, or a logical vector of the same length as <code>statistics</code> or, in general, any vector such that <code>statistic[index]</code> gives a subset of the statistic values. Can be omitted if <code>gene.weights</code> has same length as <code>statistics</code> , in which case positive values of <code>gene.weights</code> indicate to members of the positive set and negative weights correspond to members of the negative set. |
| <code>index2</code> | optional index vector for a second (negative) gene set. If specified, then <code>index</code> and <code>index2</code> specify positive and negative genes respectively. Usually used to distinguish down-regulated genes from up-regulated genes. |
| <code>gene.weights</code> | numeric vector giving directional weights for the genes in the (first) set. Positive and negative weights correspond to positive and negative genes. Ignored if <code>index2</code> is non-null. |
| <code>weights.label</code> | label describing the entries in <code>gene.weights</code> . |
| <code>labels</code> | character vector of labels for low and high statistics. First label is associated with low statistics or negative statistics and is displayed at the left end of the plot. Second label is associated with high or positive statistics and is displayed at the right end of the plot. |
| <code>quantiles</code> | numeric vector of length 2, giving cutoff values for statistics considered small or large respectively. Used to color the rectangle of the barcodeplot. |
| <code>col.bars</code> | character vector of colors for the vertical bars of the barcodeplot showing the ranks of the gene set members. Defaults to "black" for one set or <code>c("red", "blue")</code> for two sets. |
| <code>alpha</code> | transparency for vertical bars. When <code>gene.weights</code> are not NULL, values $0 < \alpha < 1$ give semitransparent colors for the vertical bars inside the rectangle. This helps distinguish position bars from the weighted bars and also helps to show the density of the bars when there are many bars. Ignored if <code>gene.weights=NULL</code> . |
| <code>worm</code> | logical, should enrichment worms be plotted? |
| <code>span.worm</code> | loess span for enrichment worms. Larger spans give smoother worms. |
| <code>xlab</code> | x-axis label for statistics. |
| <code>...</code> | other arguments are passed to <code>plot</code> . |

Details

The function displays the enrichment of a specified gene set signature in a ranked list of genes. The vector `statistics` defines the ranking of the population of genes. This vector can represent

any useful ranking but often it provides t-statistics or a log-fold-changes arising from a differential analysis. The gene set signature is defined either by `index` and `index2` or by `gene.weights`.

The signature can be either unidirectional or bidirectional. A unidirectional signature is a simple set of genes (defined by `index`), optionally accompanied by a set of positive magnitude scores (specified by `gene.weights`). Typically this is a set of genes representing a pathway or biological process that are expected to be co-regulated in the same direction. A bidirectional signature consists of a set of up-genes and a set of down-genes (specified by `index` and `index2` respectively) or, more generally, a set of genes with accompanying magnitude scores that are either positive or negative (specified by `gene.weights`).

Technically, this function plots the positions of one or two gene sets in a ranked list of statistics. If there are two sets, then one is considered to be the positive set and the other the down set. For example, the first set and second sets often correspond to genes that are expected to be up- or down-regulated respectively. The function can optionally display varying weights for different genes, for example log-fold-changes from a previous experiment.

The statistics are ranked left to right from smallest to largest. The ranked statistics are represented by a shaded bar or bed, and the positions of the specified subsets are marked by vertical bars, forming a pattern like a barcode. An enrichment worm optionally shows the relative enrichment of the vertical bars in each part of the plot. The worm is computed by the `tricubeMovingAverage` function.

Barcode plots are often used in conjunction with gene set tests, and show the enrichment of gene sets amongst high or low ranked genes. They were inspired by the set location plot of Subramanian et al (2005), with a number of enhancements, especially the ability to plot positive and negative sets simultaneously. Barcode plots first appeared in the literature in Lim et al (2009). More recent examples can be seen in Liu et al (2014), Sheikh et al (2015), Witkowski et al (2015) and Ng et al (2015).

The function can be used with any of four different calling sequences:

- `index` is specified, but not `index2` or `gene.weights`. Single direction plot.
- `index` and `index2` are specified. Two directional plot.
- `index` and `gene.weights` are specified. `gene.weights` must have same length as `statistics[index]`. Plot will be two-directional if `gene.weights` contains positive and negative values.
- `gene.weights` is specified by not `index` or `index2`. `gene.weights` must have same length as `statistics`. Plot will be two-directional if `gene.weights` contains positive and negative values.

Value

No value is returned but a plot is produced as a side effect.

Author(s)

Yifang Hu, Gordon Smyth and Di Wu

References

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by trisomy of Erg leads to myeloproliferation in a down syndrome model. *PLOS Genetics* 11, e1005211. doi:10.1371/journal.pgen.1005211

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Liu, GJ, Cimmino, L, Jude, JG, Hu, Y, Witkowski, MT, McKenzie, MD, Kartal-Kaess, M, Best, SA, Tuohey, L, Liao, Y, Shi, W, Mullighan, CG, Farrar, MA, Nutt, SL, Smyth, GK, Zuber, J, and Dickins, RA (2014). Pax5 loss imposes a reversible differentiation block in B progenitor acute lymphoblastic leukemia. *Genes & Development* 28, 1337-1350. doi:10.1101/gad.240416.114

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Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, and Mesirov JP (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 102, 15545-15550.

Witkowski, MT, Cimmino, L, Hu, Y, Trimarchi, T, Tagoh, H, McKenzie, MD, Best, SA, Tuohey, L, Willson, TA, Nutt, SL, Meinrad Busslinger, M, Aifantis, I, Smyth, GK, and Dickins, RA (2015). Activated Notch counteracts Ikaros tumor suppression in mouse and human T cell acute lymphoblastic leukemia. *Leukemia* 29, 1301-1311. doi:10.1038/leu.2015.27

See Also

[tricubeMovingAverage](#), [roast](#), [camera](#), [romer](#), [geneSetTest](#)

There is a topic page on [10.GeneSetTests](#).

Examples

```
stat <- rnorm(100)
sel <- 1:10
sel2 <- 11:20
stat[sel] <- stat[sel]+1
stat[sel2] <- stat[sel2]-1

# One directional
barcodeplot(stat, index = sel)

# Two directional
barcodeplot(stat, index = sel, index2 = sel2)

# Second set can be indicated by negative weights
barcodeplot(stat, index = c(sel,sel2), gene.weights = c(rep(1,10), rep(-1,10)))

# Two directional with unequal weights
w <- rep(0,100)
```

```

w[sel] <- runif(10)
w[sel2] <- -runif(10)
barcodeplot(stat, gene.weights = w, weights.label = "logFC")

# One directional with unequal weights
w <- rep(0,100)
w[sel2] <- -runif(10)
barcodeplot(stat, gene.weights = w, weights.label = "logFC", col.bars = "dodgerblue")

```

| | |
|------------------|--|
| beadCountWeights | <i>Bead Count Weights for Illumina BeadChips</i> |
|------------------|--|

Description

Estimates weights which account for biological variation and technical variation resulting from varying bead numbers.

Usage

```
beadCountWeights(y, x, design = NULL, bead.stdev = NULL, bead.stderr = NULL,
                 nbeads = NULL, array.cv = TRUE, scale = FALSE)
```

Arguments

| | |
|-------------|---|
| y | an "EList" object or a numeric matrix containing normalized log ₂ -expression values. |
| x | an "EListRaw" object or a numeric matrix of raw expression values, with same dimensions as y. |
| design | the design matrix of the microarray experiment, with rows corresponding to arrays and columns to coefficients to be estimated. Defaults to y\$design or, if that is NULL, then to a column of ones meaning that the arrays are treated as replicates. |
| bead.stdev | numeric matrix of bead-level standard deviations. |
| bead.stderr | numeric matrix of bead-level standard errors. Not required if bead.stdev is set. |
| nbeads | numeric matrix containing number of beads. |
| array.cv | logical, should technical variation for each observation be calculated from a constant or array-specific coefficient of variation? The default is to use array-specific coefficients of variation. |
| scale | logical, should weights be scaled so that the average weight size is the mean of the inverse technical variance along a probe? By default, weights are scaled so that the average weight size along a probe is 1. |

Details

This function estimates optimum weights using the bead statistics for each probe for an Illumina expression BeadChip. It can be used with any Illumina expression BeadChip, but is most likely to be useful with HumanHT-12 BeadChips.

Arguments `x` and `y` are both required. `x` contains the raw expression values and `y` contains the corresponding \log_2 values for the same probes and the same arrays after background correction and normalization. `x` and `y` be any type of object that can be coerced to a matrix, with rows corresponding to probes and columns to arrays. `x` and `y` must contain the same rows and columns in the same order.

The reliability of the normalized expression value for each probe on each array is measured by estimating its technical and biological variability. The bead number weights are the inverse sum of the technical and biological variances.

The technical variance for each probe on each array is inversely proportional to the number of beads and is estimated using array-specific bead-level coefficients of variation.

Coefficients of variation are calculated using raw expression values.

The biological variance for each probe across the arrays are estimated using a Newton iteration, with the assumption that the total residual deviance for each probe from `lmFit` is inversely proportional to the sum of the technical variance and biological variance.

Only one of `bead.stdev` or `bead.stdev` needs to be set. If `bead.stdev` is not provided, then it will be computed as `bead.stdev * sqrt(nbeads)`.

If arguments `bead.stdev` and `nbeads` are not set explicitly in the call, then they will be extracted from `my$other$BEAD_STDEV` and `y$other$Avg_NBEADS`. An `EList` object containing these components can be created by `read.idat` or `read.ilmn`, see the example code below.

Value

A list object with the following components:

| | |
|-----------------------------|--|
| <code>weights</code> | numeric matrix of bead number weights |
| <code>cv.constant</code> | numeric value of constant bead-level coefficient of variation |
| <code>cv.array</code> | numeric vector of array-specific bead-level coefficient of variation |
| <code>var.technical</code> | numeric matrix of technical variances |
| <code>var.biological</code> | numeric vector of biological variances |

Author(s)

Charity Law and Gordon Smyth

References

Law CW (2013). *Precision weights for gene expression analysis*. PhD Thesis. University of Melbourne, Australia. <http://hdl.handle.net/11343/38150>

See Also

[read.ilmn](#), [read.idat](#), [neqc](#).

An overview of linear model functions in limma is given by [06.LinearModels](#).

Examples

```
## Not run:
z <- read.ilmn(files="probesummaryprofile.txt",
              ctrfiles="controlprobesummary.txt",
              other.columns=c("BEAD_STDEV", "Avg_NBEADS"))
y <- neqc(z)
x <- z[z$genes$Status=="regular",]
bcw <- beadCountWeights(y,x,design)
fit <- lmFit(y,design,weights=bcw$weights)
fit <- eBayes(fit)

## End(Not run)
```

blockDiag

Block Diagonal Matrix

Description

Form a block diagonal matrix from the given blocks.

Usage

```
blockDiag(...)
```

Arguments

... numeric matrices

Details

This function is sometimes useful for constructing a design matrix for a disconnected two-color microarray experiment in conjunction with `modelMatrix`.

Value

A block diagonal matrix with dimensions equal to the sum of the input dimensions

Author(s)

Gordon Smyth

See Also

[modelMatrix](#)

Examples

```
a <- matrix(1,3,2)
b <- matrix(2,2,2)
blockDiag(a,b)
```

bwss

Between and within sums of squares

Description

Sums of squares between and within groups. Allows for missing values.

Usage

```
bwss(x, group)
```

Arguments

| | |
|-------|--|
| x | a numeric vector giving the responses. |
| group | a vector or factor giving the grouping variable. |

Details

This is equivalent to one-way analysis of variance.

Value

A list with components

| | |
|-----|--|
| bss | sums of squares between the group means. |
| wss | sums of squares within the groups. |
| bdf | degrees of freedom corresponding to bss. |
| wdf | degrees of freedom corresponding to wss. |

Author(s)

Gordon Smyth

See Also

[bwss.matrix](#)

| | |
|-------------|--|
| bwss.matrix | <i>Between and within sums of squares for matrix</i> |
|-------------|--|

Description

Sums of squares between and within the columns of a matrix. Allows for missing values. This function is called by the [anova](#) method for `MAList` objects.

Usage

```
bwss.matrix(x)
```

Arguments

`x` a numeric matrix.

Details

This is equivalent to a one-way analysis of variance where the columns of the matrix are the groups. If `x` is a matrix then `bwss.matrix(x)` is the same as `bwss(x, col(x))` except for speed of execution.

Value

A list with components

| | |
|------------------|--|
| <code>bss</code> | sums of squares between the column means. |
| <code>wss</code> | sums of squares within the column means. |
| <code>bdf</code> | degrees of freedom corresponding to <code>bss</code> . |
| <code>wdf</code> | degrees of freedom corresponding to <code>wss</code> . |

Author(s)

Gordon Smyth

See Also

[bwss](#), [anova](#), [MAList](#)

Description

Test whether a set of genes is highly ranked relative to other genes in terms of differential expression, accounting for inter-gene correlation.

Usage

```
## Default S3 method:
camera(y, index, design, contrast = ncol(design), weights = NULL,
       use.ranks = FALSE, allow.neg.cor=FALSE, inter.gene.cor=0.01, trend.var = FALSE,
       sort = TRUE, directional = TRUE, ...)
## Default S3 method:
cameraPR(statistic, index, use.ranks = FALSE, inter.gene.cor=0.01,
         sort = TRUE, directional = TRUE, ...)
interGeneCorrelation(y, design)
```

Arguments

| | |
|-----------------------------|--|
| <code>y</code> | a numeric matrix of log-expression values or log-ratios of expression values, or any data object containing such a matrix. Rows correspond to probes and columns to samples. Any type of object that can be processed by getEAWP is acceptable. NA or infinite values are not allowed. |
| <code>statistic</code> | a numeric vector of genewise statistics. If <code>index</code> contains gene IDs, then <code>statistic</code> should be a named vector with the gene IDs as names. |
| <code>index</code> | an index vector or a list of index vectors. Can be any vector such that <code>y[index,]</code> of <code>statistic[index]</code> selects the rows corresponding to the test set. The list can be made using ids2indices . |
| <code>design</code> | design matrix. |
| <code>contrast</code> | contrast of the linear model coefficients for which the test is required. Can be an integer specifying a column of <code>design</code> , or else a numeric vector of same length as the number of columns of <code>design</code> . |
| <code>weights</code> | numeric matrix of precision weights. Can be a matrix of the same size as <code>y</code> , or a numeric vector of array weights with length equal to <code>ncol(y)</code> , or a numeric vector of gene weights with length equal to <code>nrow(y)</code> . |
| <code>use.ranks</code> | do a rank-based test (TRUE) or a parametric test (FALSE)? |
| <code>allow.neg.cor</code> | should reduced variance inflation factors be allowed for negative correlations? |
| <code>inter.gene.cor</code> | numeric, optional preset value for the inter-gene correlation within tested sets. If NA or NULL, then an inter-gene correlation will be estimated for each tested set. |
| <code>trend.var</code> | logical, should an empirical Bayes trend be estimated? See eBayes for details. |
| <code>sort</code> | logical, should the results be sorted by p-value? |

`directional` are the gene sets directional? If TRUE, will test for genes changing in the same direction within each set. If FALSE, will test for large effects without regard to direction in each set.

... other arguments are not currently used

Details

`camera` and `interGeneCorrelation` implement methods proposed by Wu and Smyth (2012). `camera` performs a *competitive* test in the sense defined by Goeman and Buhlmann (2007). It tests whether the genes in the set are highly ranked in terms of differential expression relative to genes not in the set. It has similar aims to `geneSetTest` but accounts for inter-gene correlation. See [roast](#) for an analogous *self-contained* gene set test.

The function can be used for any microarray experiment which can be represented by a linear model. The design matrix for the experiment is specified as for the `lmFit` function, and the contrast of interest is specified as for the `contrasts.fit` function. This allows users to focus on differential expression for any coefficient or contrast in a linear model by giving the vector of test statistic values.

`camera` estimates p-values after adjusting the variance of test statistics by an estimated variance inflation factor. The inflation factor depends on estimated genewise correlation and the number of genes in the gene set.

By default, `camera` uses `interGeneCorrelation` to estimate the mean pair-wise correlation within each set of genes. `camera` can alternatively be used with a preset correlation specified by `inter.gene.cor` that is shared by all sets. This usually works best with a small value, say `inter.gene.cor=0.01`.

If `inter.gene.cor=NA`, then `camera` will estimate the inter-gene correlation for each set. In this mode, `camera` gives rigorous error rate control for all sample sizes and all gene sets. However, in this mode, highly co-regulated gene sets that are biological interpretable may not always be ranked at the top of the list.

With the default value `inter.gene.cor=0.01`, `camera` will rank biologically interpretable sets more highly. This gives a useful compromise between strict error rate control and interpretable gene set rankings.

`cameraPR` is a "pre-ranked" version of `camera` where the genes are pre-ranked according to a pre-computed statistic.

If `direction=TRUE`, then the gene sets are assumed to contain genes changing in the same direction and the functions will do two-sided directional tests for each gene set. If `direction=FALSE`, then the gene sets are assumed to be non-directional, so that significant genes in each set could be changing in different directions. In this case, the functions will test for large changes in each set without regard to direction of change. If `direction=FALSE`, then use `.ranks` should be TRUE and `statistic` should be non-negative.

Value

`camera` and `cameraPR` return a `data.frame` with a row for each set and the following columns:

| | |
|--------------------------|---|
| <code>NGenes</code> | number of genes in set. |
| <code>Correlation</code> | inter-gene correlation (only included if the <code>inter.gene.cor</code> was not preset). |
| <code>Direction</code> | direction of change ("Up" or "Down"). |

PValue two-tailed p-value.
 FDR Benjamini and Hochberg FDR adjusted p-value.

interGeneCorrelation returns a list with components:

vif variance inflation factor.
 correlation inter-gene correlation.

Note

The default settings for `inter.gene.cor` and `allow.neg.cor` were changed to the current values in `limma 3.29.6`. Previously, the default was to estimate an inter-gene correlation for each set. To reproduce the earlier default, use `allow.neg.cor=TRUE` and `inter.gene.cor=NA`.

Author(s)

Di Wu and Gordon Smyth

References

Wu D, Smyth GK (2012). Camera: a competitive gene set test accounting for inter-gene correlation. *Nucleic Acids Research* 40, e133. doi:10.1093/nar/gks461

Goeman JJ, Buhlmann P (2007). Analyzing gene expression data in terms of gene sets: methodological issues. *Bioinformatics* 23, 980-987.

See Also

[getEAWP](#)
[rankSumTestWithCorrelation](#), [geneSetTest](#), [roast](#), [fry](#), [romer](#), [ids2indices](#).
 There is a topic page on [10.GeneSetTests](#).

Examples

```
y <- matrix(rnorm(1000*6),1000,6)
design <- cbind(Intercept=1,Group=c(0,0,0,1,1,1))

# First set of 20 genes are genuinely differentially expressed
index1 <- 1:20
y[index1,4:6] <- y[index1,4:6]+1

# Second set of 20 genes are not DE
index2 <- 21:40

camera(y, index1, design)
camera(y, index2, design)

camera(y, list(set1=index1,set2=index2), design, inter.gene.cor=NA)
camera(y, list(set1=index1,set2=index2), design, inter.gene.cor=0.01)

# Pre-ranked version
```

```
fit <- eBayes(lmFit(y, design))
cameraPR(fit$t[,2], list(set1=index1,set2=index2))

# Non-directional tests
cameraPR(abs(fit$t[,2]), list(set1=index1,set2=index2), use.ranks=TRUE, directional=FALSE)
```

cbind*Combine RGList, MAList, EList or EListRaw Objects*

Description

Combine a set of RGList, MAList, EList or EListRaw objects.

Usage

```
## S3 method for class 'RGList'
cbind(..., deparse.level=1)
## S3 method for class 'RGList'
rbind(..., deparse.level=1)
```

Arguments

... RGList, MAList, EList or EListRaw objects.
deparse.level not currently used, see [cbind](#) in the base package

Details

cbind combines data objects assuming the same probes in the same order but different arrays. rbind combines data objects assuming equivalent arrays, i.e., the same RNA targets, but different probes.

For cbind, the matrices of expression data from the individual objects are cbinded. The data.frames of target information, if they exist, are rbinded. The combined data object will preserve any additional components or attributes found in the first object to be combined. For rbind, the matrices of expression data are rbinded while the target information, in any, is unchanged.

Value

An [RGList](#), [MAList](#), [EList](#) or [EListRaw](#) object holding data from all the arrays and all genes from the individual objects.

Author(s)

Gordon Smyth

See Also

[cbind](#) in the base package.

[03.ReadingData](#) gives an overview of data input and manipulation functions in LIMMA.

Examples

```
M <- A <- matrix(11:14,4,2)
rownames(M) <- rownames(A) <- c("a","b","c","d")
colnames(M) <- colnames(A) <- c("A1","A2")
MA1 <- new("MAList",list(M=M,A=A))
```

```
M <- A <- matrix(21:24,4,2)
rownames(M) <- rownames(A) <- c("a","b","c","d")
colnames(M) <- colnames(A) <- c("B1","B2")
MA2 <- new("MAList",list(M=M,A=A))
```

```
cbind(MA1,MA2)
```

changeLog

Change Log

Description

Show the most recent changes from a package change log or NEWS file.

Usage

```
changeLog(n = 30, package = "limma")
```

Arguments

| | |
|---------|--|
| n | integer, number of lines to write of change log. |
| package | character string giving name of package. |

Details

The function will look for a file `changeLog.txt` or `ChangeLog` in the top-level or doc directories of the installed package. Failing that, it will look for `NEWS` or `NEWS.md` in the top-level directory.

Note that `changeLog` does not write the content of `NEWS.Rd`, which is a structured file. Use `news(package="limma")` for that instead.

Value

No value is produced, but a number of lines of text are written to standard output.

Author(s)

Gordon Smyth

See Also

[01.Introduction, news.](#)

Examples

```
changeLog()
changeLog(package="statmod")
```

chooseLowessSpan *Choose Span for Local-Weighted Regression Smoothing*

Description

Choose an optimal span, depending on the number of points, for lowess smoothing of variance trends.

Usage

```
chooseLowessSpan(n=1000, small.n=50, min.span=0.3, power=1/3)
```

Arguments

| | |
|----------|--|
| n | the number of points the lowess curve will be applied to. |
| small.n | the span will be set to 1 for any n less than or equal to this value. |
| min.span | the minimum span for large n. |
| power | numeric power between 0 and 1 that determines how fast the chosen span decreases with n. |

Details

The span is the proportion of points used for each of the local regressions. When there are a few points, a large span should be used to ensure a smooth curve. When there are a large number of points, smaller spans can be used because each span window still contains good coverage. By default, the chosen span decreases as the cube-root of the number of points, a rule that is motivated by analogous rules to choose the number of bins for a histogram (Scott, 1979; Freedman & Diaconis, 1981; Hyndman, 1995).

The span returned is $\text{min.span} + (1 - \text{min.span}) * (\text{small.n}/n)^{\text{power}}$ except that the span is set to 1 for any n less than small.n. Note that the fitted lowess curve will still estimate a trend (i.e., will not be constant) even if span=1.

The function is tuned for smoothing of mean-variance trends, for which the trend is usually monotonic, so preference is given to moderately large spans. Even for the very large datasets, the span is always greater than min.span.

This function is used to create adaptive spans for voom, vooma and voomaLmFit where n is the number of genes in the analysis.

Value

A numeric vector of length 1 containing the span value.

Author(s)

Gordon Smyth

References

- Freedman, D. and Diaconis, P. (1981). On the histogram as a density estimator: L₂ theory. *Zeitschrift für Wahrscheinlichkeitstheorie und verwandte Gebiete* 57, 453-476.
- Hyndman, R. J. (1995). The problem with Sturges' rule for constructing histograms. <https://robjhyndman.com/papers/sturges.pdf>.
- Scott, D. W. (1979). On optimal and data-based histograms. *Biometrika* 66, 605-610.

See Also

[loessFit](#), [weightedLowess](#), [lowess](#), [loess](#).
[vooma](#), [eBayes](#), [squeezeVar](#), [fitFDistRobustly](#).

Examples

```
chooseLowessSpan(100)
chooseLowessSpan(1e6)

n <- 10:5000
span <- chooseLowessSpan(n)
plot(n, span, type="l", log="x")
```

classifyTestsF

Genewise Nested F-Tests

Description

For each gene, classify a series of related t-statistics as significantly up or down using nested F-tests.

Usage

```
classifyTestsF(object, cor.matrix = NULL, df = Inf, p.value = 0.01, fstat.only = FALSE)
```

Arguments

| | |
|-------------------------|--|
| <code>object</code> | numeric matrix of t-statistics or an MArrayLM object from which the t-statistics may be extracted. |
| <code>cor.matrix</code> | covariance matrix of each row of t-statistics. Will be extracted automatically from an MArrayLM object but otherwise defaults to the identity matrix. |
| <code>df</code> | numeric vector giving the degrees of freedom for the t-statistics. May have length 1 or length equal to the number of rows of <code>tstat</code> . Will be extracted automatically from an MArrayLM object but otherwise default to <code>Inf</code> . |
| <code>p.value</code> | numeric value between 0 and 1 giving the desired size of the test. |
| <code>fstat.only</code> | logical, if TRUE then return the overall F-statistic as for <code>FStat</code> instead of classifying the test results. |

Details

classifyTestsF implements the "nestedF" multiple testing option offered by `decideTests`. Users should generally use `decideTests` rather than calling `classifyTestsF` directly because, by itself, `classifyTestsF` does not incorporate any multiple testing adjustment across genes. Instead it simply tests across contrasts for each gene individually.

`classifyTestsF` uses a nested F-test approach giving particular attention to correctly classifying genes that have two or more significant t-statistics, i.e., which are differentially expressed in two or more conditions. For each row of `tstat`, the overall F-statistics is constructed from the t-statistics as for `FStat`. At least one contrast will be classified as significant if and only if the overall F-statistic is significant. If the overall F-statistic is significant, then the function makes a best choice as to which t-statistics contributed to this result. The methodology is based on the principle that any t-statistic should be called significant if the F-test is still significant for that row when all the larger t-statistics are set to the same absolute size as the t-statistic in question.

Compared to conventional multiple testing methods, the nested F-test approach achieves better consistency between related contrasts. (For example, if B is judged to be different from C, then at least one of B or C should be different to A.) The approach was first used by Michaud et al (2008). The nested F-test approach provides *weak* control of the family-wise error rate, i.e., it correctly controls the type I error rate of calling any contrast as significant if all the null hypotheses are true. In other words, it provides error rate control at the overall F-test level but does not provide strict error rate control at the individual contrast level.

Usually `object` is a `limma` linear model fitted object, from which a matrix of t-statistics can be extracted, but it can also be a numeric matrix of t-statistics. In either case, rows correspond to genes and columns to coefficients or contrasts. If `object` is a matrix, then it may be necessary to supply values for `cor.matrix` and `df`. The `cor.matrix` is the same as the correlation matrix of the coefficients from which the t-statistics were calculated and `df` is the degrees of freedom of the t-statistics. All statistics for the same gene must have the same degrees of freedom.

If `fstat.only=TRUE`, the `classifyTestsF` just returns the vector of overall F-statistics for each gene.

Value

If `fstat.only=FALSE`, then an object of class `TestResults` is returned. This is essentially a numeric matrix with elements -1, 0 or 1 depending on whether each t-statistic is classified as significantly negative, not significant or significantly positive respectively.

If `fstat.only=TRUE`, then a numeric vector of F-statistics is returned with attributes `df1` and `df2` giving the corresponding degrees of freedom.

Author(s)

Gordon Smyth

References

Michaud, J, Simpson, KM, Escher, R, Buchet-Poyau, K, Beissbarth, T, Carmichael, C, Ritchie, ME, Schutz, F, Cannon, P, Liu, M, Shen, X, Ito, Y, Raskind, WH, Horwitz, MS, Osato, M, Turner, DR, Speed, TP, Kavallaris, M, Smyth, GK, and Scott, HS (2008). Integrative analysis of RUNX1 downstream pathways and target genes. *BMC Genomics* 9, 363.

See Also

An overview of multiple testing functions is given in [08.Tests](#).

Examples

```
TStat <- matrix(c(0,10,0, 0,5,0, -4,-4,4, 2,2,2), 4, 3, byrow=TRUE)
colnames(TStat) <- paste0("Contrast",1:3)
rownames(TStat) <- paste0("Gene",1:4)
classifyTestsF(TStat, df=20)
FStat <- classifyTestsF(TStat, df=20, fstat.only=TRUE)
P <- pf(FStat, df1=attr(FStat,"df1"), df2=attr(FStat,"df2"), lower.tail=FALSE)
data.frame(F.Statistic=FStat,P.Value=P)
```

 contrastAsCoef

Reform a Design Matrix so that Contrasts Become Coefficients

Description

Reform a design matrix so that one or more coefficients from the new matrix correspond to specified contrasts of coefficients from the old matrix.

Usage

```
contrastAsCoef(design, contrast=NULL, first=TRUE)
```

Arguments

| | |
|----------|--|
| design | numeric design matrix. |
| contrast | numeric matrix with rows corresponding to columns of the design matrix (coefficients) and columns containing contrasts. May be a vector if there is only one contrast. |
| first | logical, should coefficients corresponding to contrasts be the first columns (TRUE) or last columns (FALSE) of the output design matrix. |

Details

If the contrasts contained in the columns of `contrast` are not linearly dependent, then superfluous columns are dropped until the remaining matrix has full column rank. The number of retained contrasts is stored in `qr$rank` and the retained columns are given by `qr$pivot`.

Value

A list with components

| | |
|--------|---|
| design | reformed design matrix |
| coef | columns of design matrix which hold the meaningful coefficients |
| qr | QR-decomposition of contrast matrix |

Author(s)

Gordon Smyth

See Also[model.matrix](#) in the stats package.An overview of linear model functions in limma is given by [06.LinearModels](#).**Examples**

```

design <- cbind(1,c(0,0,1,1,0,0),c(0,0,0,0,1,1))
cont <- c(0,-1,1)
design2 <- contrastAsCoef(design, cont)$design

# Original coef[3]-coef[2] becomes coef[1]
y <- rnorm(6)
fit1 <- lm(y~0+design)
fit2 <- lm(y~0+design2)
coef(fit1)
coef(fit1)
coef(fit2)

```

contrasts.fit

Compute Contrasts from Linear Model Fit

Description

Given a linear model fit to microarray data, compute estimated coefficients and standard errors for a given set of contrasts.

Usage

```
contrasts.fit(fit, contrasts=NULL, coefficients=NULL)
```

Arguments

| | |
|---------------------------|--|
| <code>fit</code> | an MArrayLM object or a list object produced by the function <code>lm.series</code> or equivalent. Must contain components <code>coefficients</code> and <code>stdev.unscaled</code> . |
| <code>contrasts</code> | numeric matrix with rows corresponding to coefficients in <code>fit</code> and columns containing contrasts. May be a vector if there is only one contrast. NAs are not allowed. |
| <code>coefficients</code> | vector indicating which coefficients are to be kept in the revised fit object. An alternative way to specify the contrasts. |

Details

This function accepts input from any of the functions `lmFit`, `lm.series`, `mrlm`, `gls.series` or `lmscFit`. The function re-orientates the fitted model object from the coefficients of the original design matrix to any set of contrasts of the original coefficients. The coefficients, unscaled standard deviations and correlation matrix are re-calculated in terms of the contrasts.

The idea of this function is to fit a full-rank model using `lmFit` or equivalent, then use `contrasts.fit` to obtain coefficients and standard errors for any number of contrasts of the coefficients of the original model. Unlike the design matrix input to `lmFit`, which normally has one column for each treatment in the experiment, the matrix `contrasts` may have any number of columns and these are not required to be linearly independent. Methods of assessing differential expression, such as `eBayes` or `classifyTestsF`, can then be applied to fitted model object.

The `coefficients` argument provides a simpler way to specify the contrasts matrix when the desired contrasts are just a subset of the original coefficients.

Value

An list object of the same class as `fit`, usually `MArrayLM`. This is a list with components

| | |
|-------------------------------|---|
| <code>coefficients</code> | numeric matrix containing the estimated coefficients for each contrast for each probe. |
| <code>stdev.unscaled</code> | numeric matrix conformal with <code>coef</code> containing the unscaled standard deviations for the coefficient estimators. |
| <code>cov.coefficients</code> | numeric matrix giving the unscaled covariance matrix of the estimable coefficients. |

Most other components found in `fit` are passed through unchanged, but `t`, `p.value`, `lods`, `F` and `F.p.value` will all be removed.

Note

For efficiency reasons, this function does not re-factorize the design matrix for each probe. A consequence is that, if the design matrix is non-orthogonal and the original fit included precision weights or missing values, then the unscaled standard deviations produced by this function are approximate rather than exact. The approximation is usually acceptable. If not, then the issue can be avoided by redefining the design matrix to fit the contrasts directly.

Even with precision weights or missing values, the results from `contrasts.fit` are always exact if the coefficients being compared are statistically independent. This will be true, for example, if the original fit was a oneway model without blocking and the group-means (no-intercept) parametrization was used for the design matrix.

Author(s)

Gordon Smyth

See Also

An overview of linear model functions in `limma` is given by [06.LinearModels](#).

Examples

```
# Simulate gene expression data: 6 microarrays and 100 genes
# with one gene differentially expressed in first 3 arrays
M <- matrix(rnorm(100*6,sd=0.3),100,6)
M[1,1:3] <- M[1,1:3] + 2
# Design matrix corresponds to oneway layout, columns are orthogonal
design <- cbind(First3Arrays=c(1,1,1,0,0,0),Last3Arrays=c(0,0,0,1,1,1))
fit <- lmFit(M,design=design)
# Would like to consider original two estimates plus difference between first 3 and last 3 arrays
contrast.matrix <- cbind(First3=c(1,0),Last3=c(0,1),"Last3-First3"=c(-1,1))
fit2 <- contrasts.fit(fit,contrast.matrix)
fit2 <- eBayes(fit2)
# Large values of eb$t indicate differential expression
results <- decideTests(fit2, method="nestedF")
vennCounts(results)
```

controlStatus

Set Status of each Spot from List of Spot Types

Description

Determine the type (or status) of each spot in the gene list.

Usage

```
controlStatus(types, genes, spottypecol="SpotType", regexpcol, verbose=TRUE)
```

Arguments

| | |
|-------------|--|
| types | dataframe containing spot type specifiers, usually input using readSpotTypes. |
| genes | dataframe containing gene annotation, or an object of class RGList, MAList, EListRaw, EList or MArrayLM from which the gene annotation can be extracted. |
| spottypecol | integer or name specifying column of types containing spot type names. |
| regexpcol | vector of integers or column names specifying columns of types containing regular expressions. Defaults to any column names in common between types and genes. |
| verbose | logical, if TRUE then progress on pattern matching is reported to the standard output channel. |

Details

This function constructs a vector of status codes by searching for patterns in the gene list. The data frame genes contains gene IDs and should have as many rows as there are spots on the microarrays. Such a data frame is often read using readGAL. The data frame types has as many rows as you want to distinguish types of spots in the gene list. This data frame should contain a column or columns, the regexpcol columns, which have the same names as columns in genes and which contain patterns to match in the gene list. Another column, the spottypecol, contains the names

of the spot types. Any other columns are assumed to contain plotting parameters, such as colors or symbols, to be associated with the spot types.

The patterns in the `regexpcol` columns are simplified regular expressions. For example, `AA*` means any string starting with `AA`, `*AA` means any code ending with `AA`, `AA` means exactly these two letters, `*AA*` means any string containing `AA`, `AA.` means `AA` followed by exactly one other character and `AA\.` means exactly `AA` followed by a period and no other characters. Any other regular expressions are allowed but the codes `^` for beginning of string and `$` for end of string should not be included.

Note that the patterns are matched sequentially from first to last, so more general patterns should be included first. For example, it is often a good idea to include a default spot-type as the first line in types with pattern `*` for all `regexpcol` columns and default plotting parameters.

Value

Character vector specifying the type (or status) of each spot on the array. Attributes contain plotting parameters associated with each spot type.

Author(s)

Gordon Smyth

See Also

An overview of LIMMA functions for reading data is given in [03.ReadingData](#).

Examples

```
genes <- data.frame(
  ID=c("Control", "Control", "Control", "Control", "AA1", "AA2", "AA3", "AA4"),
  Name=c("Ratio 1", "Ratio 2", "House keeping 1", "House keeping 2",
        "Gene 1", "Gene 2", "Gene 3", "Gene 4"))
types <- data.frame(
  SpotType=c("Gene", "Ratio", "Housekeeping"),
  ID=c("*", "Control", "Control"),
  Name=c("*", "Ratio*", "House keeping*"),
  col=c("black", "red", "blue"))
status <- controlStatus(types, genes)
```

coolmap

Heatmap of gene expression values

Description

Create a heatmap of a matrix of log-expression values.

Usage

```
coolmap(x, cluster.by="de pattern", col=NULL,
        linkage.row="complete", linkage.col="complete", show.dendrogram="both", ...)
```

Arguments

| | |
|------------------------------|---|
| <code>x</code> | any data object that can be coerced to a matrix of log-expression values, for example an ExpressionSet or EList. Rows represent genes and columns represent RNA samples. |
| <code>cluster.by</code> | choices are "de pattern" or "expression level". In the former case, the intention is to cluster by relative changes in expression, so genes are clustered by Pearson correlation and log-expression values are mean-corrected by rows for the plot. In the latter case, the intention is to cluster by absolute expression, so genes are clustered by Euclidean and log-expression values are not mean-corrected. |
| <code>col</code> | character vector specifying the color panel. Can be either the name of the panel or a vector of R colors that can be passed directly to the heatmap.2 function. Possible panel names are "redblue", "redgreen", "yellowblue" or "whitered". Defaults to "redblue" if cluster.by="de pattern" or "yellowblue" if cluster.by="expression level". |
| <code>linkage.row</code> | linkage criterion used to cluster the rows. Choices are "none", "ward", "ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median" or "centroid", with "ward" treated as "ward.D2". |
| <code>linkage.col</code> | linkage criterion used to cluster the columns. Choices are the same as for linkage.row. |
| <code>show.dendrogram</code> | choices are "row", "column", "both" or "none". |
| <code>...</code> | any other arguments are passed to heatmap.2. See details for which arguments are reserved. |

Details

This function calls the heatmap.2 function in the gplots package with sensible argument settings for genomic log-expression data. The default settings for heatmap.2 are often not ideal for expression data, and overriding the defaults requires explicit calls to hclust and as.dendrogram as well as prior standardization of the data values. The coolmap function implements our preferred defaults for the two most common types of heatmaps. When clustering by relative expression (cluster.by="de pattern"), it implements a row standardization that takes account of NA values and standard deviations that might be zero.

coolmap sets the following heatmap.2 arguments internally: Rowv, Colv, scale, density.info, trace, col, symbreaks, symkey, dendrogram, key.title and key.xlab. These arguments are therefore reserved and cannot be varied. Other than these reserved arguments, any other heatmap.2 argument can be included in the coolmap call, thereby giving full access to heatmap.2 functionality.

Value

A plot is created on the current graphics device. A list is also invisibly returned, see heatmap.2 for details.

Author(s)

Gordon Smyth

See Also

[heatmap.2](#), [hclust](#), [dist](#).

An overview of diagnostic functions available in LIMMA is given in [09.Diagnostics](#).

Examples

```
# Simulate gene expression data for 50 genes and 6 microarrays.
# Samples are in two groups
# First 50 probes are differentially expressed in second group
ngenes <- 50
sd <- 0.3*sqrt(4/rchisq(ngenes,df=4))
x <- matrix(rnorm(ngenes*6,sd=sd),ngenes,6)
rownames(x) <- paste("Gene",1:ngenes)
x <- x + seq(from=0, to=16, length=ngenes)
x[,4:6] <- x[,4:6] + 2
coolmap(x)
```

cumOverlap

Cumulative Overlap Analysis of Ordered Lists

Description

Test whether the leading members of ordered lists significantly overlap.

Usage

```
cumOverlap(ol1, ol2)
```

Arguments

| | |
|-----|--|
| ol1 | vector containing first ordered list. Duplicate values not allowed. |
| ol2 | vector containing second ordered list. Should contain the same values as found in ol1 but in a possibly different order. Duplicate values not allowed. |

Details

The function compares the top n members of each list, for every possible n , and conducts a hypergeometric test for overlap. The function returns the value of n giving the smallest p -value.

The p -values are adjusted for multiple testing in a similar way to Bonferroni's method, but starting from the top of the ranked list instead of from the smallest p -values. This approach is designed to be sensitive to contexts where the number of Ids involved in the significant overlap are a small proportion of the total.

The vectors `ol1` and `ol2` do not need to be of the same length, but only values in common between the two vectors will be used in the calculation.

This method was described in Chapter 4 of Wu (2011).

Value

List containing the following components:

| | |
|-------------|---|
| n.total | integer, total number of values in common between o11 and o12. |
| n.min | integer, top table length leading to smallest adjusted p-value. |
| p.min | smallest adjusted p-value. |
| n.overlap | integer, number of overlapping IDs in first n.min. |
| id.overlap | vector giving the overlapping IDs in first n.min. |
| p.value | numeric, vector of p-values for each possible top table length. |
| adj.p.value | numeric, vector of Bonferroni adjusted p-values for each possible top table length. |

Author(s)

Gordon Smyth and Di Wu

References

Wu, D (2011). Finding hidden relationships between gene expression profiles with application to breast cancer biology. PhD thesis, University of Melbourne. <http://hdl.handle.net/11343/36278>

Examples

```
GeneIds <- paste0("Gene", 1:50)
o11 <- GeneIds
o12 <- c(sample(GeneIds[1:5]), sample(GeneIds[6:50]))
coa <- cumOverlap(o11, o12)
coa$p.min
coa$id.overlap
```

decideTests

Multiple Testing Across Genes and Contrasts

Description

Identify which genes are significantly differentially expressed for each contrast from a fit object containing p-values and test statistics. A number of different multiple testing strategies are offered that adjust for multiple testing down the genes as well as across contrasts for each gene.

Usage

```
## S3 method for class 'MArrayLM'
decideTests(object, method = "separate", adjust.method = "BH", p.value = 0.05,
            lfc = 0, ...)
## Default S3 method:
decideTests(object, method = "separate", adjust.method = "BH", p.value = 0.05,
            lfc = 0, coefficients = NULL, cor.matrix = NULL, tstat = NULL, df = Inf,
            genewise.p.value = NULL, ...)
```

Arguments

| | |
|-------------------------------|---|
| <code>object</code> | a numeric matrix of p-values or an <code>MArrayLM</code> object from which p-values and t-statistics can be extracted. |
| <code>method</code> | character string specifying how genes and contrasts are to be combined in the multiple testing scheme. Choices are "separate", "global", "hierarchical" or "nestedF". |
| <code>adjust.method</code> | character string specifying p-value adjustment method. Possible values are "none", "BH", "fdr" (equivalent to "BH"), "BY" and "holm". See p.adjust for details. |
| <code>p.value</code> | numeric value between 0 and 1 giving the required family-wise error rate or false discovery rate. |
| <code>lfc</code> | numeric, minimum absolute log2-fold-change required. |
| <code>coefficients</code> | numeric matrix of coefficients or log2-fold-changes. Of same dimensions as <code>object</code> . |
| <code>cor.matrix</code> | correlation matrix of coefficients. Square matrix of dimension <code>ncol(object)</code> . |
| <code>tstat</code> | numeric matrix of t-statistics. Of same dimensions as <code>object</code> . |
| <code>df</code> | numeric vector of length <code>nrow(object)</code> giving degrees of freedom for the t-statistics. |
| <code>genewise.p.value</code> | numeric vector of length <code>nrow(object)</code> containing summary gene-level p-values for use with <code>method="hierarchical"</code> . |
| <code>...</code> | other arguments are not used. |

Details

This function can be applied to a matrix of p-values but is more often applied to an `MArrayLM` fit object produced by `eBayes` or `treat`. In either case, rows of `object` correspond to genes and columns to coefficients or contrasts.

This function applies a multiple testing procedure and a significance level cutoff to the statistics contained in `object`. It implements a number of multiple testing procedures for determining whether each statistic should be considered significantly different from zero.

`method="separate"` will apply multiple testing adjustments to each column of p-values separately. Setting `method="separate"` is equivalent to using `topTable` separately for each coefficient in the linear model fit and will identify the same probes as significantly differentially expressed if `adjust.method` is the same. `method="global"` will treat the entire matrix of t-statistics as a single vector of unrelated tests. `method="hierarchical"` adjusts down genes and then across contrasts. `method="nestedF"` adjusts down genes according to overall F-tests and then uses `classifyTestsF` to classify contrasts as significant or not for the selected genes.

The default `method="separate"` and `adjust.method="BH"` settings are appropriate for most analyses. `method="global"` is useful when it is important that the same t-statistic cutoff should correspond to statistical significance for all the contrasts. The "nestedF" method was proposed by Michaud et al (2008) and achieves better consistency between contrasts than the other methods. It provides formal error rate control at the gene level but not for individual contrasts. See the [classifyTestsF](#) help page for more detail about the "nestedF" method.

If `object` is a `MArrayLM` linear model fit, then the "hierarchical" method conducts row-wise F-tests and then proceeds to t-tests for those rows with significant F-tests. The multiple testing

adjustment is applied initially to the F-tests and then, with an adjusted level, to the t-tests for each significant row.

Also see the limma User's Guide for a discussion of the statistical properties of the various adjustment methods.

Value

An object of class `TestResults`. This is essentially a numeric matrix with elements -1, 0 or 1 depending on whether each t-statistic is classified as significantly negative, not significant or significantly positive.

If $lfc > 0$ then contrasts are judged significant only when the log₂-fold change is at least this large in absolute value. For example, one might choose $lfc = \log_2(1.5)$ to restrict to 50% changes or $lfc = 1$ for 2-fold changes. In this case, contrasts must satisfy both the p-value and the fold-change cutoff to be judged significant.

Note

Although this function enables users to set p-value and lfc cutoffs simultaneously, this combination criterion is not recommended. logFC cutoffs tend to favor low expressed genes and thereby reduce rather than increase biological significance. Unless the fold changes and p-values are very highly correlated, the addition of a fold change cutoff can increase the family-wise error rate or false discovery rate above the nominal level. Users wanting to use fold change thresholding are recommended to use `treat` instead of `eBayes` and to leave `lfc` at the default value when using `decideTests`.

Author(s)

Gordon Smyth

References

Michaud J, Simpson KM, Escher R, Buchet-Poyau K, Beissbarth T, Carmichael C, Ritchie ME, Schutz F, Cannon P, Liu M, Shen X, Ito Y, Raskind WH, Horwitz MS, Osato M, Turner DR, Speed TP, Kavallaris M, Smyth GK, Scott HS (2008). Integrative analysis of RUNX1 downstream pathways and target genes. *BMC Genomics* 9, 363. doi:10.1186/147121649363

See Also

An overview of multiple testing functions is given in [08.Tests](#).

designI2M

Convert Individual Channel Design Matrix to M-A Format

Description

Convert a design matrix in terms of individual channels to ones in terms of M-values or A-values for two-color microarray data.

Usage

```
designI2M(design)  
designI2A(design)
```

Arguments

design numeric model matrix with one row for each channel observation, i.e., twice as many rows as arrays

Details

If `design` is a model matrix suitable for modelling individual log-intensities for two color microarray data, then `designI2M` computes the corresponding model matrix for modelling M-values (log-ratios) and `designI2A` computes the model matrix for modelling A-values (average log-intensities).

Note that the matrices `designI2M(design)` or `designI2A(design)` may be singular if not all of the coefficients are estimable from the M or A-values. In that case there will be columns containing entirely zeros.

Value

numeric model matrix with half as many rows as `design`

Author(s)

Gordon Smyth

See Also

[model.matrix](#) in the stats package.

An overview of individual channel linear model functions in limma is given by [07.SingleChannel](#).

Examples

```
X <- cbind(1,c(1,1,1,1,0,0,0,0),c(0,0,0,0,1,1,1,1))  
designI2M(X)  
designI2A(X)
```

detectionPValues

Detection P-Values from Negative Controls

Description

Compute the proportion of negative controls greater than each observed expression value. Particularly useful for Illumina BeadChips.

Usage

```
## S3 method for class 'EListRaw'
detectionPValues(x, status = NULL, ...)
## Default S3 method:
detectionPValues(x, status, negctrl = "negative", ...)
```

Arguments

| | |
|---------|--|
| x | object of class EListRaw or a numeric matrix containing raw intensities for regular and control probes from a series of microarrays. |
| status | character vector giving probe types. Defaults to x\$genes\$Status if x is an EListRaw object. |
| negctrl | character string identifier for negative control probes. |
| ... | other arguments are not currently used. |

Details

The rows of x for which status == negctrl are assumed to correspond to negative control probes.

For each column of x, the detection p-values are defined as $(N.eq/2 + N.gt) / N.neg$, where N.gt is the number of negative controls with expression greater than the observed value, N.eq is the number of negative controls with expression equal to the observed value, and N.neg is the total number of negative controls.

When used on Illumina BeadChip data, this function produces essentially the same detection p-values as returned by Illumina's GenomeStudio software.

Value

numeric matrix of same dimensions as x containing detection p-values.

Author(s)

Gordon Smyth

References

Shi W, de Graaf C, Kinkel S, Achtman A, Baldwin T, Schofield L, Scott H, Hilton D, Smyth GK (2010). Estimating the proportion of microarray probes expressed in an RNA sample. *Nucleic Acids Research* 38(7), 2168-2176. doi:10.1093/nar/gkp1204

See Also

An overview of LIMMA functions to read expression data is given in [03.ReadingData](#).

[read.idat](#) reads Illumina BeadChip expression data from binary IDAT files.

[neqc](#) performs normexp background correction and quantile normalization aided by control probes.

Examples

```
## Not run:
# Read Illumina binary IDAT files
x <- read.idat(idat, bgx)
x$other$Detection <- detectionPValues(x)
y <- neqc(x)

## End(Not run)
```

diffSplice *Test for Differential Splicing*

Description

Given a linear model fit at the exon (transcript) level, test for differences in exon (transcript) usage between experimental conditions.

Usage

```
## S3 method for class 'MArrayLM'
diffSplice(fit, geneid, exonid = NULL,
           robust = FALSE, legacy = FALSE, verbose = TRUE, ...)
```

Arguments

| | |
|---------|--|
| fit | an MArrayLM fitted model object produced by <code>lmFit</code> or <code>contrasts.fit</code> . Rows should correspond to exons, exon-exon junctions or transcripts. |
| geneid | gene identifiers. Either a vector of length <code>nrow(fit)</code> or the name of the column of <code>fit\$genes</code> containing the gene identifiers. Rows with the same ID are assumed to belong to the same gene. |
| exonid | exon identifiers. Either a vector of length <code>nrow(fit)</code> or the name of the column of <code>fit\$genes</code> containing the exon identifiers. |
| robust | logical, should the estimation of the empirical Bayes prior parameters be robustified against outlier sample variances? |
| legacy | logical. If FALSE then the new empirical Bayes hyperparameter estimation (introduced in <code>limma</code> 3.61.8) will be used, if TRUE the earlier hyperparameter estimation will be used. The new method is particularly appropriate when the residual degrees of freedom are not all equal, which is likely to be the case for <code>diffSplice</code> . |
| verbose | logical, if TRUE some diagnostic information about the number of genes and exons is output. |
| ... | other arguments are not currently used. |

Details

This function tests for differential usage of the row-wise genomic features for each gene and for each column of `fit`. The genomic features are usually transcripts, exons, or exon-exon junctions.

Testing for differential exon (transcript) usage is equivalent to testing whether the log-fold-changes in the `fit` differ between exons for the same gene. Two different tests are provided. The first is a F-test for differences between the log-fold-changes for each gene. This is equivalent to testing for interaction between the exons for that gene and the coefficient of the linear model. The other is a series of t-tests in which each exon is compared to the weighted average of all other exons for the same gene. The exon-level t-tests are converted into genewise tests by adjusting the p-values for the same gene by Simes method. Alternatively, the exon-level t-tests are also converted into genewise tests by adjusting the smallest p-value for each gene by Bonferroni's method.

This function can be used on data from an exon microarray or can be used in conjunction with `voom` or `voomLmFit` for exon-level RNA-seq counts.

Value

An object of class `MArrayLM` containing both exon level and gene level tests. Results are sorted by `geneid` and by `exonid` within gene.

| | |
|--------------------------------------|--|
| <code>coefficients</code> | numeric matrix of coefficients of same dimensions as <code>fit</code> . Each coefficient is the difference between the log-fold-change for that exon versus the average log-fold-change for all other exons for the same gene. |
| <code>t</code> | numeric matrix of moderated t-statistics, of same dimensions as <code>fit</code> . |
| <code>p.value</code> | numeric vector of p-values corresponding to the t-statistics |
| <code>genes</code> | data.frame of exon annotation |
| <code>geneColname</code> | character string giving the name of the column of genes containing gene IDs |
| <code>gene.F</code> | numeric matrix of moderated F-statistics, one row for each gene. |
| <code>gene.F.p.value</code> | numeric matrix of p-values corresponding to <code>gene.F</code> |
| <code>gene.simes.p.value</code> | numeric matrix of Simes adjusted p-values, one row for each gene. |
| <code>gene.bonferroni.p.value</code> | numeric matrix of Bonferroni adjusted p-values, one row for each gene. |
| <code>gene.genes</code> | data.frame of gene annotation. |

Author(s)

Gordon Smyth and Charity Law

See Also

[topSplice](#), [plotSplice](#)

A summary of functions available in LIMMA for RNA-seq analysis is given in [11.RNAseq](#).

Examples

```
## Not run:
# Consider using edgeR::voomLmFit() instead of voom() and lmFit()
v <- voom(dge,design)
fit <- lmFit(v,design)
ex <- diffSplice(fit, geneid="EntrezID")
topSplice(ex)
plotSplice(ex)

## End(Not run)
```

dim

Retrieve the Dimensions of an RGList, MAList or MArrayLM Object

Description

Retrieve the number of rows (genes) and columns (arrays) for an RGList, MAList or MArrayLM object.

Usage

```
## S3 method for class 'RGList'
dim(x)
```

Arguments

x an object of class RGList, MAList or MArrayLM

Details

Microarray data objects share many analogies with ordinary matrices in which the rows correspond to spots or genes and the columns to arrays. These methods allow one to extract the size of microarray data objects in the same way that one would do for ordinary matrices.

A consequence is that row and column commands `nrow(x)`, `ncol(x)` and so on also work.

Value

Numeric vector of length 2. The first element is the number of rows (genes) and the second is the number of columns (arrays).

Author(s)

Gordon Smyth

See Also

[dim](#) in the base package.

[02.Classes](#) gives an overview of data classes used in LIMMA.

Examples

```
M <- A <- matrix(11:14,4,2)
rownames(M) <- rownames(A) <- c("a","b","c","d")
colnames(M) <- colnames(A) <- c("A1","A2")
MA <- new("MAList",list(M=M,A=A))
dim(M)
ncol(M)
nrow(M)
```

| | |
|----------|--|
| dimnames | <i>Retrieve the Dimension Names of an RGList, MAList, EList, EListRaw or MArrayLM Object</i> |
|----------|--|

Description

Retrieve the dimension names of a microarray data object.

Usage

```
## S3 method for class 'RGList'
dimnames(x)
## S3 replacement method for class 'RGList'
dimnames(x) <- value
```

Arguments

| | |
|-------|---|
| x | an object of class RGList, MAList, EList, EListRaw or (not for assignment) MArrayLM |
| value | a possible value for dimnames(x): see dimnames |

Details

The dimension names of a microarray object are the same as those of the most important matrix component of that object.

A consequence is that rownames and colnames will work as expected.

Value

Either NULL or a list of length 2. If a list, its components are either NULL or a character vector the length of the appropriate dimension of x.

Author(s)

Gordon Smyth

See Also

[dimnames](#) in the base package.

[02.Classes](#) gives an overview of data classes used in LIMMA.

dupcor

Correlation Between Duplicates or Within Blocks

Description

Estimate the intra-block correlation given a block structure for the arrays or samples.

Usage

```
duplicateCorrelation(object, design=NULL, ndups=2, spacing=1, block=NULL,
                    trim=0.15, weights=NULL)
```

Arguments

| | |
|---------|---|
| object | A matrix-like data object containing log-ratios or log-expression values for a series of samples, with rows corresponding to genes and columns to samples. Any type of data object that can be processed by getEAWP is acceptable. |
| design | the design matrix of the microarray experiment, with rows corresponding to arrays and columns to comparisons to be estimated. The number of rows must match the number of columns of object. Defaults to the unit vector meaning that the arrays are treated as replicates. |
| ndups | a positive integer giving the number of times each gene is printed on an array. <code>nrow(object)</code> must be divisible by <code>ndups</code> . Ignored if <code>block</code> is specified. |
| spacing | the spacing between the rows of object corresponding to duplicate spots, <code>spacing=1</code> for consecutive spots |
| block | vector or factor specifying a blocking variable |
| trim | the fraction of observations to be trimmed from each end of <code>tanh(all.correlations)</code> when computing the trimmed mean. |
| weights | an optional numeric matrix of the same dimension as object containing weights for each spot. If smaller than object then it will be filled out to the same size. |

Details

When `block=NULL`, this function estimates the correlation between duplicate spots (regularly spaced within-array replicate spots). If `block` is not null, this function estimates the correlation between repeated observations on the blocking variable. Typically the blocks are biological replicates and repeated observations on the same block may be correlated. In either case, the correlation is estimated by fitting a mixed linear model by REML individually for each gene. The function also returns a consensus correlation, which is a robust average of the individual correlations, intended for input to functions such as `lmFit`, `gls.series` or `voom`.

It is not possible to estimate correlations between duplicate spots and with sample blocks simultaneously. If `block` is not null, then the function will set `ndups=1`, which is equivalent to ignoring duplicate spots.

For this function to return statistically useful results, there must be at least two more arrays than the number of coefficients to be estimated, i.e., two more than the column rank of design.

The function may take long time to execute as it fits a mixed linear model for each gene using an iterative algorithm.

If present, `ndups` and `spacing` will be extracted from `object$printer$ndups` and `object$printer$spacing`.

Value

A list with components

`consensus.correlation`

the average estimated inter-duplicate correlation. The average is the trimmed mean of the individual correlations on the `atanh`-transformed scale.

`cor`

same as `consensus.correlation`, for compatibility with earlier versions of the software

`atanh.correlations`

numeric vector of length `nrow(object)/ndups` giving the individual genewise `atanh`-transformed correlations.

Author(s)

Gordon Smyth

References

Smyth, G. K., Michaud, J., and Scott, H. (2005). The use of within-array replicate spots for assessing differential expression in microarray experiments. *Bioinformatics* 21(9), 2067-2075. [<http://bioinformatics.oxfordjournals.org/content/21/9/2067>] [Preprint with corrections: <https://gksmyth.github.io/pubs/dupcor.pdf>]

See Also

These functions use `mixedModel2Fit` from the `statmod` package.

An overview of linear model functions in `limma` is given by [06.LinearModels](#).

Examples

```
# Simulate a paired experiment with incomplete blocks
Block <- c(1,1,2,2,3,3,4,4,5,6,7,8)
Treat <- factor(c(1,2,1,2,1,2,1,2,1,2,1,2))
design <- model.matrix(~Treat)

ngenes <- 50
nsamples <- 12
y <- matrix(rnorm(ngenes*nsamples),ngenes,nsamples)
rownames(y) <- paste0("Gene",1:ngenes)
```

```
# Estimate the within-block correlation
dupcor <- duplicateCorrelation(y,design,block=Block)
dupcor$consensus.correlation

# Estimate the treatment effect using both complete and incomplete blocks
fit <- lmFit(y,design,block=Block,correlation=dupcor$consensus)
fit <- eBayes(fit)
topTable(fit,coef=2)
```

eBayes

*Empirical Bayes Statistics for Differential Expression***Description**

Given a linear model fit from `lmFit`, compute moderated t-statistics, moderated F-statistic, and log-odds of differential expression by empirical Bayes moderation of the standard errors towards a global value.

Usage

```
eBayes(fit, proportion = 0.01, stdev.coef.lim = c(0.1,4),
       trend = FALSE, robust = FALSE, winsor.tail.p = c(0.05,0.1), legacy = NULL)
treat(fit, fc = 1.2, lfc = NULL, trend = FALSE,
      robust = FALSE, winsor.tail.p = c(0.05,0.1), legacy = NULL)
```

Arguments

| | |
|-----------------------------|--|
| <code>fit</code> | an MArrayLM fitted model object produced by <code>lmFit</code> or <code>contrasts.fit</code> . For <code>eBayes</code> only, <code>fit</code> can alternatively be an unclassed list produced by <code>lm.series</code> , <code>gls.series</code> or <code>mr1m</code> containing components <code>coefficients</code> , <code>stdev.unscaled</code> , <code>sigma</code> and <code>df.residual</code> . |
| <code>proportion</code> | numeric value between 0 and 1, assumed proportion of genes which are differentially expressed |
| <code>stdev.coef.lim</code> | numeric vector of length 2, assumed lower and upper limits for the standard deviation of log ₂ -fold-changes for differentially expressed genes |
| <code>trend</code> | logical, should an intensity-dependent trend be allowed for the prior variance? If FALSE then the prior variance is constant. Alternatively, <code>trend</code> can be a row-wise numeric vector, which will be used as the covariate for the prior variance. |
| <code>robust</code> | logical, should the estimation of <code>df.prior</code> and <code>var.prior</code> be robustified against outlier sample variances? |
| <code>winsor.tail.p</code> | numeric vector of length 1 or 2, giving left and right tail proportions of <code>x</code> to Winsorize (if <code>robust=TRUE</code> and <code>legacy=TRUE</code>). |
| <code>legacy</code> | logical. If FALSE then the new hyperparameter estimation (introduced in <code>limma</code> 3.61.8) will be used, if TRUE the earlier hyperparameter estimation will be used. If NULL, then the new method will be used when the residual degrees of freedom are not all equal and the old method will be used otherwise. |

| | |
|------------------|--|
| <code>fc</code> | minimum fold-change below which changes are not considered scientifically meaningful. |
| <code>lfc</code> | minimum log ₂ -fold-change below which changes not considered scientifically meaningful. Defaults to $\log_2(fc)$. If specified then takes precedence over <code>fc</code> . |

Details

These functions are used to rank genes in order of evidence for differential expression. They use an empirical Bayes method to squeeze the genewise-wise residual variances towards a common value (or towards a global trend) (Smyth, 2004; Phipson et al, 2016). The degrees of freedom for the individual variances are increased to reflect the extra information gained from the empirical Bayes moderation, resulting in increased statistical power to detect differential expression.

These functions accept as input an `MArrayLM` fitted model object `fit` produced by `lmFit`. The columns of `fit` define a set of contrasts which are to be tested equal to zero. The fitted model object may have been processed by `contrasts.fit` before being passed to `eBayes` to convert the coefficients of the original design matrix into an arbitrary number of contrasts.

The empirical Bayes moderated t-statistics test each individual contrast equal to zero. For each gene (row), the moderated F-statistic tests whether all the contrasts are zero. The F-statistic is an overall test computed from the set of t-statistics for that probe. This is exactly analogous the relationship between t-tests and F-statistics in conventional anova, except that the residual mean squares have been moderated between genes.

The estimates `s2.prior` and `df.prior` are computed by one of `fitFDist`, `fitFDistRobustly` or `fitFDistUnequalDF1` (depending on settings for robust and legacy). `s2.post` is the weighted average of `s2.prior` and σ^2 with weights proportional to `df.prior` and `df.residual` respectively. The log-odds of differential expression `lods` was called the *B-statistic* by Loennstedt and Speed (2002). The F-statistics `F` are computed by `classifyTestsF` with `fstat.only=TRUE`.

`eBayes` does not compute ordinary t-statistics because they always have worse performance than the moderated versions. The ordinary (unmoderated) t-statistics can, however, be easily extracted from the linear model output for comparison purposes—see the example code below.

`treat` computes empirical Bayes moderated-t p-values relative to a minimum fold-change threshold. Instead of testing for genes that have true log-fold-changes different from zero, it tests whether the true log₂-fold-change is greater than `lfc` in absolute value (McCarthy and Smyth, 2009). In other words, it uses an interval null hypothesis, where the interval is $[-lfc, lfc]$. When the number of DE genes is large, `treat` is often useful for giving preference to larger fold-changes and for prioritizing genes that are biologically important. `treat` is concerned with p-values rather than posterior odds, so it does not compute the B-statistic `lods`. The idea of thresholding doesn't apply to F-statistics in a straightforward way, so moderated F-statistics are also not computed. When `fc=1` and `lfc=0`, `treat` is identical to `eBayes`, except that F-statistics and B-statistics are not computed. The `fc` threshold is usually chosen relatively small, because genes need to have fold changes substantially greater than the testing threshold in order to be considered statistically significant. Typical values for `fc` are 1.1, 1.2 or 1.5. The top genes chosen by `treat` can be examined using [topTreat](#).

The `treat` threshold can be specified either as a fold-change via `fc` or as a log₂-fold-change via `lfc`, with $lfc = \log_2(fc)$. Note that the `treat` testing procedure is considerably more rigorous and conservative than simply applying same `fc` values as a fold-change cutoff to the list of differentially expressed genes. Indeed, the observed log₂-fold-change needs to be substantially larger than `lfc` for a gene to be called as statistically significant by `treat`. The threshold should be chosen as a small value below which results should be ignored rather than as a target fold-change. In practice,

modest values for `fc` such as 1.1, 1.2 or 1.5 are usually the most useful. Setting `fc=1.2` or `fc=1.5` will usually cause most differentially expressed genes to have estimated fold-changes of 2-fold or greater, depending on the sample size and precision of the experiment. Larger thresholds are usually overly conservative and counter productive. In general, the `fc` threshold should be chosen sufficiently small so that a worthwhile number of DE genes remain, otherwise the purpose of prioritizing genes with larger fold-changes will be defeated.

The use of `eBayes` or `treat` with `trend=TRUE` is known as the *limma-trend* method (Law et al, 2014; Phipson et al, 2016). With this option, an intensity-dependent trend is fitted to the prior variances `s2.prior`. Specifically, `squeezeVar` is called with the covariate equal to `Amean`, the average log₂-intensity for each gene. The trend that is fitted can be examined by `plotSA`. *limma-trend* is useful for processing expression values that show a mean-variance relationship. This is often useful for microarray data, and it can also be applied to RNA-seq counts that have been converted to log₂-counts per million (logCPM) values (Law et al, 2014). When applied to RNA-seq logCPM values, *limma-trend* give similar results to the `voom` method. The `voom` method incorporates the mean-variance trend into the precision weights, whereas *limma-trend* incorporates the trend into the empirical Bayes moderation. *limma-trend* is somewhat simpler than `voom` because it assumes that the sequencing depths (library sizes) are not wildly different between the samples and it applies the mean-variance trend on a gene-wise basis instead to individual observations. *limma-trend* is recommended for RNA-seq analysis when the library sizes are reasonably consistent (less than 3-fold difference from smallest to largest) because of its simplicity and speed.

If `robust=TRUE` then the robust empirical Bayes procedure of Phipson et al (2016) is used. This is frequently useful to protect the empirical Bayes procedure against hyper-variable or hypo-variable genes, especially when analysing RNA-seq data. See `squeezeVar` for more details.

In *limma* 3.61.8 (August 2024), the new function `fitFDistUnequalDF1` was introduced to improve estimation of the hyperparameters `s2.prior` and `df.prior`, especially when not all genes have the same residual degrees of freedom. `fitFDistUnequalDF1` is a potential replacement for the original functions `fitFDist` and `fitFDistRobustly` and the argument `legacy` is provided to control backward compatibility. The new hyperparameter estimation will be used if `legacy=FALSE` and the original methods will be used if `legacy=TRUE`. If `legacy=NULL`, then the new method will be used if the residual degrees of freedom are unequal and the original methods otherwise. Unequal residual degrees of freedom arise in *limma* pipelines when the expression matrix includes missing values or from the quasi-likelihood pipeline in *edgeR* v4.

Value

`eBayes` produces an object of class `MArrayLM` (see [MArrayLM-class](#)) containing everything found in `fit` plus the following added components:

| | |
|-----------------------|--|
| <code>t</code> | numeric matrix of moderated t-statistics. |
| <code>p.value</code> | numeric matrix of two-sided p-values corresponding to the t-statistics. |
| <code>lods</code> | numeric matrix giving the log-odds of differential expression (on the natural log scale). |
| <code>s2.prior</code> | estimated prior value for σ^2 . A row-wise vector if <code>covariate</code> is non-NULL, otherwise a single value. |
| <code>df.prior</code> | degrees of freedom associated with <code>s2.prior</code> . A row-wise vector if <code>robust=TRUE</code> , otherwise a single value. |

| | |
|------------------------|---|
| <code>df.total</code> | row-wise numeric vector giving the total degrees of freedom associated with the t-statistics for each gene. Equal to <code>df.prior+df.residual</code> or <code>sum(df.residual)</code> , whichever is smaller. |
| <code>s2.post</code> | row-wise numeric vector giving the posterior values for σ^2 . |
| <code>var.prior</code> | column-wise numeric vector giving estimated prior values for the variance of the log ₂ -fold-changes for differentially expressed gene for each contrast. Used for evaluating lods. |
| <code>F</code> | row-wise numeric vector of moderated F-statistics for testing all contrasts defined by the columns of <code>fit</code> simultaneously equal to zero. |
| <code>F.p.value</code> | row-wise numeric vector giving p-values corresponding to <code>F</code> . |

The matrices `t`, `p.value` and `lods` have the same dimensions as the input object `fit`, with rows corresponding to genes and columns to coefficients or contrasts. The vectors `s2.prior`, `df.prior`, `df.total`, `F` and `F.p.value` correspond to rows, with length equal to the number of genes. The vector `var.prior` corresponds to columns, with length equal to the number of contrasts. If `s2.prior` or `df.prior` have length 1, then the same value applies to all genes.

`s2.prior`, `df.prior` and `var.prior` contain empirical Bayes hyperparameters used to obtain `df.total`, `s2.post` and `lods`.

`treat` produces an `MArrayLM` object similar to that from `eBayes` but without `lods`, `var.prior`, `F` or `F.p.value`.

Note

The algorithm used by `eBayes` and `treat` with `robust=TRUE` was revised slightly in `limma` 3.27.6. The minimum `df.prior` returned may be slightly smaller than previously.

Author(s)

Gordon Smyth and Davis McCarthy

References

- Law CW, Chen Y, Shi W, Smyth GK (2014). Voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biology* 15, R29. doi:10.1186/gb2014152r29. See also the Preprint Version at <https://gksmyth.github.io/pubs/VoomPreprint.pdf> incorporating some notational corrections.
- Loennstedt I, and Speed TP (2002). Replicated microarray data. *Statistica Sinica* 12, 31-46.
- McCarthy D J, Smyth GK (2009). Testing significance relative to a fold-change threshold is a TREAT. *Bioinformatics* 25, 765-771. doi:10.1093/bioinformatics/btp053
- Phipson B, Lee S, Majewski IJ, Alexander WS, Smyth GK (2016). Robust hyperparameter estimation protects against hypervariable genes and improves power to detect differential expression. *Annals of Applied Statistics* 10, 946-963. doi:10.1214/16AOAS920
- Smyth GK (2004). Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Statistical Applications in Genetics and Molecular Biology* Volume 3, Issue 1, Article 3. doi:10.2202/15446115.1027. See also the Preprint Version <https://gksmyth.github.io/pubs/ebayes.pdf> incorporating corrections to 30 June 2009.

See Also

[squeezeVar](#), [fitFDist](#), [tmixture.matrix](#), [plotSA](#).

An overview of linear model functions in limma is given by [06.LinearModels](#).

Examples

```
# See also lmFit examples

# Simulate gene expression data,
# 6 microarrays and 100 genes with one gene differentially expressed
set.seed(2016)
sigma2 <- 0.05 / rchisq(100, df=10) * 10
y <- matrix(rnorm(100*6, sd=sqrt(sigma2)), 100, 6)
design <- cbind(Intercept=1, Group=c(0,0,0,1,1,1))
y[1,4:6] <- y[1,4:6] + 1
fit <- lmFit(y, design)

# Moderated t-statistic
fit <- eBayes(fit)
topTable(fit, coef=2)

# Ordinary t-statistic
ordinary.t <- fit$coef[,2] / fit$stdev.unscaled[,2] / fit$sigma

# Treat relative to a 10% fold-change
tfit <- treat(fit, fc=1.1)
topTreat(tfit, coef=2)
```

EList-class

Expression List (EList) class

Description

A list-based S4 classes for storing expression values (E-values), for example for a set of one-channel microarrays or a set of RNA-seq samples. EListRaw holds expression values on the raw scale. EList holds expression values on the log scale, usually after background correction and normalization.

EListRaw objects are often created by [read.maimages](#), while EList objects are often created by [normalizeBetweenArrays](#) or by [voom](#). Alternatively, an EList object can be created directly by `new("EList", x)`, where x is a list.

Required Components

These classes contains no slots (other than `.Data`), but objects should contain a list component E:

E numeric matrix containing expression values. In an EListRaw object, the expression values are unlogged, while in an EList object, they are log₂ values. Rows correspond to probes and columns to samples.

Optional Components

Optional components include:

`Eb` numeric matrix containing unlogged background expression values, of same dimensions as `E`.
For an `EListRaw` object only.

`weights` numeric matrix of same dimensions as `E` containing relative spot quality weights. Elements should be non-negative.

`other` list containing other matrices, all of the same dimensions as `E`.

`genes` data.frame containing probe information. Should have one row for each probe. May have any number of columns.

`targets` data.frame containing information on the target RNA samples. Rows correspond to samples. May have any number of columns.

Valid `EList` or `EListRaw` objects may contain other optional components, but all probe or sample information should be contained in the above components.

Methods

These classes inherit directly from class `list` so any operation appropriate for lists will work on objects of this class. In addition, `EList` objects can be [subsetting](#) and [combined](#). `EList` objects will return dimensions and hence functions such as `dim`, `nrow` and `ncol` are defined. `ELists` also inherit a `show` method from the virtual class `LargeDataObject`, which means that `ELists` will print in a compact way.

Author(s)

Gordon Smyth

See Also

[02.Classes](#) gives an overview of all the classes defined by this package.

[ExpressionSet](#) is a more formal class in the `Biobase` package used for the same purpose.

Examples

```
# Two ways to make an EList object:

y <- matrix(rnorm(10,5),10,5)
rownames(y) <- paste0("Gene",1:10)
colnames(y) <- LETTERS[1:5]
Genes <- data.frame(Chr=sample(1:21,10))
row.names(Genes) <- row.names(y)

# Create the object, than add components:
E <- new("EList")
E$E <- y
E$genes <- Genes

# Create with components:
E <- new("EList", list(E=y, genes=Genes))
```

`exprs.MA`*Extract Log-Expression Matrix from MAList*

Description

Extract the matrix of log-expression values from an `MAList` object.

Usage

```
exprs.MA(MA)
```

Arguments

`MA` an `MAList` object.

Details

Converts `M` and `A`-values to log-expression values. The output matrix will have two columns for each array, in the order green, red for each array.

This contrasts with `as.matrix.MAList` which extracts the `M`-values only, or `RG.MA` which converts to expression values in `RGList` form.

Value

A numeric matrix with twice the columns of the input.

Author(s)

Gordon Smyth

See Also

[02.Classes](#) gives an overview of data classes used in LIMMA.

`fitFDist`*Moment Estimation of Scaled F-Distribution*

Description

Moment estimation of the parameters of a scaled `F`-distribution given one of the degrees of freedom. These functions are called internally by `eBayes` and `squeezeVar` and is not usually called directly by a user.

Usage

```
fitFDist(x, df1, covariate = NULL)
fitFDistRobustly(x, df1, covariate = NULL, winsor.tail.p = c(0.05,0.1), trace = FALSE)
fitFDistUnequalDF1(x, df1, covariate = NULL, robust = FALSE, prior.weights = NULL)
```

Arguments

| | |
|----------------------------|--|
| <code>x</code> | numeric vector or array of positive values representing a sample from a scaled F-distribution. |
| <code>df1</code> | the first degrees of freedom of the F-distribution. Can be a single value, or else a vector of the same length as <code>x</code> . |
| <code>covariate</code> | if non-NULL, the estimated scale value will depend on this numeric covariate. |
| <code>winsor.tail.p</code> | numeric vector of length 1 or 2, giving left and right tail proportions of <code>x</code> to Winsorize. |
| <code>trace</code> | logical value indicating whether a trace of the iteration progress should be printed. |
| <code>robust</code> | logical. Should outlier values of <code>x</code> be down-weighted with results similar to <code>fitFDistRobustly</code> ? |
| <code>prior.weights</code> | numeric vector of (non-negative) prior weights. |

Details

`fitFDist()` implements an algorithm proposed by Smyth (2004) and Phipson et al (2016). It estimates `scale` and `df2` under the assumption that `x` is distributed as `scale` times an F-distributed random variable on `df1` and `df2` degrees of freedom. The parameters are estimated using the method of moments, specifically from the mean and variance of the `x` values on the log-scale.

When `covariate` is supplied, a spline curve trend will be estimated for the `x` values and the estimation will be adjusted for this trend (Phipson et al, 2016).

`fitFDistRobustly` is similar to `fitFDist` except that it computes the moments of the Winsorized values of `x`, making it robust against left and right outliers. Larger values for `winsor.tail.p` produce more robustness but less efficiency. When `covariate` is supplied, a loess trend is estimated for the `x` values. The robust method is described by Phipson et al (2016).

As well as estimating the F-distribution for the bulk of the cases, i.e., with outliers discounted, `fitFDistRobustly` also returns an estimated F-distribution with reduced `df2` that might be appropriate for each outlier case.

`fitFDistUnequalDF1` was introduced in `limma` 3.61.8 and gives special attention to the possibility that the degrees of freedom `df1` might be unequal and might include non-integer values. The most important innovation of `fitFDistUnequalDF1` is downweighting of observations with lower degrees of freedom, to give more precise estimation overall. It also allows the possibility of prior weights, which can be used to downweight unreliable `x` values for reasons other than small `df1`. `fitFDistUnequalDF1` implements a different robust estimation strategy to `fitFDistRobustly`. Instead of Winsorizing the `x` values, potential outliers are instead downweighted using the prior weights. Whereas `fitFDist` and `fitFDistRobustly` use unweighted moment estimation for both `scale` and `df2`, `fitFDistUnequalDF1` uses weighted moment estimation for `scale` and profile maximum likelihood for `df2`.

fitFDistUnequalDF1 gives improved performance over fitFDist and fitFDistRobustly, especially when the degrees of freedom are unequal but also to a lesser extent when the degrees of freedom are equal. Unequal residual degrees of freedom arise in limma pipelines when the expression matrix includes missing values, or from edgeR: :vroomLmFit or from the quasi-likelihood pipeline in edgeR v4 (Chen et al 2024). The edgeR v4 pipeline produces fractional degrees of freedom including, potentially, degrees of freedom less than 1.

Value

fitFDist or fitFDistUnequalDF1 with robust=FALSE produces a list with the following components:

| | |
|-------|---|
| scale | scale factor for F-distribution. A vector if covariate is non-NULL, otherwise a scalar. |
| df2 | the second degrees of freedom of the fitted F-distribution. |

fitFDistRobustly returns the following components as well:

| | |
|--------------|--|
| tail.p.value | right tail probability of the scaled F-distribution for each x value. |
| prob.outlier | posterior probability that each case is an outlier relative to the scaled F-distribution with degrees of freedom df1 and df2. |
| df2.outlier | the second degrees of freedom associated with extreme outlier cases. |
| df2.shrunk | numeric vector of values for the second degrees of freedom, with shrunk values for outliers. Most values are equal to df2, but outliers have reduced values depending on how extreme each case is. All values lie between df2.outlier and df2. |

Note

The algorithm used by fitFDistRobustly was revised slightly in limma 3.27.6. The prob.outlier value, which is the lower bound for df2.shrunk, may be slightly smaller than previously.

Author(s)

Gordon Smyth, Belinda Phipson (fitFDistRobustly) and Lizhong Chen (fitFDistUnequalDF1).

References

- Smyth GK (2004). Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Statistical Applications in Genetics and Molecular Biology* Volume 3, Issue 1, Article 3. doi:10.2202/15446115.1027 <https://gksmyth.github.io/pubs/ebayes.pdf>
- Phipson B, Lee S, Majewski IJ, Alexander WS, Smyth GK (2016). Robust hyperparameter estimation protects against hypervariable genes and improves power to detect differential expression. *Annals of Applied Statistics* 10, 946-963. doi:10.1214/16AOAS920
- Chen Y, Chen L, Lun ATL, Baldoni PL, Smyth GK (2024). edgeR 4.0: powerful differential analysis of sequencing data with expanded functionality and improved support for small counts and larger datasets. *bioRxiv* 2024.01.21.576131. doi:10.1101/2024.01.21.576131

See Also

This function is called by [squeezeVar](#), which in turn is called by [eBayes](#) and [treat](#).

This function calls [trigammaInverse](#).

Examples

```
x <- rf(100,df1=8,df2=16)
fitFDist(x,df1=8)
```

fitGammaIntercept *Fit Intercept to Vector of Gamma Distributed Variates*

Description

Fit Intercept to Vector of Gamma Distributed Variates

Usage

```
fitGammaIntercept(y,offset=0,maxit=1000)
```

Arguments

| | |
|---------------------|---|
| <code>y</code> | numeric vector of positive response values. |
| <code>offset</code> | numeric vector giving known part of the expected value of <code>y</code> . Can be a single value, or else a vector of the same length as <code>y</code> . |
| <code>maxit</code> | maximum number of Newton iterations to be done. |

Details

The values `y` are assumed to follow a gamma distribution with common shape parameter and with expected values given by `x+offset`. The function implements a globally convergent Newton iteration to estimate `x`.

Value

Numeric value giving intercept.

Author(s)

Gordon Smyth and Belinda Phipson

References

Phipson, B. (2013). *Empirical Bayes modelling of expression profiles and their associations*. PhD Thesis. University of Melbourne, Australia.

See Also

This function is called by [genas](#).

Examples

```
offset <- runif(10)
x <- 9
mu <- x+offset
y <- rgamma(10,shape=20,scale=mu/20)
fitGammaIntercept(y,offset=offset)
```

fitmixture

Fit Mixture Model by Non-Linear Least Squares

Description

Fit Mixture Model by Non-Linear Least Squares

Usage

```
fitmixture(log2e, mixprop, niter = 4, trace = FALSE)
```

Arguments

| | |
|---------|---|
| log2e | a numeric matrix containing log2 expression values. Rows correspond to probes for genes and columns to RNA samples. |
| mixprop | a vector of length ncol(log2e) giving the mixing proportion (between 0 and 1) for each sample. |
| niter | integer number of iterations. |
| trace | logical. If TRUE, summary working estimates are output from each iteration. |

Details

A mixture experiment is one in which two reference RNA sources are mixed in different proportions to create experimental samples. Mixture experiments have been used to evaluate genomic technologies and analysis methods (Holloway et al, 2006). This function uses all the data for each gene to estimate the expression level of the gene in each of two pure samples.

The function fits a nonlinear mixture model to the log2 expression values for each gene. The expected values of log2e for each gene are assumed to be of the form $\log_2(\text{mixprop} \cdot Y_1 + (1 - \text{mixprop}) \cdot Y_2)$ where Y_1 and Y_2 are the expression levels of the gene in the two reference samples being mixed. The mixprop values are the same for each gene but Y_1 and Y_2 are specific to the gene. The function returns the estimated values $A = 0.5 \cdot \log_2(Y_1 \cdot Y_2)$ and $M = \log_2(Y_2 / Y_1)$ for each gene.

The nonlinear estimation algorithm implemented in `fitmixture` uses a nested Gauss-Newton iteration (Smyth, 1996). It is fully vectorized so that the estimation is done for all genes simultaneously.

Value

List with three components:

| | |
|-------|--|
| A | numeric vector giving the estimated average log ₂ expression of the two reference samples for each gene |
| M | numeric vector giving estimated log-ratio of expression between the two reference samples for each gene |
| stdev | standard deviation of the residual term in the mixture model for each gene |

Author(s)

Gordon K Smyth

References

Holloway, A. J., Oshlack, A., Diyagama, D. S., Bowtell, D. D. L., and Smyth, G. K. (2006). Statistical analysis of an RNA titration series evaluates microarray precision and sensitivity on a whole-array basis. *BMC Bioinformatics* 7, Article 511. doi:10.1186/147121057511

Smyth, G. K. (1996). Partitioned algorithms for maximum likelihood and other nonlinear estimation. *Statistics and Computing*, 6, 201-216. <https://gksmyth.github.io/pubs/partitio.pdf>

Examples

```
ngenes <- 100
TrueY1 <- rexp(ngenes)
TrueY2 <- rexp(ngenes)
mixprop <- matrix(c(0,0.25,0.75,1),1,4)
TrueExpr <- TrueY1

log2e <- log2(TrueExpr) + matrix(rnorm(ngenes*4),ngenes,4)*0.1
out <- fitmixture(log2e,mixprop)

# Plot true vs estimated log-ratios
plot(log2(TrueY1/TrueY2), out$M)
```

fitted.MArrayLM

Fitted Values Method for MArrayLM Fits

Description

Obtains fitted values from a fitted microarray linear model object.

Usage

```
## S3 method for class 'MArrayLM'
fitted(object, ...)
```

Arguments

object a fitted object of class inheriting from "MArrayLM".
 ... other arguments are not currently used.

Value

A numeric matrix of fitted values.

Author(s)

Gordon Smyth

See Also

[fitted](#)

genas

Genuine Association of Gene Expression Profiles

Description

Calculates biological correlation between two gene expression profiles.

Usage

```
genas(fit, coef=c(1,2), subset="all", plot=FALSE, alpha=0.4)
```

Arguments

fit an MArrayLM fitted model object produced by `lmFit` or `contrasts.fit` and followed by `eBayes`.

coef numeric vector of length 2 indicating which columns in the fit object are to be correlated.

subset character string indicating which subset of genes to include in the correlation analysis. Choices are "all", "Fpval", "p.union", "p.int", "logFC" or "predFC".

plot logical, should a scatterplot be produced summarizing the correlation analysis?

alpha numeric value between 0 and 1 determining the transparency of the technical and biological ellipses if a plot is produced. `alpha=0` indicates fully transparent and `alpha=1` indicates fully opaque.

Details

The function estimates the biological correlation between two different contrasts in a linear model. By biological correlation, we mean the correlation that would exist between the log₂-fold changes (logFC) for the two contrasts, if measurement error could be eliminated and the true log-fold-changes were known. This function is motivated by the fact that different contrasts for a linear model are often strongly correlated in a technical sense. For example, the estimated logFC for multiple treatment conditions compared back to the same control group will be positively correlated even in the absence of any biological effect. This function aims to separate the biological from the technical components of the correlation. The method is explained briefly in Majewski et al (2010) and in full detail in Phipson (2013).

The subset argument specifies whether and how the fit object should be subsetted. Ideally, only genes that are truly differentially expressed for one or both of the contrasts should be used estimate the biological correlation. The default is "all", which uses all genes in the fit object to estimate the biological correlation. The option "Fpval" chooses genes based on how many F-test p-values are estimated to be truly significant using the function `propTrueNull`. This should capture genes that display any evidence of differential expression in either of the two contrasts. The options "p.union" and "p.int" are based on the moderated t p-values from both contrasts. From the `propTrueNull` function an estimate of the number of p-values truly significant in either of the two contrasts can be obtained. "p.union" takes the union of these genes and "p.int" takes the intersection of these genes. The other options, "logFC" and "predFC" subsets on genes that attain a logFC or predFC at least as large as the 90th percentile of the log fold changes or predictive log fold changes on the absolute scale.

The plot option is a logical argument that specifies whether or not to plot a scatter plot of log-fold-changes for the two contrasts. The biological and technical correlations are overlaid on the scatterplot using semi-transparent ellipses. `library(ellipse)` is required to enable the plotting of ellipses.

Value

`genas` produces a list with the following components:

| | |
|-------------------------------------|---|
| <code>technical.correlation</code> | estimate of the technical correlation |
| <code>biological.correlation</code> | estimate of the biological correlation |
| <code>covariance.matrix</code> | estimate of the covariance matrix from which the biological correlation is obtained |
| <code>deviance</code> | the likelihood ratio test statistic used to test whether the biological correlation is equal to 0 |
| <code>p.value</code> | the p.value associated with deviance |
| <code>n</code> | the number of genes used to estimate the biological correlation |

Note

As present, `genas` assumes that technical correlations between coefficients are the same for all genes, and hence it only works with fit objects that were created without observation weights or missing values. It does not work with voom pipelines, because these involve observation weights.

Author(s)

Belinda Phipson and Gordon Smyth

References

Majewski, IJ, Ritchie, ME, Phipson, B, Corbin, J, Pakusch, M, Ebert, A, Busslinger, M, Koseki, H, Hu, Y, Smyth, GK, Alexander, WS, Hilton, DJ, and Blewitt, ME (2010). Opposing roles of polycomb repressive complexes in hematopoietic stem and progenitor cells. *Blood* 116, 731-739. <http://www.bloodjournal.org/content/116/5/731>

Phipson, B. (2013). *Empirical Bayes modelling of expression profiles and their associations*. PhD Thesis. University of Melbourne, Australia. <http://hdl.handle.net/11343/38162>

Ritchie, ME, Phipson, B, Wu, D, Hu, Y, Law, CW, Shi, W, and Smyth, GK (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* 43, e47. <http://nar.oxfordjournals.org/content/43/7/e47>

See Also

[lmFit](#), [eBayes](#), [contrasts.fit](#)

Examples

```
# Simulate gene expression data

# Three conditions (Control, A and B) and 1000 genes
ngene <- 1000
mu.A <- mu.B <- mu.ctrl <- rep(5,ngene)

# 200 genes are differentially expressed.
# All are up in condition A and down in B
# so the biological correlation is negative.
mu.A[1:200] <- mu.ctrl[1:200]+2
mu.B[1:200] <- mu.ctrl[1:200]-2

# Two microarrays for each condition
mu <- cbind(mu.ctrl,mu.ctrl,mu.A,mu.A,mu.B,mu.B)
y <- matrix(rnorm(6000,mean=mu,sd=1),ngene,6)

# two experimental groups and one control group with two replicates each
group <- factor(c("Ctrl","Ctrl","A","A","B","B"), levels=c("Ctrl","A","B"))
design <- model.matrix(~group)

# fit a linear model
fit <- lmFit(y,design)
fit <- eBayes(fit)

# Estimate biological correlation between the logFC profiles
# for A-vs-Ctrl and B-vs-Ctrl
genas(fit, coef=c(2,3), plot=TRUE, subset="F")
```

geneSetTest

*Mean-rank Gene Set Test***Description**

Test whether a set of genes is highly ranked relative to other genes in terms of a given statistic. Genes are assumed to be independent.

Usage

```
geneSetTest(index, statistics, alternative = "mixed", type = "auto",
            ranks.only = TRUE, nsim=9999)
wilcoxGST(index, statistics, ...)
```

Arguments

| | |
|-------------|---|
| index | index vector for the gene set. This can be a vector of indices, or a logical vector of the same length as <code>statistics</code> or, in general, any vector such that <code>statistic[index]</code> gives the statistic values for the gene set to be tested. |
| statistics | vector, any genewise statistic by which genes can be ranked. |
| alternative | character string specifying the alternative hypothesis, must be one of "mixed", "either", "up" or "down". "two.sided", "greater" and "less" are also permitted as synonyms for "either", "up" and "down" respectively. |
| type | character string specifying whether the statistics are signed (t-like, "t") or unsigned (F-like, "f") or whether the function should make an educated guess ("auto"). If the statistic is unsigned, then it assume that larger statistics are more significant. |
| ranks.only | logical, if TRUE only the ranks of the statistics are used. |
| nsim | number of random samples to take in computing the p-value. Not used if <code>ranks.only=TRUE</code> . |
| ... | other arguments are passed to <code>geneSetTest</code> . |

Details

These functions compute a p-value to test the hypothesis that the indexed test set of genes tends to be more highly ranked in terms of some test statistic compared to randomly chosen genes. The statistic might be any statistic of interest, for example a t-statistic or F-statistic for differential expression. Like all gene set tests, these functions can be used to detect differential expression for a group of genes, even when the effects are too small or there is too little data to detect the genes individually.

`wilcoxGST` is a synonym for `geneSetTest` with `ranks.only=TRUE`. This version of the test procedure was developed by Michaud et al (2008), who called it *mean-rank gene-set enrichment*.

`geneSetTest` performs a *competitive* test in the sense that genes in the test set are compared to other genes (Goeman and Buhlmann, 2007). If the `statistic` is a genewise test statistic for differential expression, then `geneSetTest` tests whether genes in the set are more differentially expressed than genes not in the set. By contrast, a *self-contained* gene set test such as `roast` tests whether genes in

the test set are differentially expressed, in an absolute sense, without regard to any other genes on the array.

Because it is based on permuting genes, `geneSetTest` assumes that the different genes (or probes) are statistically independent. (Strictly speaking, it assumes that the genes in the set are no more correlated on average than randomly chosen genes.) If inter-gene correlations are present, then a statistically significant result from `geneSetTest` indicates either that the set is highly ranked or that the genes in the set are positively correlated on average (Wu and Smyth, 2012). Unless gene sets with positive correlations are particularly of interest, it may be advisable to use `camera` or `cameraPR` instead to adjust the test for inter-gene correlations. Inter-gene correlations are likely to be present in differential expression experiments with biologically heterogeneous experimental units. On the other hand, the assumption of independence between genes should hold when the replicates are purely technical, i.e., when there is no biological variability between the replicate arrays in each experimental condition.

The statistics are usually a set of probe-wise statistics arising from some comparison from a microarray experiment. They may be t-statistics, meaning that the genewise null hypotheses would be rejected for large positive or negative values, or they may be F-statistics, meaning that only large values are significant. Any set of signed statistics, such as log-ratios, M-values or moderated t-statistics, are treated as t-like. Any set of unsigned statistics, such as F-statistics, posterior probabilities or chi-square tests are treated as F-like. If `type="auto"` then the statistics will be taken to be t-like if they take both positive and negative values and will be taken to be F-like if they are all of the same sign.

There are four possible alternatives to test for. `alternative=="up"` means the genes in the set tend to be up-regulated, with positive t-statistics. `alternative=="down"` means the genes in the set tend to be down-regulated, with negative t-statistics. `alternative=="either"` means the set is either up or down-regulated as a whole. `alternative=="mixed"` test whether the genes in the set tend to be differentially expressed, without regard for direction. In this case, the test will be significant if the set contains mostly large test statistics, even if some are positive and some are negative.

The latter three alternatives are appropriate when there is a prior expectation that all the genes in the set will react in the same direction. The "mixed" alternative is appropriate if you know only that the genes are involved in the relevant pathways, possibly in different directions. The "mixed" is the only meaningful alternative with F-like statistics.

The test statistic used for the gene-set-test is the mean of the statistics in the set. If `ranks.only` is TRUE the only the ranks of the statistics are used. In this case the p-value is obtained from a Wilcoxon test. If `ranks.only` is FALSE, then the p-value is obtained by simulation using `nsim` random sets of genes.

Value

numeric value giving the estimated p-value.

Note

Wu and Smyth (2012) show that `geneSetTest` does not does correct for inter-gene correlations and is more likely to assign small p-values to sets containing positive correlated genes. The function `cameraPR` is recommended as a alternative.

Author(s)

Gordon Smyth and Di Wu

References

Wu, D, and Smyth, GK (2012). Camera: a competitive gene set test accounting for inter-gene correlation. *Nucleic Acids Research* 40(17), e133. doi:10.1093/nar/gks461

Goeman, JJ, and Buhlmann P (2007). Analyzing gene expression data in terms of gene sets: methodological issues. *Bioinformatics* 23, 980-987.

Michaud, J, Simpson, KM, Escher, R, Buchet-Poyau, K, Beissbarth, T, Carmichael, C, Ritchie, ME, Schutz, F, Cannon, P, Liu, M, Shen, X, Ito, Y, Raskind, WH, Horwitz, MS, Osato, M, Turner, DR, Speed, TP, Kavallaris, M, Smyth, GK, and Scott, HS (2008). Integrative analysis of RUNX1 downstream pathways and target genes. *BMC Genomics* 9, 363. doi:10.1186/147121649363

See Also

[cameraPR](#), [camera](#), [roast](#), [barcodeplot](#), [wilcox.test](#).

There is a topic page on [10.GeneSetTests](#).

Examples

```
stat <- rnorm(100)
sel <- 1:10; stat[sel] <- stat[sel]+1
wilcoxGST(sel,stat)
```

getEAWP

Extract Basic Data from Expression Data Objects

Description

Given an expression data object of any known class, get the expression values, weights, probe annotation and A-values that are needed for linear modelling. This function is called by the linear modelling functions in LIMMA.

Usage

```
getEAWP(object)
```

Arguments

object any matrix-like object containing log-expression values. Can be an object of class `MAList`, `EList`, `marrayNorm`, `PLMset`, `vsn`, or any class inheriting from `ExpressionSet`, or any object that can be coerced to a numeric matrix.

Details

Rows correspond to probes and columns to RNA samples.

In the case of two-color microarray data objects (MList or marrayNorm), Amean is the vector of row means of the matrix of A-values. For other data objects, Amean is the vector of row means of the matrix of expression values.

From April 2013, the rownames of the output exprs matrix are required to be unique. If object has no row names, then the output rownames of exprs are 1:nrow(object). If object has row names but with duplicated names, then the rownames of exprs are set to 1:nrow(object) and the original row names are preserved in the ID column of probes.

object should be a normalized data object. getEAWP will return an error if object is a non-normalized data object such as RGList or EListRaw, because these do not contain log-expression values.

Value

A list with components

| | |
|---------|--|
| exprs | numeric matrix of log-ratios, log-intensities or log-expression values |
| weights | numeric matrix of weights |
| probes | data.frame of probe-annotation |
| Amean | numeric vector of average log-expression for each probe |

exprs is the only required component. The other components will be NULL if not found in the input object.

Author(s)

Gordon Smyth

See Also

[02.Classes](#) gives an overview of data classes used in LIMMA.

getLayout

Extract the Print Layout of an Array from the GAL File

Description

From the Block, Row and Column information in a genelist, determine the number of grid rows and columns on the array and the number of spot rows and columns within each grid.

Usage

```
getLayout(gal, guessdups=FALSE)
getLayout2(galfile)
getDupSpacing(ID)
```

Arguments

| | |
|-----------|---|
| gal | data.frame containing the GAL, i.e., giving the position and gene identifier of each spot |
| galfile | name or path of GAL file |
| guessdups | logical, if TRUE then try to determine number and spacing of duplicate spots, i.e., within-array replicates |
| ID | vector or factor of gene IDs |

Details

A GenePix Array List (GAL) file is a list of genes and associated information produced by an Axon microarray scanner. The function `getLayout` determines the print layout from a data frame created from a GAL file or gene list. The data.frame must contain columns `Block`, `Column` and `Row`. (The number of tip columns is assumed to be either one or four.)

On some arrays, each probe may be duplicated a number of times (`ndups`) at regular intervals (spacing) in the GAL file. `getDupSpacing` determines valid values for `ndups` and `spacing` from a vector of IDs. If `guessdups=TRUE`, then `getLayout` calls `getDupSpacing`.

The function `getLayout2` attempts to determine the print layout from the header information of an actual GAL file.

Value

A `printlayout` object, which is a list with the following components. The last two components are present only if `guessdups=TRUE`.

| | |
|----------------------|---|
| <code>ngrid.r</code> | integer, number of grid rows on the arrays |
| <code>ngrid.c</code> | integer, number of grid columns on the arrays |
| <code>nspot.r</code> | integer, number of rows of spots in each grid |
| <code>nspot.c</code> | integer, number of columns of spots in each grid |
| <code>ndups</code> | integer, number of times each probe is printed on the array |
| <code>spacing</code> | integer, spacing between multiple printings of each probe |

Author(s)

Gordon Smyth and James Wettenhall

See Also

An overview of LIMMA functions for reading data is given in [03.ReadingData](#).

Examples

```
# gal <- readGAL()
# layout <- getLayout(gal)
```

| | |
|------------|------------------------------|
| getSpacing | <i>Get Numerical Spacing</i> |
|------------|------------------------------|

Description

Convert character to numerical spacing measure for within-array replicate spots.

Usage

```
getSpacing(spacing, layout)
```

Arguments

| | |
|---------|--|
| spacing | character string or integer. Acceptable character strings are "columns", "rows", "subarrays" or "topbottom". Integer values are simply passed through. |
| layout | list containing printer layout information |

Details

"rows" means that duplicate spots are printed side-by-side by rows. These will be recorded in consecutive rows in the data object.

"columns" means that duplicate spots are printed side-by-side by columns. These will be separated in the data object by `layout$nspot.r` rows.

"subarrays" means that a number of sub-arrays, with identical probes in the same arrangement, are printed on each array. The spacing therefore will be the size of a sub-array.

"topbottom" is the same as "subarrays" when there are two sub-arrays.

Value

Integer giving spacing between replicate spots in the gene list.

Author(s)

Gordon Smyth

See Also

An overview of LIMMA functions for reading data is given in [03.ReadingData](#).

Examples

```
getSpacing("columns", list(ngrid.r=2, ngrid.c=2, nspot.r=20, nspot.c=19))
getSpacing("rows", list(ngrid.r=2, ngrid.c=2, nspot.r=20, nspot.c=19))
getSpacing("topbottom", list(ngrid.r=2, ngrid.c=2, nspot.r=20, nspot.c=19))
```

gls.series

*Fit Linear Model to Microarray Data by Generalized Least Squares***Description**

Fit a linear model genewise to expression data from a series of microarrays. The fit is by generalized least squares allowing for correlation between duplicate spots or related arrays. This is a utility function for `lmFit`.

Usage

```
gls.series(M, design=NULL, ndups=2, spacing=1, block=NULL, correlation=NULL, weights=NULL, ...)
```

Arguments

| | |
|--------------------------|---|
| <code>M</code> | numeric matrix containing log-ratio or log-expression values for a series of microarrays, rows correspond to genes and columns to arrays. |
| <code>design</code> | numeric design matrix defining the linear model, with rows corresponding to arrays and columns to comparisons to be estimated. The number of rows must match the number of columns of <code>M</code> . Defaults to the unit vector meaning that the arrays are treated as replicates. |
| <code>ndups</code> | positive integer giving the number of times each gene is printed on an array. <code>nrow(M)</code> must be divisible by <code>ndups</code> . Ignored if <code>block</code> is not <code>NULL</code> . |
| <code>spacing</code> | the spacing between the rows of <code>M</code> corresponding to duplicate spots, <code>spacing=1</code> for consecutive spots. Ignored if <code>block</code> is not <code>NULL</code> . |
| <code>block</code> | vector or factor specifying a blocking variable on the arrays. Same length as <code>ncol(M)</code> . |
| <code>correlation</code> | numeric value specifying the inter-duplicate or inter-block correlation. |
| <code>weights</code> | an optional numeric matrix of the same dimension as <code>M</code> containing weights for each spot. If it is of different dimension to <code>M</code> , it will be filled out to the same size. |
| <code>...</code> | other optional arguments to be passed to <code>dupcor.series</code> . |

Details

This is a utility function used by the higher level function `lmFit`. Most users should not use this function directly but should use `lmFit` instead.

This function is for fitting gene-wise linear models when some of the expression values are correlated. The correlated groups may arise from replicate spots on the same array (duplicate spots) or from a biological or technical replicate grouping of the arrays. This function is normally called by `lmFit` and is not normally called directly by users.

Note that the correlation is assumed to be constant across genes. If `correlation=NULL` then a call is made to `duplicateCorrelation` to estimate the correlation.

Value

A list with components

| | |
|-----------------------------|--|
| <code>coefficients</code> | numeric matrix containing the estimated coefficients for each linear model. Same number of rows as M, same number of columns as design. |
| <code>stdev.unscaled</code> | numeric matrix conformal with <code>coef</code> containing the unscaled standard deviations for the coefficient estimators. The standard errors are given by <code>stdev.unscaled * sigma</code> . |
| <code>sigma</code> | numeric vector containing the residual standard deviation for each gene. |
| <code>df.residual</code> | numeric vector giving the degrees of freedom corresponding to <code>sigma</code> |
| <code>correlation</code> | inter-duplicate or inter-block correlation |
| <code>qr</code> | QR decomposition of the generalized linear squares problem, i.e., the decomposition of design standardized by the Choleski-root of the correlation matrix defined by <code>correlation</code> |

Author(s)

Gordon Smyth

See Also

[duplicateCorrelation](#).

An overview of linear model functions in limma is given by [06.LinearModels](#).

goana

Gene Ontology or KEGG Pathway Analysis

Description

Test for over-representation of gene ontology (GO) terms or KEGG pathways in one or more sets of genes, optionally adjusting for abundance or gene length bias.

Usage

```
## S3 method for class 'MArrayLM'
goana(de, coef = ncol(de), geneid = rownames(de), FDR = 0.05, trend = FALSE, ...)
## S3 method for class 'MArrayLM'
kegga(de, coef = ncol(de), geneid = rownames(de), FDR = 0.05, trend = FALSE, ...)
## Default S3 method:
goana(de, universe = NULL, species = "Hs", null.prob = NULL, covariate=NULL,
      plot=FALSE, ...)
## Default S3 method:
kegga(de, universe = NULL, restrict.universe = FALSE, species = "Hs", species.KEGG = NULL,
      convert = FALSE, gene.pathway = NULL, pathway.names = NULL,
      null.prob = NULL, covariate=NULL, plot=FALSE, ...)
getGeneKEGGLinks(species.KEGG = "hsa", convert = FALSE)
getKEGGPathwayNames(species.KEGG = NULL, remove.qualifier = FALSE)
```

Arguments

| | |
|--------------------------------|--|
| <code>de</code> | a character vector of Entrez Gene IDs, or a list of such vectors, or an MArrayLM fit object. |
| <code>coef</code> | column number or column name specifying for which coefficient or contrast differential expression should be assessed. |
| <code>geneid</code> | Entrez Gene identifiers. Either a vector of length <code>nrow(de)</code> or the name of the column of <code>de\$genes</code> containing the Entrez Gene IDs. |
| <code>FDR</code> | false discovery rate cutoff for differentially expressed genes. Numeric value between 0 and 1. |
| <code>species</code> | character string specifying the species. Possible values include "Hs" (human), "Mm" (mouse), "Rn" (rat), "Dm" (fly) or "Pt" (chimpanzee), but other values are possible if the corresponding organism package is available. See alias2Symbol for other possible values. Ignored if <code>species.KEGG</code> or is not NULL or if <code>gene.pathway</code> and <code>pathway.names</code> are not NULL. |
| <code>species.KEGG</code> | three-letter KEGG species identifier. See https://www.kegg.jp/kegg/catalog/org_list.html or https://rest.kegg.jp/list/organism for possible values. Alternatively, if <code>de</code> contains KEGG ortholog IDs ("k00001" etc) instead of gene IDs, then set <code>species.KEGG="ko"</code> . This argument is ignored if <code>gene.pathway</code> and <code>pathway.names</code> are both not NULL. |
| <code>convert</code> | if TRUE then KEGG gene identifiers will be converted to NCBI Entrez Gene identifiers. Note that KEGG IDs are the same as Entrez Gene IDs for most species anyway. |
| <code>gene.pathway</code> | data.frame linking genes to pathways. First column gives gene IDs, second column gives pathway IDs. By default this is obtained automatically by <code>getGeneKEGGLinks(species.KEGG)</code> |
| <code>remove.qualifier</code> | if TRUE, the species qualifier will be removed from the pathway names. |
| <code>pathway.names</code> | data.frame giving full names of pathways. First column gives pathway IDs, second column gives pathway names. By default this is obtained automatically using <code>getKEGGPathwayNames(species.KEGG, remove=TRUE)</code> . |
| <code>trend</code> | adjust analysis for gene length or abundance? Can be logical, or a numeric vector of covariate values, or the name of the column of <code>de\$genes</code> containing the covariate values. If TRUE, then <code>de\$Amean</code> is used as the covariate. |
| <code>universe</code> | vector specifying the set of Entrez Gene identifiers to be the background universe. If NULL then all Entrez Gene IDs associated with any gene ontology term will be used as the universe. |
| <code>restrict.universe</code> | logical, should the universe be restricted to gene identifiers found in at least one pathway in <code>gene.pathway</code> ? |
| <code>null.prob</code> | optional numeric vector of the same length as <code>universe</code> giving the null probability that each gene in the universe will appear in a gene set without enrichment. Will be computed from <code>covariate</code> if the latter is provided. Ignored if <code>universe</code> is NULL. |
| <code>covariate</code> | optional numeric vector of the same length as <code>universe</code> giving a covariate against which <code>null.prob</code> should be computed. Ignored if <code>universe</code> is NULL. |

plot logical, should the null.prob vs covariate trend be plotted?
 ... any other arguments in a call to the MArrayLM methods are passed to the corresponding default method.

Details

These functions perform over-representation analyses for Gene Ontology terms or KEGG pathways. The default methods accept a gene set as a vector of Entrez Gene IDs or multiple gene sets as a list of such vectors. An over-representation analysis is then done for each set. The MArrayLM method extracts the gene sets automatically from a linear model fit object.

The p-values returned by goana and kegger are unadjusted for multiple testing. The authors have chosen not to correct automatically for multiple testing because GO terms and KEGG pathways are often overlapping, so standard methods of p-value adjustment may be very conservative. Users should be aware though that p-values are unadjusted, meaning that only very small p-values should be used for published results.

goana uses annotation from the appropriate Bioconductor organism package. The species can be any character string XX for which an organism package org.XX.eg.db is installed. Examples are "Hs" for human or "Mm" for mouse. See [alias2Symbol](#) for other possible values for species.

kegger reads KEGG pathway annotation from the KEGG website. For kegger, the species name can be provided in either Bioconductor or KEGG format. Examples of KEGG format are "hsa" for human, "mmu" for mouse or "dme" for fly. kegger can be used for any species supported by KEGG, of which there are more than 14,000 possibilities. By default, kegger obtains the KEGG annotation for the specified species from the <https://rest.kegg.jp> website using getGeneKEGGLinks and getKEGGPathwayNames. Alternatively one can supply the required pathway annotation to kegger in the form of two data.frames. If this is done, then an internet connection is not required.

The gene ID system used by kegger for each species is determined by KEGG. For human and mouse, the default (and only choice) is Entrez Gene ID. For Drosophila, the default is FlyBase CG annotation symbol. The format of the IDs can be seen by typing head(getGeneKEGGLinks(species)), for example head(getGeneKEGGLinks("hsa")) or head(getGeneKEGGLinks("dme")). Entrez Gene IDs can always be used. If Entrez Gene IDs are not the default, then conversion can be done by specifying "convert=TRUE".

Another possibility is to use KEGG orthology IDs as the gene IDs, and these can be used for any species. In that case, set species.KEGG="ko".

The ability to supply data.frame annotation to kegger means that kegger can in principle be used in conjunction with any user-supplied set of annotation terms.

The default goana and kegger methods accept a vector null.prob giving the prior probability that each gene in the universe appears in a gene set. This vector can be used to correct for unwanted trends in the differential expression analysis associated with gene length, gene abundance or any other covariate (Young et al, 2010). The MArrayLM object computes the null.prob vector automatically when trend is non-NULL.

If null.prob=NULL, the function computes one-sided hypergeometric tests equivalent to Fisher's exact test. If prior probabilities are specified, then a test based on the Wallenius' noncentral hypergeometric distribution is used to adjust for the relative probability that each gene will appear in a gene set, following the approach of Young et al (2010).

The MArrayLM methods performs over-representation analyses for the up and down differentially expressed genes from a linear model analysis. In this case, the universe is all the genes found in the fit object.

trend=FALSE is equivalent to null.prob=NULL. If trend=TRUE or a covariate is supplied, then a trend is fitted to the differential expression results and this is used to set null.prob.

The statistical approach provided here is the same as that provided by the goseq package, with one methodological difference and a few restrictions. Unlike the goseq package, the gene identifiers here must be Entrez Gene IDs and the user is assumed to be able to supply gene lengths if necessary. The goseq package has additional functionality to convert gene identifiers and to provide gene lengths. The only methodological difference is that goana and kegg computes gene length or abundance bias using `tricubeMovingAverage` instead of monotonic regression. While `tricubeMovingAverage` does not enforce monotonicity, it has the advantage of numerical stability when it contains only a small number of genes. The trend is estimated by the `goanaTrend` function.

Value

The goana default method produces a data frame with a row for each GO term and the following columns:

| | |
|------|--|
| Term | GO term. |
| Ont | ontology that the GO term belongs to. Possible values are "BP", "CC" and "MF". |
| N | number of genes in the GO term. |
| DE | number of genes in the DE set. |
| P.DE | p-value for over-representation of the GO term in the set. |

The last two column names above assume one gene set with the name DE. In general, there will be a pair of such columns for each gene set and the name of the set will appear in place of "DE".

The goana method for MArrayLM objects produces a data frame with a row for each GO term and the following columns:

| | |
|--------|--|
| Term | GO term. |
| Ont | ontology that the GO term belongs to. Possible values are "BP", "CC" and "MF". |
| N | number of genes in the GO term. |
| Up | number of up-regulated differentially expressed genes. |
| Down | number of down-regulated differentially expressed genes. |
| P.Up | p-value for over-representation of GO term in up-regulated genes. |
| P.Down | p-value for over-representation of GO term in down-regulated genes. |

The row names of the data frame give the GO term IDs.

The output from kegg is the same except that row names become KEGG pathway IDs, Term becomes Pathway and there is no Ont column.

Note

kegga requires an internet connection unless `gene.pathway` and `pathway.names` are both supplied. The default for kegga with `species="Dm"` changed from `convert=TRUE` to `convert=FALSE` in limma 3.27.8. Users wanting to use Entrez Gene IDs for *Drosophila* should set `convert=TRUE`, otherwise fly-base CG annotation symbol IDs are assumed (for example "Dme1_CG4637").

The default for `restrict.universe=TRUE` in kegga changed from TRUE to FALSE in limma 3.33.4.

Bug fix: results from kegga with `trend=TRUE` or with non-NULL covariate were incorrect prior to limma 3.32.3. The results were biased towards significant Down p-values and against significant Up p-values.

Author(s)

Gordon Smyth and Yifang Hu

References

Young MD, Wakefield MJ, Smyth GK, Oshlack A (2010). Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome Biology* 11, R14. doi:10.1186/gb2010112r14

See Also

[topGO](#), [topKEGG](#), [goana](#)

The goseq package provides an alternative implementation of methods from Young et al (2010). Unlike the limma functions documented here, goseq will work with a variety of gene identifiers and includes a database of gene length information for various species.

The gostat package also does GO analyses without adjustment for bias but with some other options.

See [10.GeneSetTests](#) for a description of other functions used for gene set testing.

Examples

```
## Not run:
## Linear model usage:

fit <- lmFit(y, design)
fit <- eBayes(fit)

# Standard GO analysis

go.fisher <- goana(fit, species="Hs")
topGO(go.fisher, sort = "up")
topGO(go.fisher, sort = "down")

# GO analysis adjusting for gene abundance

go.abund <- goana(fit, geneid = "GeneID", trend = TRUE)
topGO(go.abund, sort = "up")
topGO(go.abund, sort = "down")
```

```

# GO analysis adjusting for gene length bias
# (assuming that y$genes$Length contains gene lengths)

go.len <- goana(fit, geneid = "GeneID", trend = "Length")
topGO(go.len, sort = "up")
topGO(go.len, sort = "down")

## Default usage with a list of gene sets:

go.de <- goana(list(DE1 = EG.DE1, DE2 = EG.DE2, DE3 = EG.DE3))
topGO(go.de, sort = "DE1")
topGO(go.de, sort = "DE2")
topGO(go.de, ontology = "BP", sort = "DE3")
topGO(go.de, ontology = "CC", sort = "DE3")
topGO(go.de, ontology = "MF", sort = "DE3")

## Standard KEGG analysis

k <- kegga(fit, species="Hs")
k <- kegga(fit, species.KEGG="hsa") # equivalent to previous
topKEGG(k, sort = "up")
topKEGG(k, sort = "down")

## End(Not run)

```

goanaTrend

Estimate DE Trend for Gene Ontology or KEGG Pathway Analysis

Description

Given a list of differentially expressed (DE) genes and a covariate, estimate the probability of a gene being called significant as a function of the covariate. This function is typically used to estimate the gene length or gene abundance bias for a pathway analysis.

Usage

```

goanaTrend(index.de, covariate, n.prior = 10, plot = FALSE,
           xlab = "Covariate Rank", ylab = "Probability gene is DE",
           main="DE status vs covariate")

```

Arguments

| | |
|-----------|---|
| index.de | an index vector specifying which genes are significantly DE. Can be a vector of integer indices, or a logical vector of length <code>nrow(covariate)</code> , or any vector such as <code>covariate[index]</code> selects the DE genes. |
| covariate | numeric vector, length equal to the number of genes in the analysis. Usually equal to gene length or average log-expression but can be any meaningful gene-wise covariate. |

| | |
|----------------------|---|
| <code>n.prior</code> | prior number of genes using for moderating the trend towards constancy, for stability when the number of DE genes is small. |
| <code>plot</code> | if TRUE, plot the estimated trend. |
| <code>xlab</code> | label for x-axis of plot. |
| <code>ylab</code> | label for y-axis of plot. |
| <code>main</code> | main title for the plot. |

Details

goanaTrend is called by goana and kegga when the trend argument is used to correct for unwanted trends in the differential expression analysis associated with gene length, gene abundance or any other covariate (Young et al, 2010).

This function is analogous to the nullp function of the goseq package but the trend is estimated using [tricubeMovingAverage](#) instead of by monotonic regression. While [tricubeMovingAverage](#) does not enforce strict monotonicity, it has the advantage of numerical stability and statistical robustness when there are only a small number of DE genes.

This function also moderates the estimated trend slightly towards constancy to provide more stability. The degree of moderation is determined by the `n.prior` argument relative to the number of DE genes.

Value

Numeric vector of same length as `covariate` giving estimated probabilities.

Author(s)

Gordon Smyth and Yifang Hu

References

Young MD, Wakefield MJ, Smyth GK, Oshlack A (2010). Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome Biology* 11, R14. [doi:10.1186/gb2010112r14](https://doi.org/10.1186/gb2010112r14)

See Also

[goana](#), [kegga](#)

See [10.GeneSetTests](#) for a description of other functions used for gene set testing.

Examples

```
x <- runif(100)
i <- 1:10
goanaTrend(i, x, plot=TRUE)
```

`gridr`*Row and Column Positions on Microarray*

Description

Grid and spot row and column positions.

Usage

```
gridr(layout)
gridc(layout)
spotr(layout)
spotc(layout)
```

Arguments

`layout` list with the components `ngrid.r`, `ngrid.c`, `nspot.r` and `nspot.c`

Value

Vector of length `prod(unlist(layout))` giving the grid rows (`gridr`), grid columns (`gridc`), spot rows (`spotr`) or spot columns (`spotc`).

Author(s)

Gordon Smyth

`head`*Return the First to Last Part of a Data Object*

Description

Retrieve the first or last parts of an `RGList`, `MAList`, `EListRaw`, `EList`, `MArrayLM` or `TestResults` object.

Usage

```
## S3 method for class 'EList'
head(x, n = 6L, ...)
## S3 method for class 'EList'
tail(x, n = 6L, ...)
```

Arguments

x an object of class RGList, MAList, EListRaw, EList, MArrayLM or TestResults.
 n a single integer. If positive or zero, number rows of resulting object. If negative, all but the n last/first rows of x.
 ... other arguments are not currently used.

Details

head (tail) returns the first (last) n rows when $n \geq 0$ or all but the last (first) n rows when $n < 0$.

Value

An object like x but generally with fewer rows.

Author(s)

Gordon Smyth

See Also

[head](#) in the utils package.

[02.Classes](#) gives an overview of data classes used in LIMMA.

Examples

```
E <- matrix(rnorm(40),20,2)
rownames(E) <- paste0("Gene",1:20)
colnames(E) <- c("A","B")
y <- new("EList",list(E=E))
head(y)
tail(y)
```

heatdiagram

Stemmed Heat Diagram

Description

Creates a heat diagram showing the co-regulation of genes under one condition with a range of other conditions.

Usage

```
heatDiagram(results, coef, primary=1, names=NULL, treatments=colnames(coef), limit=NULL,
  orientation="landscape", low="green", high="red", cex=1, mar=NULL,
  ncolors=123, ...)
heatdiagram(stat, coef, primary=1, names=NULL, treatments=colnames(stat),
  critical.primary=4, critical.other=3, limit=NULL, orientation="landscape",
  low="green", high="red", cex=1, mar=NULL, ncolors=123, ...)
```

Arguments

| | |
|-------------------------------|--|
| <code>results</code> | TestResults matrix, containing elements -1, 0 or 1, from <code>decideTests</code> |
| <code>stat</code> | numeric matrix of test statistics. Rows correspond to genes and columns to treatments or contrasts between treatments. |
| <code>coef</code> | numeric matrix of the same size as <code>stat</code> . Holds the coefficients to be displayed in the plot. |
| <code>primary</code> | number or name of the column to be compared to the others. Genes are included in the diagram according to this column of <code>stat</code> and are sorted according to this column of <code>coef</code> . If <code>primary</code> is a name, then <code>stat</code> and <code>coef</code> must have the same column names. |
| <code>names</code> | optional character vector of gene names |
| <code>treatments</code> | optional character vector of treatment names |
| <code>critical.primary</code> | critical value above which the test statistics for the primary column are considered significant and included in the plot |
| <code>critical.other</code> | critical value above which the other test statistics are considered significant. Should usually be no larger than <code>critical.primary</code> although larger values are permitted. |
| <code>limit</code> | optional value for <code>coef</code> above which values will be plotted in extreme color. Defaults to <code>max(abs(coef))</code> . |
| <code>orientation</code> | "portrait" for upright plot or "landscape" for plot orientated to be wider than high. "portrait" is likely to be appropriate for inclusion in printed document while "landscape" may be appropriate for a presentation on a computer screen. |
| <code>low</code> | color associated with repressed gene regulation |
| <code>high</code> | color associated with induced gene regulation |
| <code>ncolors</code> | number of distinct colors used for each of up and down regulation |
| <code>cex</code> | factor to increase or decrease size of column and row text |
| <code>mar</code> | numeric vector of length four giving the size of the margin widths. Default is <code>cex*c(5, 6, 1, 1)</code> for landscape and <code>cex*c(1, 1, 4, 3)</code> for portrait. |
| <code>...</code> | any other arguments will be passed to the <code>image</code> function |

Details

Users are encouraged to use `heatDiagram` rather than `heatdiagram` as the later function may be removed in future versions of `limma`.

This function plots an image of gene expression profiles in which rows (or columns for portrait orientation) correspond to treatment conditions and columns (or rows) correspond to genes. Only genes which are significantly differentially expressed in the primary condition are included. Genes are sorted by differential expression under the primary condition.

Note: the plot produced by this function is unique to the `limma` package. It should not be confused with "heatmaps" often used to display results from cluster analyses.

Value

An image is created on the current graphics device. A matrix with named rows containing the coefficients used in the plot is also invisibly returned.

Author(s)

Gordon Smyth

See Also

[image.](#)

Examples

```
## Not run:
MA <- normalizeWithinArrays(RG)
design <- cbind(c(1,1,1,0,0,0),c(0,0,0,1,1,1))
fit <- lmFit(MA,design=design)
contrasts.mouse <- cbind(Control=c(1,0),Mutant=c(0,1),Difference=c(-1,1))
fit <- eBayes(contrasts.fit(fit,contrasts=contrasts.mouse))
results <- decideTests(fit,method="global",p=0.1)
heatDiagram(results,fit$coef,primary="Difference")

## End(Not run)
```

helpMethods

Prompt for Method Help Topics

Description

For any S4 generic function, find all methods defined in currently loaded packages. Prompt the user to choose one of these to display the help document.

Usage

```
helpMethods(genericFunction)
```

Arguments

genericFunction
a generic function or a character string giving the name of a generic function

Author(s)

Gordon Smyth

See Also

[showMethods](#)

Examples

```
## Not run: helpMethods(show)
```

ids2indices

Convert Gene Identifiers to Indices for Gene Sets

Description

Make a list of gene identifiers into a list of indices for gene sets.

Usage

```
ids2indices(gene.sets, identifiers, remove.empty=TRUE)
```

Arguments

| | |
|---------------------------|--|
| <code>gene.sets</code> | list of character vectors, each vector containing the gene identifiers for a set of genes. |
| <code>identifiers</code> | character vector of gene identifiers. |
| <code>remove.empty</code> | logical, should sets of size zero be removed from the output? |

Details

This function used to create input for `romer`, `mroast` and `camera` function. Typically, `identifiers` is the vector of Entrez Gene IDs, and `gene.sets` is obtained constructed from a database of gene sets, for example a representation of the Molecular Signatures Database (MSigDB) downloaded from <https://bioinf.wehi.edu.au/software/MSigDB/>.

Value

list of integer vectors, each vector containing the indices of a gene set in the vector `identifiers`.

Author(s)

Gordon Smyth and Yifang Hu

See Also

[romer](#), [mroast](#), [camera](#)

There is a topic page on [10.GeneSetTests](#).

Examples

```
## Not run:

download.file("https://bioinf.wehi.edu.au/software/MSigDB/human_c2_v5p2.rdata",
             "human_c2_v5p2.rdata", mode = "wb")

load("human_c2_v5p2.rdata")
c2.indices <- ids2indices(Hs.c2, y$genes$GeneID)
camera(y, c2.indices, design)

## End(Not run)
```

imageplot

Image Plot of Microarray Statistics

Description

Creates an image of colors or shades of gray that represent the values of a statistic for each spot on a spotted microarray. This function can be used to explore any spatial effects across the microarray.

Usage

```
imageplot(z, layout, low = NULL, high = NULL, ncolors = 123, zerocenter = NULL,
          zlim = NULL, mar=c(2,1,1,1), legend=TRUE, ...)
```

Arguments

| | |
|------------|---|
| z | numeric vector or array. This vector can contain any spot statistics, such as log intensity ratios, spot sizes or shapes, or t-statistics. Missing values are allowed and will result in blank spots on the image. Infinite values are not allowed. |
| layout | a list specifying the dimensions of the spot matrix and the grid matrix. |
| low | color associated with low values of z. May be specified as a character string such as "green", "white" etc, or as a rgb vector in which c(1, 0, 0) is red, c(0, 1, 0) is green and c(0, 0, 1) is blue. The default value is "green" if zerocenter=T or "white" if zerocenter=F. |
| high | color associated with high values of z. The default value is "red" if zerocenter=T or "blue" if zerocenter=F. |
| ncolors | number of color shades used in the image including low and high. |
| zerocenter | should zero values of z correspond to a shade exactly halfway between the colors low and high? The default is TRUE if z takes positive and negative values, otherwise FALSE. |
| zlim | numerical vector of length 2 giving the extreme values of z to associate with colors low and high. By default zlim is the range of z. Any values of z outside the interval zlim will be truncated to the relevant limit. |

| | |
|--------|--|
| mar | numeric vector of length 4 specifying the width of the margin around the plot. This argument is passed to <code>par</code> . |
| legend | logical, if TRUE the range of z and <code>zlim</code> is shown in the bottom margin |
| ... | any other arguments will be passed to the function <code>image</code> |

Details

This function may be used to plot the values of any spot-specific statistic, such as the log intensity ratio, background intensity or a quality measure such as spot size or shape. The image follows the layout of an actual microarray slide with the bottom left corner representing the spot (1,1,1). The color range is used to represent the range of values for the statistic. When this function is used to plot the red/green log-ratios, it is intended to be an in silico version of the classic false-colored red-yellow-green image of a scanned two-color microarray.

This function is related to the earlier `plot.spatial` function in the `sma` package and to the later `maImage` function in the `marray` package. It differs from `plot.spatial` most noticeably in that all the spots are plotted and the image is plotted from bottom left rather than from top left. It is intended to display spatial patterns and artefacts rather than to highlight only the extreme values as does `plot.spatial`. It differs from `maImage` in that any statistic may be plotted and in its use of a red-yellow-green color scheme for log-ratios, similar to the classic false-colored jpeg image, rather than the red-black-green color scheme associated with heat maps.

Value

An plot is created on the current graphics device.

Author(s)

Gordon Smyth

See Also

`maImage` in the `marray` package, `image` in the `graphics` package.

An overview of diagnostic functions available in LIMMA is given in [09.Diagnostics](#).

Examples

```
M <- rnorm(8*4*16*16)
imageplot(M, layout=list(ngrid.r=8, ngrid.c=4, nspot.r=16, nspot.c=16))
```

imageplot3by2

Write Imageplots to Files

Description

Write imageplots to files in PNG format, six plots to a file in a 3 by 2 grid arrangement.

Usage

```
imageplot3by2(RG, z="Gb", prefix=paste("image",z,sep="-"), path=NULL,  
              xlim=NULL, common.lim=TRUE, ...)
```

Arguments

| | |
|------------|---|
| RG | an RGList or MAList object, or any list with component named by z |
| z | character string giving name of component of RG to plot |
| prefix | character string giving prefix to attach to file names |
| path | character string specifying directory for output files |
| xlim | numeric vector of length 2, giving limits of response vector to be associated with saturated colors |
| common.lim | logical, should all plots on a page use the same axis limits |
| ... | any other arguments are passed to imageplot |

Details

At the time of writing, this function writes plots in PNG format in an arrangement optimized for A4-sized paper.

Value

No value is returned, but one or more files are written to the working directory. The number of files is determined by the number of columns of RG.

Author(s)

Gordon Smyth

See Also

An overview of diagnostic functions available in LIMMA is given in [09.Diagnostics](#).

intraspotCorrelation *Intra-Spot Correlation for Two Color Data*

Description

Estimate the within-block correlation associated with spots for spotted two color microarray data.

Usage

```
intraspotCorrelation(object, design, trim=0.15)
```

Arguments

| | |
|--------|---|
| object | an <code>MAList</code> object or a list from which M and A values may be extracted |
| design | a numeric matrix containing the design matrix for linear model in terms of the individual channels. The number of rows should be twice the number of arrays. The number of columns will determine the number of coefficients estimated for each gene. |
| trim | the fraction of observations to be trimmed from each end of the atanh-correlations when computing the consensus correlation. See <code>mean</code> . |

Details

This function estimates the correlation between two channels observed on each spot. The correlation is estimated by fitting a heteroscedastic regression model to the M and A-values of each gene. The function also returns a consensus correlation, which is a robust average of the individual correlations, which can be used as input for functions `lmscFit`.

The function may take long time to execute.

Value

A list with components

| | |
|-----------------------|--|
| consensus.correlation | robust average of the estimated inter-duplicate correlations. The average is the trimmed mean of the correlations for individual genes on the atanh-transformed scale. |
| atanh.correlations | a numeric vector giving the individual genewise correlations on the atanh scale |
| df | numeric matrix of degrees of freedom associated with the correlations. The first column gives the degrees of freedom for estimating the within-spot or M-value mean square while the second gives the degrees of freedom for estimating the between spot or A-value mean square. |

Author(s)

Gordon Smyth

References

Smyth, G. K. (2005). Individual channel analysis of two-colour microarray data. *Proceedings of the 55th Session of the International Statistics Institute*, 5-12 April 2005, Sydney, Australia, Paper 116. <https://gksmyth.github.io/pubs/ISI2005-116.pdf>

See Also

This function uses `remIscore` from the `statmod` package.

An overview of methods for single channel analysis in `limma` is given by [07.SingleChannel](#).

Examples

```
# See lmscFit
## Not run:
corfit <- intraspotCorrelation(MA, design)
all.correlations <- tanh(corfit$atanh.correlations)
boxplot(all.correlations)

## End(Not run)
```

| | |
|-------------|-----------------------------------|
| is.fullrank | <i>Check for Full Column Rank</i> |
|-------------|-----------------------------------|

Description

Test whether a numeric matrix has full column rank.

Usage

```
is.fullrank(x)
nonEstimable(x)
```

Arguments

x a numeric matrix or vector

Details

is.fullrank is used to check the integrity of design matrices in limma, for example after [subsetting](#) operations.

nonEstimable is used by [lmFit](#) to report which coefficients in a linear model cannot be estimated.

Value

is.fullrank returns TRUE or FALSE.

nonEstimable returns a character vector of names for the columns of x which are linearly dependent on previous columns. If x has full column rank, then the value is NULL.

Author(s)

Gordon Smyth

Examples

```
# TRUE
is.fullrank(1)
is.fullrank(cbind(1,0:1))

# FALSE
is.fullrank(0)
is.fullrank(matrix(1,2,2))
nonEstimable(matrix(1,2,2))
```

`isNumeric`*Test for Numeric Argument*

Description

Test whether argument is numeric or a data.frame with numeric columns.

Usage

```
isNumeric(x)
```

Arguments

x any object

Details

This function is used to check the validity of arguments for numeric functions. It is an attempt to emulate the behavior of internal generic math functions.

`isNumeric` differs from `is.numeric` in that data.frames with all columns numeric are accepted as numeric.

Value

TRUE or FALSE

Author(s)

Gordon Smyth

See Also

[is.numeric](#), [Math](#)

Examples

```
isNumeric(3)
isNumeric("a")
x <- data.frame(a=c(1,1),b=c(0,1))
isNumeric(x) # TRUE
is.numeric(x) # FALSE
```

 kooperberg

Kooperberg Model-Based Background Correction for GenePix data

Description

This function uses a Bayesian model to background correct GenePix microarray data.

Usage

```
kooperberg(RG, a = TRUE, layout = RG$printer, verbose = TRUE)
```

Arguments

| | |
|---------|--|
| RG | an RGList of GenePix data, read in using <code>read.maimages</code> , with other <code>.columns=c("F635 SD", "B635 SD", "F532 SD", "B532 SD", "B532 Mean", "B635 Mean", "F Pixels", "B Pixels")</code> . |
| a | logical. If TRUE, the 'a' parameters in the model (equation 3 and 4) are estimated for each slide. If FALSE the 'a' parameters are set to unity. |
| layout | list containing print layout with components <code>ngrid.r</code> , <code>ngrid.c</code> , <code>nspot.r</code> and <code>nspot.c</code> . Defaults to <code>RG\$printer</code> . |
| verbose | logical. If TRUE, progress is reported to standard output. |

Details

This function is for use with GenePix data and is designed to cope with the problem of large numbers of negative intensities and hence missing values on the log-intensity scale. It avoids missing values in most cases and at the same time dampens down the variability of log-ratios for low intensity spots. See Kooperberg et al (2002) for more details.

`kooperberg` uses the foreground and background intensities, standard deviations and number of pixels to compute empirical estimates of the model parameters as described in equation 2 of Kooperberg et al (2002).

Value

An RGList containing the components

| | |
|---------|--|
| R | matrix containing the background adjusted intensities for the red channel for each spot for each array |
| G | matrix containing the background adjusted intensities for the green channel for each spot for each array |
| printer | list containing print layout |

Author(s)

Matthew Ritchie

References

Kooperberg, C., Fazio, T. G., Delrow, J. J., and Tsukiyama, T. (2002) Improved background correction for spotted DNA microarrays. *Journal of Computational Biology* **9**, 55-66.

Ritchie, M. E., Silver, J., Oshlack, A., Silver, J., Holmes, M., Diyagama, D., Holloway, A., and Smyth, G. K. (2007). A comparison of background correction methods for two-colour microarrays. *Bioinformatics* **23**, 2700-2707. doi:10.1093/bioinformatics/btm412

See Also

[04.Background](#) gives an overview of background correction functions defined in the LIMMA package.

Examples

```
# This is example code for reading and background correcting GenePix data
# given GenePix Results (gpr) files in the working directory (data not
# provided).
## Not run:
# get the names of the GenePix image analysis output files in the current directory
genepixFiles <- dir(pattern="*\\.gpr$")
RG <- read.maimages(genepixFiles, source="genepix", other.columns=c("F635 SD", "B635 SD",
" F532 SD", "B532 SD", "B532 Mean", "B635 Mean", "F Pixels", "B Pixels"))
RGmodel <- kooperberg(RG)
MA <- normalizeWithinArrays(RGmodel)

## End(Not run)
```

LargeDataObject-class *Large Data Object - class*

Description

A virtual class including the data classes RGList, MAList and MArrayLM, all of which typically contain large quantities of numerical data in vector, matrices and data.frames.

Methods

A show method is defined for objects of class LargeDataObject which uses printHead to print only the leading elements or rows of components or slots which contain large quantities of data.

Author(s)

Gordon Smyth

See Also

[02.Classes](#) gives an overview of all the classes defined by this package.

Examples

```
# see normalizeBetweenArrays
```

| | |
|-----------------|--------------------------------|
| limmaUsersGuide | <i>View Limma User's Guide</i> |
|-----------------|--------------------------------|

Description

Finds the location of the Limma User's Guide and optionally opens it.

Usage

```
limmaUsersGuide(view=TRUE)
```

Arguments

`view` logical, should the document be opened using the default PDF document reader?

Details

The function `vignette("limma")` will find the short limma Vignette which describes how to obtain the Limma User's Guide. The User's Guide is not itself a true vignette because it is not automatically generated using [Sweave](#) during the package build process. This means that it cannot be found using `vignette`, hence the need for this special function.

If the operating system is other than Windows, then the PDF viewer used is that given by `Sys.getenv("R_PDFVIEWER")`. The PDF viewer can be changed using `Sys.putenv(R_PDFVIEWER=)`.

This function is used by drop-down Vignettes menu when the Rgui interface for Windows is used.

Value

Character string giving the file location.

Author(s)

Gordon Smyth

See Also

[vignette](#), [openPDF](#), [openVignette](#), [Sys.getenv](#), [Sys.putenv](#)

Examples

```
limmaUsersGuide(view=FALSE)
```

lm.series

*Fit Linear Model to Microarray Data by Ordinary Least Squares***Description**

Fit a linear model genewise to expression data from a series of arrays. This function uses ordinary least squares and is a utility function for `lmFit`.

Usage

```
lm.series(M,design=NULL,ndups=1,spacing=1,weights=NULL)
```

Arguments

| | |
|----------------------|--|
| <code>M</code> | numeric matrix containing log-ratio or log-expression values for a series of microarrays, rows correspond to genes and columns to arrays |
| <code>design</code> | numeric design matrix defining the linear model. The number of rows should agree with the number of columns of <code>M</code> . The number of columns will determine the number of coefficients estimated for each gene. |
| <code>ndups</code> | number of duplicate spots. Each gene is printed <code>ndups</code> times in adjacent spots on each array. |
| <code>spacing</code> | the spacing between the rows of <code>M</code> corresponding to duplicate spots, <code>spacing=1</code> for consecutive spots |
| <code>weights</code> | an optional numeric matrix of the same dimension as <code>M</code> containing weights for each spot. If it is of different dimension to <code>M</code> , it will be filled out to the same size. |

Details

This is a utility function used by the higher level function `lmFit`. Most users should not use this function directly but should use `lmFit` instead.

The linear model is fit for each gene by calling the function `lm.fit` or `lm.wfit` from the base library.

Value

A list with components

| | |
|-----------------------------|--|
| <code>coefficients</code> | numeric matrix containing the estimated coefficients for each linear model. Same number of rows as <code>M</code> , same number of columns as <code>design</code> . |
| <code>stdev.unscaled</code> | numeric matrix conformal with <code>coef</code> containing the unscaled standard deviations for the coefficient estimators. The standard errors are given by <code>stdev.unscaled * sigma</code> . |
| <code>sigma</code> | numeric vector containing the residual standard deviation for each gene. |
| <code>df.residual</code> | numeric vector giving the degrees of freedom corresponding to <code>sigma</code> . |
| <code>qr</code> | QR-decomposition of <code>design</code> |

Author(s)

Gordon Smyth

See Also[lm.fit](#).An overview of linear model functions in limma is given by [06.LinearModels](#).**Examples**

```
# See lmFit for examples
```

`lmFit`*Linear Model for Series of Arrays*

Description

Fit linear model for each gene given a series of arrays

Usage

```
lmFit(object, design = NULL, ndups = NULL, spacing = NULL, block = NULL, correlation,
      weights = NULL, method = "ls", ...)
```

Arguments

| | |
|--------------------------|--|
| <code>object</code> | A matrix-like data object containing log-ratios or log-expression values for a series of arrays, with rows corresponding to genes and columns to samples. Any type of data object that can be processed by getEAWP is acceptable. |
| <code>design</code> | the design matrix of the microarray experiment, with rows corresponding to samples and columns to coefficients to be estimated. Defaults to <code>object\$design</code> if that is non-NULL, otherwise to the unit vector meaning that all samples will be treated as replicates of a single treatment group. |
| <code>ndups</code> | positive integer giving the number of times each distinct probe is printed on each array. |
| <code>spacing</code> | positive integer giving the spacing between duplicate occurrences of the same probe, <code>spacing=1</code> for consecutive rows. |
| <code>block</code> | vector or factor specifying a blocking variable on the arrays. Has length equal to the number of arrays. Must be NULL if <code>ndups>2</code> . |
| <code>correlation</code> | the inter-duplicate or inter-technical replicate correlation |
| <code>weights</code> | non-negative precision weights. Can be a numeric matrix of individual weights of same size as the object expression matrix, or a numeric vector of array weights with length equal to <code>ncol</code> of the expression matrix, or a numeric vector of gene weights with length equal to <code>nrow</code> of the expression matrix. |
| <code>method</code> | fitting method; "ls" for least squares or "robust" for robust regression. Note that the <code>block</code> and <code>correlation</code> arguments will be ignored if <code>method="robust"</code> . |
| <code>...</code> | other optional arguments to be passed to <code>lm.series</code> , <code>gls.series</code> or <code>mr1m</code> |

Details

This function fits multiple linear models by weighted or generalized least squares. It accepts data from an experiment involving a series of microarrays with the same set of probes. A linear model is fitted to the expression data for each probe. The expression data should be log-ratios for two-color array platforms or log-expression values for one-channel platforms. (To fit linear models to the individual channels of two-color array data, see `lmScFit`.) The coefficients of the fitted models describe the differences between the RNA sources hybridized to the arrays. The probe-wise fitted model results are stored in a compact form suitable for further processing by other functions in the `limma` package.

The function allows for missing values and accepts quantitative precision weights through the `weights` argument. It also supports two different correlation structures. If `block` is not `NULL` then different arrays are assumed to be correlated. If `block` is `NULL` and `ndups` is greater than one then replicate spots on the same array are assumed to be correlated. It is not possible at this time to fit models with both a `block` structure and a duplicate-spot correlation structure simultaneously.

If `object` is a matrix then it should contain log-ratios or log-expression data with rows corresponding to probes and columns to arrays. (A numeric vector is treated the same as a matrix with one column.) For objects of other classes, a matrix of expression values is taken from the appropriate component or slot of the object. If `object` is of class `MAList` or `marrayNorm`, then the matrix of log-ratios (M-values) is extracted. If `object` is of class `ExpressionSet`, then the expression matrix is extracted. (This may contain log-expression or log-ratio values, depending on the platform.) If `object` is of class `PLMset` then the matrix of chip coefficients `chip.coefs` is extracted.

The arguments `design`, `ndups`, `spacing` and `weights` will be extracted from the data object if available. On the other hand, if any of these are set to a non-`NULL` value in the function call then this value will over-ride the value found in `object`. If `object` is an `PLMset`, then weights are computed as $1/\text{pmax}(\text{object@se.chip.coefs}, 1e-05)^2$. If `object` is an `ExpressionSet` object, then weights are not computed.

If the argument `block` is used, then it is assumed that `ndups=1`.

The `correlation` argument has a default value of 0.75, but in normal use this default value should not be relied on and the correlation value should be estimated using the function `duplicateCorrelation`. The default value is likely to be too high in particular if used with the `block` argument.

The actual linear model computations are done by passing the data to one of the lower-level functions `lm.series`, `gls.series` or `mr.lm`. The function `mr.lm` is used if `method="robust"`. If `method="ls"`, then `gls.series` is used if a correlation structure has been specified, i.e., if `ndups>1` or `block` is non-null and `correlation` is different from zero. If `method="ls"` and there is no correlation structure, `lm.series` is used. If `method="robust"` then any correlation structure will be ignored.

Value

An `MArrayLM` object containing the result of the fits.

The rownames of `object` are preserved in the fit object and can be retrieved by `rownames(fit)` where `fit` is output from `lmFit`. The column names of `design` are preserved as column names and can be retrieved by `colnames(fit)`.

Author(s)

Gordon Smyth

See Also

lmFit uses [getEAWP](#) to extract expression values, gene annotation and so from the data object.
An overview of linear model functions in limma is given by [06.LinearModels](#).

Examples

```
# Simulate gene expression data for 100 probes and 6 microarrays
# Microarray are in two groups
# First two probes are differentially expressed in second group
# Std deviations vary between genes with prior df=4
sd <- 0.3*sqrt(4/rchisq(100,df=4))
y <- matrix(rnorm(100*6,sd=sd),100,6)
rownames(y) <- paste("Gene",1:100)
y[1:2,4:6] <- y[1:2,4:6] + 2
design <- cbind(Grp1=1,Grp2vs1=c(0,0,0,1,1,1))
options(digits=3)

# Ordinary fit
fit <- lmFit(y,design)
fit <- eBayes(fit)
topTable(fit,coef=2)
dim(fit)
colnames(fit)
rownames(fit)[1:10]
names(fit)

# Fold-change thresholding
fit2 <- treat(fit,lfc=0.1)
topTreat(fit2,coef=2)

# Volcano plot
volcanoplot(fit,coef=2,highlight=2)

# Mean-difference plot
plotMD(fit,column=2)

# Q-Q plot of moderated t-statistics
qqt(fit$t[,2],df=fit$df.residual+fit$df.prior)
abline(0,1)

# Various ways of writing results to file
## Not run: write.fit(fit,file="exampleresults.txt")
## Not run: write.table(fit,file="exampleresults2.txt")

# Fit with correlated arrays
# Suppose each pair of arrays is a block
block <- c(1,1,2,2,3,3)
dupcor <- duplicateCorrelation(y,design,block=block)
dupcor$consensus.correlation
fit3 <- lmFit(y,design,block=block,correlation=dupcor$consensus)

# Fit with duplicate probes
```

```
# Suppose two side-by-side duplicates of each gene
rownames(y) <- paste("Gene",rep(1:50,each=2))
dupcor <- duplicateCorrelation(y,design,ndups=2)
dupcor$consensus.correlation
fit4 <- lmFit(y,design,ndups=2,correlation=dupcor$consensus)
dim(fit4)
fit4 <- eBayes(fit4)
topTable(fit4,coef=2)
```

lmscFit*Fit Linear Model to Individual Channels of Two-Color Data*

Description

Fit a linear model to the individual log-intensities for each gene given a series of two-color arrays

Usage

```
lmscFit(object, design, correlation)
```

Arguments

| | |
|-------------|---|
| object | an MAList object or a list from which M and A values may be extracted |
| design | a numeric matrix containing the design matrix for linear model in terms of the individual channels. The number of rows should be twice the number of arrays. The number of columns will determine the number of coefficients estimated for each gene. |
| correlation | numeric value giving the intra-spot correlation |

Details

For two color arrays, the channels measured on the same set of arrays are correlated. The M and A however are uncorrelated for each gene. This function fits a linear model to the set of M and A-values for each gene after re-scaling the M and A-values to have equal variances. The input correlation determines the scaling required. The input correlation is usually estimated using [intraspotCorrelation](#) before using `lmscFit`.

Missing values in M or A are not allowed.

Value

An object of class [MArrayLM](#)

Author(s)

Gordon Smyth

References

Smyth, GK (2005). Individual channel analysis of two-colour microarray data. *Proceedings of the 55th Session of the International Statistics Institute*, 5-12 April 2005, Sydney, Australia; International Statistics Institute; Paper 116. <https://gksmyth.github.io/pubs/ISI2005-116.pdf>

Smyth, GK, and Altman, NS (2013). Separate-channel analysis of two-channel microarrays: recovering inter-spot information. *BMC Bioinformatics* 14, 165. doi:10.1186/1471210514165

See Also

[lm.fit](#).

An overview of methods for single channel analysis in limma is given by [07.SingleChannel](#).

Examples

```
## Not run:
# Subset of data from ApoAI case study in Limma User's Guide
# Avoid non-positive intensities
RG <- backgroundCorrect(RG,method="normexp")
MA <- normalizeWithinArrays(RG)
MA <- normalizeBetweenArrays(MA,method="Aq")
targets <- data.frame(Cy3=I(rep("Pool",6)),Cy5=I(c("WT","WT","WT","KO","KO","KO")))
targets.sc <- targetsA2C(targets)
targets.sc$Target <- factor(targets.sc$Target,levels=c("Pool","WT","KO"))
design <- model.matrix(~Target,data=targets.sc)
corfit <- intraspotCorrelation(MA,design)
fit <- lmscFit(MA,design,correlation=corfit$consensus)
cont.matrix <- cbind(KOvsWT=c(0,-1,1))
fit2 <- contrasts.fit(fit,cont.matrix)
fit2 <- eBayes(fit2)
topTable(fit2,adjust="fdr")

## End(Not run)
```

loessFit

Univariate Lowess With Prior Weights

Description

Univariate locally weighted linear regression allowing for prior weights. Returns fitted values and residuals.

Usage

```
loessFit(y, x, weights=NULL, span=0.3, iterations=4L, min.weight=1e-5, max.weight=1e5,
         equal.weights.as.null=TRUE, method="weightedLowess")
```

Arguments

| | |
|------------------------------------|---|
| <code>y</code> | numeric vector of response values. Missing values are allowed. |
| <code>x</code> | numeric vector of predictor values. Missing values are allowed. |
| <code>weights</code> | numeric vector of non-negative prior weights. Missing values are treated as zero. |
| <code>span</code> | positive numeric value between 0 and 1 specifying proportion of data to be used in the local regression moving window. Larger numbers give smoother fits. |
| <code>iterations</code> | number of local regression fits. Values greater than 1 produce robust fits. |
| <code>min.weight</code> | minimum weight. Any lower weights will be reset. |
| <code>max.weight</code> | maximum weight. Any higher weights will be reset. |
| <code>equal.weights.as.null</code> | should equal weights be treated as if weights were NULL, so that lowess is called? Applies even if all weights are all zero. |
| <code>method</code> | method used for weighted lowess. Possibilities are "weightedLowess", "loess" or "locfit". |

Details

This function is essentially a wrapper function for `lowess` and `weightedLowess` with added error checking. The idea is to provide the classic univariate lowess algorithm of Cleveland (1979) but allowing for prior weights and missing values.

The venerable `lowess` code is fast, uses little memory and has an accurate interpolation scheme, so it is an advantage to use it when prior weights are not needed. This function calls `lowess` when `weights=NULL`, but returns values in original rather than sorted order and allows missing values. The treatment of missing values is analogous to `na.exclude`.

By default, `weights` that are all equal (even all zero) are treated as if they were NULL, so `lowess` is called in this case also.

When unequal `weights` are provided, this function calls `weightedLowess` by default, although two other possibilities are also provided. `weightedLowess` implements a similar algorithm to `lowess` except that it uses the prior weights both in the local regressions and in determining which other observations to include in the local neighbourhood of each observation.

Two alternative algorithms for weighted lowess curve fitting are provided as options. If `method="loess"`, then a call is made to `loess(y~x, weights=weights, span=span, degree=1, family="symmetric", ...)`. This method differs from `weightedLowess` in that the prior weights are ignored when determining the neighbourhood of each observation.

If `method="locfit"`, then repeated calls are made to `locfit::locfit.raw` with `deg=1`. In principle, this is similar to "loess", but "locfit" makes some approximations and is very much faster and uses much less memory than "loess" for long data vectors.

The arguments `span` and `iterations` here have the same meaning as for `weightedLowess` and `loess`. `span` is equivalent to the argument `f` of `lowess` while `iterations` is equivalent to `iter+1` for `lowess`. It gives the total number of fits rather than the number of robustifying fits.

When there are insufficient observations to estimate the loess curve, `loessFit` returns a linear regression fit. This mimics the behavior of `lowess` but not that of `loess` or `locfit.raw`.

Value

A list with components

| | |
|-----------|--|
| fitted | numeric vector of same length as y giving the loess fit |
| residuals | numeric vector of same length as x giving residuals from the fit |

Note

With unequal weights, "loess" was the default method prior to limma version 3.17.25. The default was changed to "locfit" in limma 3.17.25, and then to "weightedLowess" in limma 3.19.16. "weightedLowess" will potentially give somewhat different results to the older algorithms because the local neighbourhood of each observation is determined differently (more carefully).

Author(s)

Gordon Smyth

References

Cleveland, W. S. (1979). Robust locally weighted regression and smoothing scatterplots. *Journal of the American Statistical Association* 74, 829-836.

See Also

If weights=NULL, this function calls [lowess](#). Otherwise it calls [weightedLowess](#), [locfit.raw](#) or [loess](#). See the help pages of those functions for references and credits.

Compare with [loess](#) in the stats package.

See [05.Normalization](#) for an outline of the limma package normalization functions.

Examples

```
x <- (1:100)/101
y <- sin(2*pi*x)+rnorm(100,sd=0.4)
out <- loessFit(y,x)
plot(x,y)
lines(x,out$fitted,col="red")

# Example using weights

y <- x-0.5
w <- rep(c(0,1),50)
y[w==0] <- rnorm(50,sd=0.1)
pch <- ifelse(w>0,16,1)
plot(x,y,pch=pch)
out <- loessFit(y,x,weights=w)
lines(x,out$fitted,col="red")
```

| | |
|---------|--------------------------|
| logcosh | <i>Logarithm of cosh</i> |
|---------|--------------------------|

Description

Compute $\log(\cosh(x))$ without floating overflow or underflow

Usage

```
logcosh(x)
```

Arguments

x a numeric vector or matrix.

Details

The computation uses asymptotic expressions for very large or very small arguments. For intermediate arguments, $\log(\cosh(x))$ is returned.

Value

Numeric vector or matrix of same dimensions as x.

Author(s)

Gordon K Smyth

See Also

[logsumexp](#)

Examples

```
x <- c(1e-8, 1e-7, 1e-6, 1e-5, 1e-4, 1, 3, 50, 800)
logcosh(x)
log(cosh(x))
```

`logsumexp`*Log Sum of Exponentials*

Description

Compute $\log(\exp(x)+\exp(y))$ without floating overflow or underflow

Usage

```
logsumexp(x, y)
```

Arguments

`x` a numeric vector or matrix.
`y` a numeric vector or matrix of same size as `x`.

Details

The computation uses `logcosh()`.

Value

Numeric vector or matrix of same dimensions as `x`.

Author(s)

Gordon K Smyth

See Also

[logcosh](#)

Examples

```
x <- y <- c(1e-8,1e-7,1e-6,1e-5,1e-4,1,3,50,800)
logsumexp(x,y)
log( exp(x)+exp(y) )
```

`ma3x3`*Two dimensional Moving Averages with 3x3 Window*

Description

Apply a specified function to each to each value of a matrix and its immediate neighbors.

Usage

```
ma3x3.matrix(x,FUN=mean,na.rm=TRUE,...)
ma3x3.spottedarray(x,printer,FUN=mean,na.rm=TRUE,...)
```

Arguments

| | |
|----------------------|---|
| <code>x</code> | numeric matrix |
| <code>FUN</code> | function to apply to each window of values |
| <code>na.rm</code> | logical value, should missing values be removed when applying FUN |
| <code>...</code> | other arguments are passed to FUN |
| <code>printer</code> | list giving the printer layout, see PrintLayout-class |

Details

For `ma3x3.matrix`, `x` is an arbitrary function. for `ma3x3.spotted`, each column of `x` is assumed to contain the expression values of a spotted array in standard order. The printer layout information is used to re-arrange the values of each column as a spatial matrix before applying `ma3x3.matrix`.

Value

Numeric matrix of same dimension as `x` containing smoothed values

Author(s)

Gordon Smyth

See Also

An overview of functions for background correction are given in [04.Background](#).

Examples

```
x <- matrix(c(2,5,3,1,6,3,10,12,4,6,4,8,2,1,9,0),4,4)
ma3x3.matrix(x,FUN="mean")
ma3x3.matrix(x,FUN="min")
```

`makeContrasts`*Construct Matrix of Custom Contrasts*

Description

Construct the contrast matrix corresponding to specified contrasts of a set of parameters.

Usage

```
makeContrasts(..., contrasts=NULL, levels)
```

Arguments

| | |
|------------------------|--|
| <code>...</code> | expressions, or character strings which can be parsed to expressions, specifying contrasts |
| <code>contrasts</code> | character vector specifying contrasts |
| <code>levels</code> | character vector or factor giving the names of the parameters of which contrasts are desired, or a design matrix or other object with the parameter names as column names. |

Details

This function expresses contrasts between a set of parameters as a numeric matrix. The parameters are usually the coefficients from a linear model fit, so the matrix specifies which comparisons between the coefficients are to be extracted from the fit. The output from this function is usually used as input to `contrasts.fit`. The contrasts can be specified either as expressions using `...` or as a character vector through `contrasts`. (Trying to specify contrasts both ways will cause an error.)

The parameter names must be syntactically valid variable names in R and so, for example, must begin with a letter rather than a numeral. See `make.names` for a complete specification of what is a valid name.

Value

Matrix which columns corresponding to contrasts.

Author(s)

Gordon Smyth

See Also

An overview of linear model functions in limma is given by the help page [06.LinearModels](#).

Examples

```
makeContrasts(B-A,C-B,C-A,levels=c("A","B","C"))
makeContrasts(contrasts="A-(B+C)/2",levels=c("A","B","C"))
x <- c("B-A","C-B","C-A")
makeContrasts(contrasts=x,levels=c("A","B","C"))
```

makeUnique*Make Values of Character Vector Unique*

Description

Paste characters on to values of a character vector to make them unique.

Usage

```
makeUnique(x)
```

Arguments

x object to be coerced to a character vector

Details

Repeat values of x are labelled with suffixes "1", "2" etc.

Value

A character vector of the same length as x

Author(s)

Gordon Smyth

See Also

makeUnique is called by [merge.RGList](#). Compare with [make.unique](#) in the base package.

Examples

```
x <- c("a","a","b")
makeUnique(x)
```

| | |
|--------------|---|
| MAList-class | <i>M-value, A-value Expression List - class</i> |
|--------------|---|

Description

A simple list-based class for storing M-values and A-values for a batch of spotted microarrays. MAList objects are usually created during normalization by the functions [normalizeWithinArrays](#) or [MA.RG](#).

Slots/List Components

MAList objects can be created by `new("MAList", MA)` where MA is a list. This class contains no slots (other than `.Data`), but objects should contain the following components:

- M: numeric matrix containing the M-values (log-2 expression ratios). Rows correspond to spots and columns to arrays.
- A: numeric matrix containing the A-values (average log-2 expression values).

Optional components include:

- weights: numeric matrix of same dimensions as M containing relative spot quality weights. Elements should be non-negative.
- other: list containing other matrices, all of the same dimensions as M.
- genes: data.frame containing probe information. Should have one row for each spot. May have any number of columns.
- targets: data.frame containing information on the target RNA samples. Rows correspond to arrays. May have any number of columns.
- printer: list containing information on the process used to print the spots on the arrays. See [PrintLayout](#).

Valid MAList objects may contain other optional components, but all probe or array information should be contained in the above components.

Methods

This class inherits directly from class `list` so any operation appropriate for lists will work on objects of this class. In addition, MAList objects can be [subsetting](#) and [combined](#). RGList objects will return dimensions and hence functions such as `dim`, `nrow` and `ncol` are defined. MALists also inherit a `show` method from the virtual class [LargeDataObject](#), which means that RGLists will print in a compact way.

Other functions in LIMMA which operate on MAList objects include [normalizeWithinArrays](#), [normalizeBetweenArrays](#), [normalizeForPrintorder](#), [plotMA](#) and [plotPrintTipLoess](#).

Author(s)

Gordon Smyth

See Also

[02.Classes](#) gives an overview of all the classes defined by this package.
`marrayNorm` is the corresponding class in the `marray` package.

| | |
|----------------|--|
| MArrayLM-class | <i>Microarray Linear Model Fit - class</i> |
|----------------|--|

Description

A list-based S4 class for storing the results of fitting gene-wise linear models to a set of microarrays. Objects are normally created by `lmFit`, and additional components are added by `eBayes`.

Components

MArrayLM objects do not contain any slots (apart from `.Data`) but they should contain the following list components:

| | |
|-----------------------------|---|
| <code>coefficients</code> | matrix containing fitted coefficients or contrasts |
| <code>stdev.unscaled</code> | matrix containing unscaled standard deviations of the coefficients or contrasts |
| <code>sigma</code> | numeric vector containing residual standard deviations for each gene |
| <code>df.residual</code> | numeric vector containing residual degrees of freedom for each gene |

The following additional components may be created by `lmFit`:

| | |
|-------------------------------|--|
| <code>Amean</code> | numeric vector containing the average log-intensity for each probe over all the arrays in the original linear model |
| <code>genes</code> | data.frame containing probe annotation. |
| <code>design</code> | design matrix. |
| <code>cov.coefficients</code> | numeric matrix giving the unscaled covariance matrix of the estimable coefficients |
| <code>pivot</code> | integer vector giving the order of coefficients in <code>cov.coefficients</code> . Is computed by the QR-decomposition |
| <code>qr</code> | QR-decomposition of the design matrix (if the fit involved no weights or missing values). |
| <code>...</code> | other components returned by <code>lm.fit</code> (if the fit involved no weights or missing values). |

The following component may be added by `contrasts.fit`:

| | |
|------------------------|--|
| <code>contrasts</code> | numeric matrix defining contrasts of coefficients for which results are desired. |
|------------------------|--|

The following components may be added by `eBayes`:

| | |
|------------------------|---|
| <code>s2.prior</code> | numeric value or vector giving empirical Bayes estimated prior value for residual variances |
| <code>df.prior</code> | numeric value or vector giving empirical Bayes estimated degrees of freedom associated with <code>s2.prior</code> for each gene |
| <code>df.total</code> | numeric vector giving total degrees of freedom used for each gene, usually equal to <code>df.prior + df.residual</code> . |
| <code>s2.post</code> | numeric vector giving posterior residual variances |
| <code>var.prior</code> | numeric vector giving empirical Bayes estimated prior variance for each true coefficient |
| <code>F</code> | numeric vector giving moderated F-statistics for testing all contrasts equal to zero |
| <code>F.p.value</code> | numeric vector giving p-value corresponding to <code>F.stat</code> |
| <code>t</code> | numeric matrix containing empirical Bayes t-statistics |

Methods

MArrayLM objects will return dimensions and hence functions such as `dim`, `nrow` and `ncol` are defined. MArrayLM objects inherit a `show` method from the virtual class `LargeDataObject`.

The functions `eBayes`, `decideTests` and `classifyTestsF` accept MArrayLM objects as arguments.

Author(s)

Gordon Smyth

See Also

[02.Classes](#) gives an overview of all the classes defined by this package.

mdplot

Mean-Difference Plot

Description

Creates a mean-difference plot of two columns of a matrix.

Usage

```
mdplot(x, columns=c(1,2), xlab="Mean", ylab="Difference", main=NULL, ...)
```

Arguments

| | |
|----------------------|---|
| <code>x</code> | numeric matrix with at least two columns. |
| <code>columns</code> | which columns of <code>x</code> to compare. Plot will display second minus first. |
| <code>xlab</code> | label for the x-axis. |
| <code>ylab</code> | label for the y-axis. |
| <code>main</code> | title of the plot. Defaults to |
| <code>...</code> | any other arguments are passed to plotWithHighlights . |

Details

Plots differences vs means for a set of bivariate values. This is a generally useful approach for comparing two correlated measures of the same underlying phenomenon. Bland and Altman (1986) argue it is more information than a simple scatterplot of the two variables. The bivariate values are stored as columns of `x`.

Value

A plot is created on the current graphics device.

Author(s)

Gordon Smyth

References

Cleveland WS (1993). *Visualizing Data*. Hobart Press.

Bland JM, Altman DG (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 327, 307-310.

See also <http://www.statsci.org/micrarra/refs/maplots.html>

See Also

[plotWithHighlights](#)

[plotMD](#) is an object-oriented implementation of mean-difference plots for expression data.

An overview of diagnostic functions available in LIMMA is given in [09.Diagnostics](#).

Examples

```
x1 <- runif(100)
x2 <- (x1 + rnorm(100, sd=0.01))^1.2
oldpar <- par(mfrow=c(1,2))
plot(x1,x2)
mdplot(cbind(x1,x2),bg.pch=1,bg.cex=1)
par(oldpar)
```

merge

Merge RGList or MAList Data Objects

Description

Merge two microarray data sets represented by RGLists in possibly irregular order.

Usage

```
## S3 method for class 'RGList'
merge(x,y,...)
```

Arguments

| | |
|-----|---|
| x | data object of class RGList , MAList , EList or EListRaw . |
| y | data object of same class as x, corresponding to the same genes as for x, possibly in a different order, but with different arrays. |
| ... | other arguments are accepted but not used at present |

Details

RGList, MAList, EListRaw and EList data objects are lists containing numeric matrices all of the same dimensions. The data objects are merged by merging each of the components by row names or, if there are no row names, by IDs in the genes component. Unlike when using `cbind`, row names are not required to be in the same order or to be unique. In the case of repeated row names, the order of the rows with repeated names is preserved. This means that the first occurrence of each name in `x` is matched with the first occurrence of the same name in `y`, the second with the second, and so on. The final vector of row names is the same as in `x`.

Note: if the objects contain the same number of genes in the same order then the appropriate function to combine them is `cbind` rather than `merge`.

Value

An merged object of the same class as `x` and `y` with the same components as `x`. Component matrices have the same rows names as in `x` but columns from `y` as well as from `x`.

Author(s)

Gordon Smyth

See Also

R base provides a `merge` method for merging data.frames.

An overview of limma commands for reading, subsetting and merging data is given in [03.Reading-Data](#).

Examples

```
M <- A <- matrix(11:14,4,2)
rownames(M) <- rownames(A) <- c("a","a","b","c")
MA1 <- new("MAList",list(M=M,A=A))
```

```
M <- A <- matrix(21:24,4,2)
rownames(M) <- rownames(A) <- c("b","a","a","c")
MA2 <- new("MAList",list(M=M,A=A))
```

```
merge(MA1,MA2)
merge(MA2,MA1)
```

mergeScans

Merge two scans of two-color arrays

Description

Merge two sets of intensities of two-color arrays that are scanned twice at two different scanner settings, one at a lower gain setting with no saturated spot intensities and the other at a higher gain setting with a higher signal-to-noise ratio and some saturated spot intensities.

Usage

```
mergeScansRG(RGlow, RGhigh, AboveNoiseLowG=NULL, AboveNoiseLowR=NULL, outlierp=0.01)
```

Arguments

| | |
|----------------|---|
| RGlow | object of class <code>RGList</code> containing red and green intensities constituting two-color microarray data scanned at a lower gain setting. |
| RGhigh | object of class <code>RGList</code> containing red and green intensities constituting two-color microarray data scanned at a higher gain setting. |
| AboveNoiseLowG | matrix of 1 or 0 for low scan intensities of green color, 1 for spots above noise level or 0 otherwise. One column per array. |
| AboveNoiseLowR | matrix of 1 or 0 for low scan intensities of red color, 1 for spots above noise level or 0 otherwise. One column per array. |
| outlierp | p-value for outliers. 0 for no outlier detection or any value between 0 and 1. Default p-value is 0.01. |

Details

This function merges two separate scans of each fluorescent label on a two-color array scanned at two different scanner settings by using a nonlinear regression model consisting of two linear regression lines and a quadratic function connecting the two, which looks like a hockey stick. The changing point, i.e. the saturation point, in high scan is also estimated as part of model. Signals produced for certain spots can sometimes be very low (below noise) or too high (saturated) to be accurately read by the scanner. The proportions of spots that are below noise or above saturation are affected by the settings of the laser scanner used to read the arrays, with low scans minimizing saturation effects and high scans maximizing signal-to-noise ratios. Saturated spots can cause bias in intensity ratios that cannot be corrected for using conventional normalization methods.

Each fluorescent label on a two-color array can be scanned twice: for example, a high scan targeted at reaching saturation level for the brightest 1 percent of the spots on the array, and a low scan targeted at the lowest level of intensity which still allowed accurate grid placement on the arrays. By merging data from two separate laser scans of each fluorescent label on an array, we can avoid the potential bias in signal intensities due to below noise or above saturation and, thus provide better estimates of true differential expression as well as increase usable spots.

The merging process is designed to retain signal intensities from the high scan except when scanner saturation causes the high scan signal to be under-measured. The saturated spots are predicted from the corresponding low scans by the fitted regression model. It also checks any inconsistency between low and high scans.

Value

An object of class `RGList-class` with the following components:

| | |
|---|--|
| G | numeric matrix containing the merged green (cy3) foreground intensities. Rows correspond to spots and columns to arrays. |
| R | numeric matrix containing the merged red (cy5) foreground intensities. Rows correspond to spots and columns to arrays. |

| | |
|-------|---|
| Gb | numeric matrix containing the green (cy3) background intensities from high scan. |
| Rb | numeric matrix containing the red (cy5) background intensities from high scan. |
| other | list numeric matrices Gsaturated, Rsaturated, Goutlier and Routlier. The first two contain saturation flags (1=saturated, 0=otherwise) for the green (cy3) and red (Cy5) channels of the high scan. The second two contain outlier flags (1=outlier, 0=otherwise) for the green (cy3) and red (Cy5) channels. |

Author(s)

Dongseok Choi <choid@ohsu.edu>.

References

Choi D, O'Malley JP, Lasarev MR, Lapidus J, Lu X, Pattee P, Nagalla SR (2006). Extending the Dynamic Range of Signal Intensities in DNA Microarrays. *Online Journal of Bioinformatics*, **7**, 46-56.

Examples

```
## Not run:
#RG1: An RGList from low scan
#RG2: An RGList from high scan
RGmerged <- mergeScansRG(RG1, RG2, AboveNoiseLowG=ANc3, AboveNoiseLowR=ANc5)

#merge two scans when all spots are above noise in low scan and no outlier detection.
RGmerged <- mergeScansRG(RG1, RG2, outlierp=0)

## End(Not run)
```

modelMatrix

Construct Design Matrix

Description

Construct design matrix from RNA target information for a two colour microarray experiment.

Usage

```
modelMatrix(targets, parameters, ref, verbose=TRUE)
uniqueTargets(targets)
```

Arguments

| | |
|------------|--|
| targets | matrix or data.frame with columns Cy3 and Cy5 specifying which RNA was hybridized to each array |
| parameters | matrix specifying contrasts between RNA samples which should correspond to regression coefficients. Row names should correspond to unique RNA sample names found in targets. |
| ref | character string giving name of one of the RNA sources to be treated as reference. Exactly one argument of parameters or ref should be specified. |
| verbose | logical, if TRUE then unique names found in targets will be printed to standard output |

Details

This function computes a design matrix for input to `lmFit` when analysing two-color microarray experiments in terms of log-ratios.

If the argument `ref` is used, then the experiment is treated as a one-way layout and the coefficients measure expression changes relative to the RNA source specified by `ref`. The RNA source `ref` is often a common reference which appears on every array or is a control sample to which all the others are compared. There is no restriction however. One can choose `ref` to be any of the RNA sources appearing the Cy3 or Cy5 columns of `targets`.

If the `parameters` argument is set, then the columns of this matrix specify the comparisons between the RNA sources which are of interest. This matrix must be of size n by $(n-1)$, where n is the number of unique RNA sources found in Cy3 and Cy5, and must have row names which correspond to the RNA sources.

Value

`modelMatrix` produces a numeric design matrix with row names as in `targets` and column names as in `parameters`.

`uniqueTargets` produces a character vector of unique target names from the columns Cy3 and Cy5 of `targets`.

Author(s)

Gordon Smyth

See Also

`model.matrix` in the stats package.

An overview of linear model functions in `limma` is given by [06.LinearModels](#).

Examples

```
targets <- cbind(Cy3=c("Ref", "Control", "Ref", "Treatment"), Cy5=c("Control", "Ref", "Treatment", "Ref"))
rownames(targets) <- paste("Array", 1:4)

parameters <- cbind(C=c(-1, 1, 0), T=c(-1, 0, 1))
```

```
rownames(parameters) <- c("Ref", "Control", "Treatment")  
  
modelMatrix(targets, parameters)  
modelMatrix(targets, ref="Ref")
```

modifyWeights*Modify Matrix of Weights By Control Status of Rows*

Description

Modify weights matrix for given gene status values.

Usage

```
modifyWeights(weights=rep(1,length(status)), status, values, multipliers)
```

Arguments

| | |
|-------------|---|
| weights | numeric matrix of relative weights, rows corresponding to genes and columns to arrays |
| status | character vector giving the control status of each spot on the array, of same length as the number of rows of weights |
| values | character vector giving subset of the unique values of status |
| multipliers | numeric vector of same length as values giving factor by which weights will be modified |

Details

The function is usually used to temporarily modify the weights matrix during normalization of data. The function can be used for example to give zero weight to spike-in ratio control spots during normalization.

Value

Numeric matrix of same dimensions as weights with rows corresponding to values in status modified by the specified multipliers.

Author(s)

Gordon Smyth

See Also

An overview of normalization functions available in LIMMA is given in [05.Normalization](#).

Examples

```
w <- matrix(runif(6*3),6,3)
status <- c("Gene","Gene","Ratio_Control","Ratio_Control","Gene","Gene")
modifyWeights(w,status,values="Ratio_Control",multipliers=0)
```

mrlm

Fit Linear Model to Microarray Data by Robust Regression

Description

Fit a linear model genewise to expression data from a series of arrays. The fit is by robust M-estimation allowing for a small proportion of outliers. This is a utility function for `lmFit`.

Usage

```
mrlm(M,design=NULL,ndups=1,spacing=1,weights=NULL,...)
```

Arguments

| | |
|---------|---|
| M | numeric matrix containing log-ratio or log-expression values for a series of microarrays, rows correspond to genes and columns to arrays. |
| design | numeric design matrix defining the linear model, with rows corresponding to arrays and columns to comparisons to be estimated. The number of rows must match the number of columns of M. Defaults to the unit vector meaning that the arrays are treated as replicates. |
| ndups | a positive integer giving the number of times each gene is printed on an array. <code>nrow(M)</code> must be divisible by <code>ndups</code> . |
| spacing | the spacing between the rows of M corresponding to duplicate spots, <code>spacing=1</code> for consecutive spots. |
| weights | numeric matrix of the same dimension as M containing weights. If it is of different dimension to M, it will be filled out to the same size. NULL is equivalent to equal weights. |
| ... | any other arguments are passed to <code>r1m.default</code> . |

Details

This is a utility function used by the higher level function `lmFit`. Most users should not use this function directly but should use `lmFit` instead.

This function fits a linear model for each gene by calling the function `r1m` from the MASS library.

Warning: don't use weights with this function unless you understand how `r1m` treats weights. The treatment of weights is somewhat different from that of `lm.series` and `gls.series`.

Value

A list with components

| | |
|-----------------------------|--|
| <code>coefficients</code> | numeric matrix containing the estimated coefficients for each linear model. Same number of rows as <code>M</code> , same number of columns as <code>design</code> . |
| <code>stdev.unscaled</code> | numeric matrix conformal with <code>coef</code> containing the unscaled standard deviations for the coefficient estimators. The standard errors are given by <code>stdev.unscaled * sigma</code> . |
| <code>sigma</code> | numeric vector containing the residual standard deviation for each gene. |
| <code>df.residual</code> | numeric vector giving the degrees of freedom corresponding to <code>sigma</code> . |
| <code>qr</code> | QR decomposition of <code>design</code> . |

Author(s)

Gordon Smyth

See Also

[r1m](#).

An overview of linear model functions in `limma` is given by [06.LinearModels](#).

| | |
|------------------|---|
| <code>nec</code> | <i>NormExp Background Correction and Normalization Using Control Probes</i> |
|------------------|---|

Description

Perform `normexp` background correction using negative control probes and quantile normalization using negative and positive control probes. Particularly useful for Illumina BeadChips.

Usage

```
nec(x, status=NULL, negctrl="negative", regular="regular", offset=16,
    robust=FALSE, detection.p="Detection")
neqc(x, status=NULL, negctrl="negative", regular="regular", offset=16,
     robust=FALSE, detection.p="Detection", ...)
```

Arguments

| | |
|----------------------|--|
| <code>x</code> | object of class <code>EListRaw</code> or <code>matrix</code> containing raw intensities for regular and control probes from a series of microarrays. |
| <code>status</code> | character vector giving probe types. Defaults to <code>x\$genes\$Status</code> if <code>x</code> is an <code>EListRaw</code> object. |
| <code>negctrl</code> | character string identifier for negative control probes. |

| | |
|-------------|--|
| regular | character string identifier for regular probes, i.e., all probes other than control probes. |
| offset | numeric value added to the intensities after background correction. |
| robust | logical. Should robust estimators be used for the background mean and standard deviation? |
| detection.p | detection p-values. Only used when no negative control probes can be found in the data. Can be a numeric matrix or a character string giving the name of the component of <code>x\$other</code> containing the matrix. |
| ... | any other arguments are passed to <code>normalizeBetweenArrays</code> . |

Details

`neqc` performs background correction followed by quantile normalization, using negative control probes for background correction and both negative and positive controls for normalization (Shi et al, 2010). `nec` is similar but performs background correction only. These methods are particularly designed for Illumina BeadChip microarrays, but could be useful for other platforms for which good quality negative control probes or detection p-values are available.

When control data are available, these function call `normexp.fit.control` to estimate the parameters required by normal+exponential(`normexp`) convolution model with the help of negative control probes, followed by `normexp.signal` to perform the background correction. If `x` contains background intensities `x$Eb`, then these are first subtracted from the foreground intensities, prior to `normexp` background correction. After background correction, an `offset` is added to the data.

When expression values for negative controls are not available, the `detection.p` argument is used instead. In that case, these functions call `normexp.fit.detection.p`, which infers the negative control probe intensities from the detection p-values associated with the regular probes. The function outputs a message if this is done.

For more detailed descriptions of the arguments `x`, `status`, `negctrl`, `regular` and `detection.p`, please refer to functions `normexp.fit.control`, `normexp.fit.detection.p` and `read.ilmn`.

Both `nec` and `neqc` perform the above steps. `neqc` continues on to quantile normalize the background-corrected intensities, including control probes. After normalization, the intensities are log₂ transformed and the control probes are removed.

Value

`nec` produces a `EListRaw-class` or matrix object of the same dimensions as `x` containing background-corrected intensities, on the raw scale. `neqc` produces a `EList-class` or matrix object containing normalized log₂ intensities, with rows corresponding to control probes removed.

Author(s)

Wei Shi and Gordon Smyth

References

Shi W, Oshlack A and Smyth GK (2010). Optimizing the noise versus bias trade-off for Illumina Whole Genome Expression BeadChips. *Nucleic Acids Research* 38, e204. doi:10.1093/nar/gkq871

See Also

An overview of background correction functions is given in [04.Background](#).

An overview of LIMMA functions for normalization is given in [05.Normalization](#).

[normexp.fit.control](#) estimates the parameters in the normal+exponential convolution model using the negative control probes.

[normexp.fit.detection.p](#) estimates the parameters in the normal+exponential convolution model using negative control probe intensities inferred from regular probes by using their detection p values information.

[normexp.fit](#) estimates parameters in the normal+exponential convolution model using a saddle-point approximation or other methods.

[neqc](#) performs normexp background correction and quantile normalization aided by control probes.

Examples

```
## Not run:
# neqc normalization for data which include control probes
x <- read.ilmn(files="sample probe profile.txt", ctrlfiles="control probe profile.txt")
y <- neqc(x)
fit <- lmFit(y,design)

# Same thing but in separate steps:
x.b <- nec(x)
y <- normalizeBetweenArrays(x.b,method="quantile")
y <- y[y$genes$Status=="regular",]

# neqc normalization for data without control probes
# neqc can process detection p-values in lieu of control probes
xr <- read.ilmn(files="sample probe profile.txt")
yr <- neqc(xr)

## End(Not run)
```

normalizeBetweenArrays

Normalize Between Arrays

Description

Normalizes expression intensities so that the intensities or log-ratios have similar distributions across a set of arrays.

Usage

```
normalizeBetweenArrays(object, method=NULL, targets=NULL, cyclic.method="fast", ...)
```

Arguments

| | |
|---------------|---|
| object | a numeric matrix, EListRaw , RGList or MAList object containing un-normalized expression data. If a matrix, then it is assumed to contain log-transformed single-channel data. |
| method | character string specifying the normalization method to be used. Choices for single-channel data are "none", "scale", "quantile" or "cyclicloess". Choices for two-color data are those previously mentioned plus "Aquantile", "Gquantile", "Rquantile" or "Tquantile". A partial string sufficient to uniquely identify the choice is permitted. The default is "Aquantile" for two-color data objects or "quantile" for single-channel objects. |
| targets | vector, factor or matrix of length twice the number of arrays, used to indicate target groups if method="Tquantile" |
| cyclic.method | character string indicating the variant of <code>normalizeCyclicLoess</code> to be used if method="cyclicloess", see normalizeCyclicLoess for possible values. |
| ... | other arguments are passed to <code>normalizeQuantiles</code> or <code>normalizeCyclicLoess</code> |

Details

`normalizeBetweenArrays` normalizes expression values to achieve consistency between arrays. For two-color arrays, normalization between arrays is usually a follow-up step after normalization within arrays using `normalizeWithinArrays`. For single-channel arrays, within array normalization is not usually relevant and so `normalizeBetweenArrays` is the sole normalization step.

For single-channel data, the scale, quantile or cyclic loess normalization methods can be applied to the columns of data. Trying to apply other normalization methods when object is a matrix or `EListRaw` object will produce an error. If object is an `EListRaw` object, then normalization will be applied to the matrix object's expression values, which will then be log₂-transformed. Scale (method="scale") scales the columns to have the same median. Quantile and cyclic loess normalization was originally proposed by Bolstad et al (2003) for Affymetrix-style single-channel arrays. Quantile normalization forces the entire empirical distribution of each column to be identical. Cyclic loess normalization applies loess normalization to all possible pairs of arrays, usually cycling through all pairs several times. Cyclic loess is slower than quantile, but allows probe-wise weights and is more robust to unbalanced differential expression.

The other normalization methods are for two-color arrays. Scale normalization was proposed by Yang et al (2001, 2002) and is further explained by Smyth and Speed (2003). The idea is simply to scale the log-ratios to have the same median-absolute-deviation (MAD) across arrays. This idea has also been implemented by the `maNormScale` function in the `marray` package. The implementation here is slightly different in that the MAD scale estimator is replaced with the median-absolute-value and the A-values are normalized as well as the M-values.

Quantile normalization was explored by Yang and Thorne (2003) for two-color cDNA arrays. method="quantile" ensures that the intensities have the same empirical distribution across arrays and across channels. method="Aquantile" ensures that the A-values (average intensities) have the same empirical distribution across arrays leaving the M-values (log-ratios) unchanged. These two methods are called "q" and "Aq" respectively in Yang and Thorne (2003).

method="Tquantile" performs quantile normalization separately for the groups indicated by targets. targets may be a target frame such as read by `readTargets` or can be a vector indicating green channel groups followed by red channel groups.

method="Gquantile" ensures that the green (first) channel has the same empirical distribution across arrays, leaving the M-values (log-ratios) unchanged. This method might be used when the green channel is a common reference throughout the experiment. In such a case the green channel represents the same target throughout, so it makes compelling sense to force the distribution of intensities to be same for the green channel on all the arrays, and to adjust to the red channel accordingly. method="Rquantile" ensures that the red (second) channel has the same empirical distribution across arrays, leaving the M-values (log-ratios) unchanged. Both Gquantile and Rquantile normalization have the implicit effect of changing the red and green log-intensities by equal amounts.

See the limma User's Guide for more examples of use of this function.

Value

If object is a matrix then normalizeBetweenArrays produces a matrix of the same size. If object is an EListRaw object, then an EList object with expression values on the log2 scale is produced. For two-color data, normalizeBetweenArrays produces an MAList object with M and A-values on the log2 scale.

Author(s)

Gordon Smyth

References

- Bolstad, B. M., Irizarry R. A., Astrand, M., and Speed, T. P. (2003). A comparison of normalization methods for high density oligonucleotide array data based on bias and variance. *Bioinformatics* **19**, 185-193.
- Smyth, G. K., and Speed, T. P. (2003). Normalization of cDNA microarray data. *Methods* **31**, 265-273.
- Yang, Y. H., Dudoit, S., Luu, P., and Speed, T. P. (2001). Normalization for cDNA microarray data. In *Microarrays: Optical Technologies and Informatics*, M. L. Bittner, Y. Chen, A. N. Dorsel, and E. R. Dougherty (eds), Proceedings of SPIE, Volume 4266, pp. 141-152.
- Yang, Y. H., Dudoit, S., Luu, P., Lin, D. M., Peng, V., Ngai, J., and Speed, T. P. (2002). Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Research* **30**(4):e15.
- Yang, Y. H., and Thorne, N. P. (2003). Normalization for two-color cDNA microarray data. In: D. R. Goldstein (ed.), *Science and Statistics: A Festschrift for Terry Speed*, IMS Lecture Notes - Monograph Series, Volume 40, pp. 403-418.

See Also

An overview of LIMMA functions for normalization is given in [05.Normalization](#).

The [neqc](#) function provides a variation of quantile normalization that is customized for Illumina BeadChips. This method uses control probes to refine the background correction and normalization steps.

Note that vsn normalization, previously offered as a method of this function, is now performed by the [normalizeVSN](#) function.

See also maNormScale in the marray package and [normalize-methods](#) in the affy package.

Examples

```
ngenes <- 100
narrays <- 4
x <- matrix(rnorm(ngenes*narrays),100,4)
y <- normalizeBetweenArrays(x)
```

normalizeCyclicLoess *Normalize Columns of a Matrix by Cyclic Loess*

Description

Normalize the columns of a matrix, cyclicly applying loess normalization to normalize each pair of columns to each other.

Usage

```
normalizeCyclicLoess(x, weights = NULL, span = 0.7, adaptive.span = FALSE,
                    iterations = 3, method = "fast")
```

Arguments

| | |
|---------------|---|
| x | numeric matrix, or object which can be coerced to a numeric matrix, containing log-expression values. |
| weights | numeric vector of probe weights. Must be non-negative. |
| span | span of loess smoothing window, between 0 and 1. |
| adaptive.span | logical. If TRUE, then an optimal value for span will be chosen depending on the number of rows of x. |
| iterations | number of times to cycle through all pairs of columns. |
| method | character string specifying which variant of the cyclic loess method to use. Options are "fast", "affy" or "pairs". |

Details

This function is intended to normalize single channel or A-value microarray intensities between arrays. Cyclic loess normalization is similar effect and intention to quantile normalization, but with some advantages, in particular the ability to incorporate probe weights.

A number of variants of cyclic loess have been suggested. `method="pairs"` implements the intuitive idea that each pair of arrays is subjected to loess normalization as for two-color arrays. This process is simply cycled through all possible pairs of arrays, then repeated for several iterations. This is the method described by Ballman et al (2004) as ordinary cyclic loess normalization.

`method="affy"` implements a method similar to `normalize.loess` in the `affy` package, except that here we call `lowess` instead of `loess` and avoid the use of probe subsets and the `predict` function. In this approach, no array is modified until a complete cycle of all pairs has been completed. The adjustments are stored for a complete iteration, then averaged, and finally used to modify the arrays. The "affy" method is invariant to the order of the columns of x, whereas the "pairs" method is

not. The affy approach is presumably that used by Bolstad et al (2003), although the algorithm was not explicitly described in that article.

method="fast" implements the "fast linear loess" method of Ballman et al (2004), whereby each array is simply normalized to a reference array, the reference array being the average of all the arrays. This method is relatively fast because computational time is linear in the number of arrays, whereas "pairs" and "affy" are quadratic in the number of arrays. "fast" requires n lowess fits per iteration, where n is the number of arrays, whereas "pairs" and "affy" require $n*(n-1)/2$ lowess fits per iteration.

If adaptive.span is TRUE, then span is set to chooseLowessSpan($n=nrow(x)$, small.n=200, min.span=0.6).

Value

A matrix of the same dimensions as x containing the normalized values.

Author(s)

Yunshun (Andy) Chen and Gordon Smyth

References

Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003). A comparison of normalization methods for high density oligonucleotide array data based on bias and variance. *Bioinformatics* 19, 185-193.

Ballman KV, Grill DE, Oberg AL, Therneau TM (2004). Faster cyclic loess: normalizing RNA arrays via linear models. *Bioinformatics* 20, 2778-2786.

See Also

An overview of LIMMA functions for normalization is given in [05.Normalization](#). [normalize.loess](#) in the affy package also implements cyclic loess normalization, without weights.

normalizeForPrintorder

Print-Order Normalization

Description

Normalize intensity values on one or more spotted microarrays to adjust for print-order effects.

Usage

```
normalizeForPrintorder(object, layout, start="topleft", method = "loess",
                      separate.channels = FALSE, span = 0.1, plate.size = 32)
normalizeForPrintorder.rg(R, G, printorder, method = "loess", separate.channels = FALSE,
                        span = 0.1, plate.size = 32, plot = FALSE)
plotPrintorder(object, layout, start="topleft", slide = 1, method = "loess",
               separate.channels = FALSE, span = 0.1, plate.size = 32)
```

Arguments

| | |
|-------------------|---|
| object | an RGList or list object containing components R and G which are matrices containing the red and green channel intensities for a series of arrays |
| R | numeric vector containing red channel intensities for a single microarray |
| G | numeric vector containing the green channel intensities for a single microarray |
| layout | list specifying the printer layout, see PrintLayout-class |
| start | character string specifying where printing starts in each pin group. Choices are "topleft" or "topright". |
| printorder | numeric vector specifying order in which spots are printed. Can be computed from <code>printorder(layout, start=start)</code> . |
| slide | positive integer giving the column number of the array for which a plot is required |
| method | character string, "loess" if a smooth loess curve should be fitted through the print-order trend or "plate" if plate effects are to be estimated |
| separate.channels | logical, TRUE if normalization should be done separately for the red and green channel and FALSE if the normalization should be proportional for the two channels |
| span | numerical constant between 0 and 1 giving the smoothing span for the loess the curve. Ignored if method="plate". |
| plate.size | positive integer giving the number of consecutive spots corresponding to one plate or plate pack. Ignored if method="loess". |
| plot | logical. If TRUE then a scatter plot of the print order effect is sent to the current graphics device. |

Details

Print-order is associated with the 384-well plates used in the printing of spotted microarrays. There may be variations in DNA concentration or quality between the different plates. There may be variations in ambient conditions during the time the array is printed.

This function is intended to pre-process the intensities before other normalization methods are applied to adjust for variations in DNA quality or concentration and other print-order effects.

Printorder means the order in which spots are printed on a microarray. Spotted arrays are printed using a print head with an array of print-tips. Spots in the various tip-groups are printed in parallel. Printing is assumed to start in the top left hand corner of each tip-groups and to proceed right and down by rows, or else to start in the top right hand and to proceed left and down by rows. See [printorder](#) for more details. (WARNING: this is not always the case.) This is true for microarrays printed at the Australian Genome Research Facility but might not be true for arrays from other sources.

If object is an RGList then printorder is performed for each intensity in each array.

plotPrintorder is a non-generic function which calls normalizeForPrintorder with plot=TRUE.

Value

normalizeForPrintorder produces an RGList containing normalized intensities.

The function plotPrintorder or normalizeForPrintorder.rg with plot=TRUE returns no value but produces a plot as a side-effect.

normalizeForPrintorder.rg with plot=FALSE returns a list with the following components:

| | |
|---------|---|
| R | numeric vector containing the normalized red channel intensities |
| G | numeric vector containing the normalized red channel intensities |
| R.trend | numeric vector containing the fitted printorder trend for the red channel |
| G.trend | numeric vector containing the fitted printorder trend for the green channel |

Author(s)

Gordon Smyth

References

Smyth, G. K. Print-order normalization of cDNA microarrays. March 2002. <https://gksmyth.github.io/pubs/porder/porder.html>

See Also

[printorder](#).

An overview of LIMMA functions for normalization is given in [05.Normalization](#).

Examples

```
## Not run:
plotPrintorder(RG,layout,slide=1,separate=TRUE)
RG <- normalizeForPrintorder(mouse.data,mouse.setup)

## End(Not run)
```

normalizeMedianAbsValues

Normalize Columns of a Matrix to have the Median Absolute Value

Description

Performs scale normalization of an M-value matrix or an A-value matrix across a series of arrays. Users do not normally need to call these functions directly - use normalizeBetweenArrays instead.

Usage

```
normalizeMedianValues(x)
normalizeMedianAbsValues(x)
```

Arguments

x numeric matrix

Details

If x is a matrix of log-ratios of expression (M-values) then normalizeMedianAbsValues is very similar to scaling to equalize the median absolute deviation (MAD) as in Yang et al (2001, 2002). Here the median-absolute value is used for preference to as to not re-center the M-values.

normalizeMedianAbsValues is also used to scale the A-values when scale-normalization is applied to an MAList object.

Value

A numeric matrix of the same size as that input which has been scaled so that each column has the same median value (for normalizeMedianValues) or median-absolute value (for normalizeMedianAbsValues).

Author(s)

Gordon Smyth

See Also

An overview of LIMMA functions for normalization is given in [05.Normalization](#).

Examples

```
M <- cbind(Array1=rnorm(10),Array2=2*rnorm(10))
normalizeMedianAbsValues(M)
```

normalizeQuantiles *Normalize Columns of a Matrix to have the same Quantiles*

Description

Normalize the columns of a matrix to have the same quantiles, allowing for missing values. Users do not normally need to call this function directly - use [normalizeBetweenArrays](#) instead.

Usage

```
normalizeQuantiles(A, ties=TRUE)
```

Arguments

A numeric matrix. Missing values are allowed.
ties logical. If TRUE, ties in each column of A are treated in careful way. tied values will be normalized to the mean of the corresponding pooled quantiles.

Details

This function is intended to normalize single channel or A-value microarray intensities between arrays. Each quantile of each column is set to the mean of that quantile across arrays. The intention is to make all the normalized columns have the same empirical distribution. This will be exactly true if there are no missing values and no ties within the columns: the normalized columns are then simply permutations of one another.

If there are ties amongst the intensities for a particular array, then with `ties=FALSE` the ties are broken in an unpredictable order. If `ties=TRUE`, all the tied values for that array will be normalized to the same value, the average of the quantiles for the tied values.

Value

A matrix of the same dimensions as A containing the normalized values.

Author(s)

Gordon Smyth

References

Bolstad, B. M., Irizarry R. A., Astrand, M., and Speed, T. P. (2003), A comparison of normalization methods for high density oligonucleotide array data based on bias and variance. *Bioinformatics* **19**, 185-193.

See Also

An overview of LIMMA functions for normalization is given in [05.Normalization](#).

normalizeRobustSpline *Normalize Single Microarray Using Shrunk Robust Splines*

Description

Normalize the M-values for a single microarray using robustly fitted regression splines and empirical Bayes shrinkage.

Usage

```
normalizeRobustSpline(M,A,layout=NULL,df=5,method="M")
```

Arguments

| | |
|--------|--|
| M | numeric vector of M-values |
| A | numeric vector of A-values |
| layout | list specifying the dimensions of the spot matrix and the grid matrix. Defaults to a single group for the whole array. |

| | |
|--------|---|
| df | degrees of freedom for regression spline, i.e., the number of regression coefficients and the number of knots |
| method | choices are "M" for M-estimation or "MM" for high breakdown point regression |

Details

This function implements an idea similar to print-tip loess normalization but uses regression splines in place of the loess curves and uses empirical Bayes ideas to shrink the individual print-tip curves towards a common value. This allows the technique to introduce less noise into good quality arrays with little spatial variation while still giving good results on arrays with strong spatial variation.

The original motivation for the robustspline method was to use whole-array information to moderate the normalization curves used for the individual print-tip groups. This was an important issue for academically printed spotted two-color microarrays, especially when some of the print-tip groups contained relatively few spots. In these situations, robust spline normalization ensures stable results even for print-tip groups with few spots.

Modern commercial two colour arrays do not usually have print tips, so in effect the whole array is a single print-tip group, and so the need for moderating individual curves is gone. Robustspline normalization can still be used for data from these arrays, in which case a single normalization curve is estimated. In this situation, the method is closely analogous to global loess, with a regression spline replacing the loess curve and with robust regression replacing the loess robustifying weights. Robust spline normalization with method="MM" has potential advantages over global loess normalization when there a lot of differential expression or the differential expression is assymetric, because of the increased level of robustness. The potential advantages of this approach have not been fully explored in a refereed publication however.

Value

Numeric vector containing normalized M-values.

Author(s)

Gordon Smyth

References

Ritchie, ME, Phipson, B, Wu, D, Hu, Y, Law, CW, Shi, W, and Smyth, GK (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* 43, e47. <http://nar.oxfordjournals.org/content/43/7/e47>

See Also

normalizeRobustSpline uses ns in the splines package to specify regression splines and rlm in the MASS package for robust regression.

This function is usually accessed through [normalizeWithinArrays](#). An overview of LIMMA functions for normalization is given in [05.Normalization](#).

Examples

```
A <- 1:100
M <- rnorm(100)
normalized.M <- normalizeRobustSpline(M,A)

# Usual usage
## Not run: MA <- normalizeWithinArrays(RG, method="robustspline")
```

normalizeVSN

Variance Stabilizing Normalization (vsn)

Description

Apply variance stabilizing normalization (vsn) to limma data objects.

Usage

```
normalizeVSN(x, ...)
```

Arguments

x a numeric matrix, `EListRaw` or `RGList` object.
... other arguments are passed to `vsn`

Details

This is an interface to the `vsnMatrix` function from the `vsn` package. The input `x` should contain raw intensities. If `x` contains background and well as foreground intensities, these will be subtracted from the foreground intensities before `vsnMatrix` is called.

Note that the `vsn` algorithm performs background correction and normalization simultaneously. If the data are from two-color microarrays, then the red and green intensities are treated as if they were single channel data, i.e., red and green channels from the same array are treated as unpaired. This algorithm is therefore separate from the `backgroundCorrection`, `normalizeWithinArrays`, then `normalizeBetweenArrays` paradigm used elsewhere in the `limma` package.

Value

The class of the output depends on the input. If `x` is a matrix, then the result is a matrix of the same size. If `x` is an `EListRaw` object, then an `EList` object with expression values on the log₂ scale is produced. For `x` is an `RGList`, then an `MAList` object with M and A-values on the log₂ scale is produced.

Author(s)

Gordon Smyth

References

Huber, W, von Heydebreck, A, Suelmann, H, Poustka, A, Vingron, M (2002). Variance stabilization applied to microarray data calibration and to the quantification of differential expression. *Bioinformatics* 18 Supplement 1, S96-S104.

See Also

An overview of LIMMA functions for normalization is given in [05.Normalization](#).

See also [vsnMatrix](#) in the vsn package.

Examples

```
ngenes <- 100
narrays <- 4
x <- matrix(rnorm(ngenes*narrays),100,4)
y <- normalizeVSN(x)
```

normalizeWithinArrays *Normalize Within Arrays*

Description

Normalize the expression log-ratios for one or more two-colour spotted microarray experiments so that the log-ratios average to zero within each array or sub-array.

Usage

```
normalizeWithinArrays(object, layout, method="printtiploess", weights=object$weights,
                     span=0.3, iterations=4, controlspots=NULL, df=5, robust="M",
                     bc.method="subtract", offset=0)
MA.RG(object, bc.method="subtract", offset=0)
RG.MA(object)
```

Arguments

| | |
|---------|--|
| object | object of class <code>list</code> , <code>RGList</code> or <code>MAList</code> containing red and green intensities constituting two-color microarray data. |
| layout | list specifying the dimensions of the spot matrix and the grid matrix. For details see PrintLayout-class . |
| method | character string specifying the normalization method. Choices are "none", "median", "loess", "printtiploess", "composite", "control" and "robustspline". A partial string sufficient to uniquely identify the choice is permitted. |
| weights | numeric matrix or vector of the same size and shape as the components of object containing spot quality weights. |
| span | numeric scalar giving the smoothing parameter for the loess fit |

| | |
|--------------|--|
| iterations | number of iterations used in loess fitting. More iterations give a more robust fit. |
| controlspots | numeric or logical vector specifying the subset of spots which are non-differentially-expressed control spots, for use with <code>method="composite"</code> or <code>method="control"</code> . |
| df | degrees of freedom for spline if <code>method="robustspline"</code> . |
| robust | robust regression method if <code>method="robustspline"</code> . Choices are "M" or "MM". |
| bc.method | character string specifying background correct method, see backgroundCorrect for options. |
| offset | numeric value, intensity offset used when computing log-ratios, see backgroundCorrect . |

Details

Normalization is intended to remove from the expression measures any systematic trends which arise from the microarray technology rather than from differences between the probes or between the target RNA samples hybridized to the arrays.

This function normalizes M-values (log-ratios) for dye-bias within each array. Apart from `method="none"` and `method="median"`, all the normalization methods make use of the relationship between dye-bias and intensity. Method "none" computes M-values and A-values but does no normalization. Method "median" subtracts the weighted median from the M-values for each array.

The loess normalization methods ("`loess`", "`printtiploess`" and "`composite`") were proposed by Yang et al (2001, 2002). Smyth and Speed (2003) review these methods and describe how the methods are implemented in the `limma` package, including choices of tuning parameters. More information on the loess control parameters `span` and `iterations` can be found under [loessFit](#). The default values used here are equivalent to those for the older function `stat.ma` in the `sma` package.

Oshlack et al (2004) consider the special issues that arise when a large proportion of probes are differentially expressed. They propose an improved version of composite loess normalization, which is implemented in the "`control`" method. This fits a global loess curve through a set of control spots, such as a whole-library titration series, and applies that curve to all the other spots.

The "`robustspline`" method calls [normalizeRobustSpline](#). See that function for more documentation.

`MA.RG` converts an unlogged `RGList` object into an `MAList` object. `MA.RG(object)` is equivalent to `normalizeWithinArrays(object,method="none")`.

`RG.MA(object)` converts back from an `MAList` object to a `RGList` object with unlogged intensities.

`weights` is normally a matrix giving a quality weight for every spot on every array. If `weights` is instead a vector or a matrix with only one column, then the weights will be assumed to be the same for every array, i.e., the weights will be probe-specific rather than spot-specific.

Value

An object of class `MAList`. Any components found in `object` will be preserved except for `R`, `G`, `Rb`, `Gb` and `other`.

Author(s)

Gordon Smyth

References

- Oshlack, A., Emslie, D., Corcoran, L., and Smyth, G. K. (2007). Normalization of boutique two-color microarrays with a high proportion of differentially expressed probes. *Genome Biology* **8**, R2.
- Smyth, G. K., and Speed, T. P. (2003). Normalization of cDNA microarray data. *Methods* **31**, 265-273.
- Yang, Y. H., Dudoit, S., Luu, P., and Speed, T. P. (2001). Normalization for cDNA microarray data. In *Microarrays: Optical Technologies and Informatics*, M. L. Bittner, Y. Chen, A. N. Dorsel, and E. R. Dougherty (eds), Proceedings of SPIE, Vol. 4266, pp. 141-152.
- Yang, Y. H., Dudoit, S., Luu, P., Lin, D. M., Peng, V., Ngai, J., and Speed, T. P. (2002). Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Research* **30**(4):e15.

See Also

An overview of limma functions for normalization is given in [05.Normalization](#). In particular, see [normalizeBetweenArrays](#) for between-array normalization.

The original loess normalization function was the `statma` function in the `sma` package. `normalizeWithinArrays` is a direct generalization of that function, with more options and with support for quantitative spot quality weights.

A different implementation of loess normalization methods, with potentially different behavior, is provided by the `maNorm` in the `marray` package.

normexp.fit

Fit Normal+Exp Convolution Model to Observed Intensities

Description

Fit the normal+exponential convolution model to a vector of observed intensities. The normal part represents the background and the exponential part represents the signal intensities. This function is called by `backgroundCorrect` and is not normally called directly by users.

Usage

```
normexp.fit(x, method="saddle", n.pts=NULL, trace=FALSE)
```

Arguments

- | | |
|---------------------|--|
| <code>x</code> | numeric vector of (background corrected) intensities |
| <code>method</code> | method used to estimate the three parameters. Choices for <code>normexp.fit</code> are "mle", "saddle", "rma" and "rma75". |
| <code>n.pts</code> | number of quantiles of <code>x</code> to use for the fit. If NULL then all values of <code>x</code> will be used. |
| <code>trace</code> | logical, if TRUE, tracing information on the progress of the optimization is given. |

Details

The Normal+Exp (normexp) convolution model is a mathematical model representing microarray intensity data for the purposes of background correction. It was proposed originally as part of the RMA algorithm for Affymetrix microarray data. For two-color microarray data, the normexp background correction method was introduced and compared with other methods by Ritchie et al (2007).

This function uses maximum likelihood estimation to fit the normexp model to background-corrected intensities. The model assumes that the observed intensities are the sum of background and signal components, the background being normal and the signal being exponential distributed.

The likelihood may be computed exactly (method="mle") or approximated using a saddle-point approximation (method="saddle"). The saddle-point approximation was proposed by Ritchie et al (2007). Silver et al (2008) added some computational refinements to the saddle-point approximation, making it more reliable in practice, and developed the exact likelihood maximization algorithm. The "mle" method uses the best performing algorithm from Silver et al (2008), which calls the optimization function `nlm` with analytic first and second derivatives. Derivatives are computed with respect to the normal-mean, the log-normal-variance and the log-exponential-mean.

Two ad-hoc estimators are also available which do not require iterative estimation. "rma" results in a call to the `bg.parameters` function of the `affy` package. This provides the kernel estimation method that is part of the RMA algorithm for Affymetrix data. "rma75" uses the similar but less biased RMA-75 method from McGee and Chen (2006).

If the length `x` is very large, it may be worth saving computation time by setting `n.pts` to a value less than the total number of probes, for example `n.pts=2^14`.

Value

A list containing the components

| | |
|--------------------------|--|
| <code>par</code> | numeric vector giving estimated values of the mean and log-standard-deviation of the background-normal part and the log-mean of the signal-exponential part. |
| <code>m2loglik</code> | numeric scalar giving minus twice the maximized log-likelihood |
| <code>convergence</code> | integer code indicating successful convergence or otherwise of the optimization. |

Author(s)

Gordon Smyth and Jeremy Silver

References

McGee, M., and Chen, Z. (2006). Parameter estimation for the exponential-normal convolution model for background correction of Affymetrix GeneChip data. *Stat Appl Genet Mol Biol*, 5(1), Article 24.

Ritchie, M. E., Silver, J., Oshlack, A., Silver, J., Holmes, M., Diyagama, D., Holloway, A., and Smyth, G. K. (2007). A comparison of background correction methods for two-colour microarrays. *Bioinformatics* 23, 2700-2707. <http://bioinformatics.oxfordjournals.org/content/23/20/2700>

Silver, JD, Ritchie, ME, and Smyth, GK (2009). Microarray background correction: maximum likelihood estimation for the normal-exponential convolution. *Biostatistics* 10, 352-363. <http://biostatistics.oxfordjournals.org/content/10/2/352>

See Also

[normexp.signal](#), [normexp.fit.control](#). Also [bg.parameters](#) in the affy package.

An overview of background correction functions is given in [04.Background](#).

Examples

```
x <- c(2,3,1,10,3,20,5,6)
out <- normexp.fit(x)
normexp.signal(out$par, x=x)
```

normexp.fit.control *Normexp Model Parameter Estimation Aided by Negative Controls*

Description

The mean and log-standard-deviation of the background-normal part of the normexp+exponential convolution model is estimated as the mean and log-standard deviation of intensities from negative control probes. The log-mean of the signal-exponential part is estimated as the log of the difference between signal mean and background mean.

Usage

```
normexp.fit.control(x, status=NULL, negctrl="negative", regular="regular", robust=FALSE)
```

Arguments

| | |
|---------|--|
| x | object of class <code>EListRaw-class</code> or <code>matrix</code> containing raw intensities for regular and control probes for a series of microarrays |
| status | character vector giving probe types. |
| negctrl | character string identifier for negative control probes. |
| regular | character string identifier for regular probes. |
| robust | logical. Should robust estimators be used for the background mean and standard deviation? |

Details

x has to contain raw expression intensities from both regular probes and negative control probes.

The probe type information for an object of `EListRaw-class` is normally saved in the `Status` column of its `genes` component. However, it will be overridden by the `status` parameter if it is explicitly provided to this function. If x is a `matrix` object, the probe type information has to be

provided through the status parameter of this function. Regular probes have the status regular. Negative control probes have the status indicated by `negctrl`, which is negative by default.

This function estimates parameters of the normal+exponential convolution model with the help of negative control probes. The mean and log-standard-deviation of the background-normal part of the normexp+exponential(normexp) convolution model are estimated as the mean and log-standard deviation of intensities from negative control probes respectively. The log-mean of the signal-exponential part is estimated as the log of the difference between signal mean and background mean. The signal mean is simply the mean of intensities from regular probes.

When negative control probes are not available, the `normexp.fit.detection.p` function can be used to estimate the normexp model parameters which infers the negative control probe intensities from regular probes by taking advantage of their detection p value information.

Value

A matrix containing estimated parameters with rows being arrays and with columns being parameters. Column names are `mu`, `logsigma` and `logalpha`.

Author(s)

Wei Shi and Gordon Smyth

References

Shi W, Oshlack A and Smyth GK (2010). Optimizing the noise versus bias trade-off for Illumina Whole Genome Expression BeadChips. *Nucleic Acids Research*, 38(22):e204. Epub 2010 Oct 6. PMID: 20929874

See Also

`nec` calls this function to get the parameters of the normal+exponential convolution model and then calls `normexp.signal` to perform the background correction.

`normexp.fit.detection.p` estimates the parameters in the normal+exponential convolution model using negative control probe intensities inferred from regular probes by using their detection p values information.

`normexp.fit` estimates normexp parameters using a saddle-point approximation or other methods.

An overview of background correction functions is given in [04.Background](#).

Examples

```
## Not run:
# read in BeadChip probe profile file and control profile file
x <- read.ilmn(files="sample probe profile", ctrlfiles="control probe profile")
# estimated normexp parameters
normexp.fit.control(x)
# normalization using control data
y <- neqc(x)

## End(Not run)
```

`normexp.fit.detection.p`*Estimate Normexp Model Parameter Using Negative Controls Inferred from Regular Probes*

Description

Detection p values from Illumina BeadChip microarray data can be used to infer negative control probe intensities from regular probe intensities by using detection p value information when negative control data are not available. The inferred negative control intensities can then be used in the background correction in the same way as those control data outputted from BeadChip used in the [normexp.fit.control](#) function.

Usage

```
normexp.fit.detection.p(x, detection.p="Detection")
```

Arguments

| | |
|--------------------------|---|
| <code>x</code> | object of class <code>EListRaw-class</code> or <code>matrix</code> containing raw intensities of regular probes for a series of microarrays |
| <code>detection.p</code> | a character string giving the name of the component which contains detection p value information in <code>x</code> or a numeric matrix giving detection p values, <code>Detection</code> by default |

Details

This function estimates the normexp parameters in the same way as [normexp.fit.control](#) does, except that negative control probe intensities are inferred from regular probes by taking advantage of detection p value information rather than from the control probe profile outputted by BeadStudio.

Calculation of detection p values in Illumina BeadChip data is based on the rank of probe intensities in the list of negative control probe intensities. Therefore, the detection p values can be used to find regular probes which have expression intensities falling into the range of negative control probe intensities. These probes give a good approximation to the real negative control data and thus can be used to estimate the mean and standard deviation of background intensities when negative control data is not available.

If `x` is an `EListRaw-class` object, this function will try to look for the component which includes detection p value matrix in `x` when `detection.p` is a character string. This function assumes that this component is located within the other component in `x`. The component name specified by `detection.p` should be exactly the same as the name of the detection p value component in `x`. If `detection.p` is a matrix, then this matrix will be used as the detection p value data used in this function.

If `x` is an `matrix` object, then `detection.p` has to be a data matrix which includes detection p values.

When `detection.p` is a `matrix`, it has to have the same dimension as that of `x`.

This function will replace the detection p values with 1 subtracted by these values if high intensity probes have detection p values less than those from low intensity probes.

Note that when control data are available, the `normexp.fit.control` function should be used instead.

Value

A matrix containing estimated parameters with rows being arrays and with columns being parameters. Column names are mu, logsigma and logalpha.

Author(s)

Wei Shi and Gordon Smyth

References

Shi W, Oshlack A and Smyth GK (2010). Optimizing the noise versus bias trade-off for Illumina Whole Genome Expression BeadChips. *Nucleic Acids Research* 38, e204. <http://nar.oxfordjournals.org/content/38/22/e204>

See Also

`neq` calls this function to get the parameters of the normal+exponential convolution model when control probe profile file is not available and then calls `normexp.signal` to perform the background correction.

`normexp.fit.control` estimates normexp parameters using control data outputted by BeadStudio.

`normexp.fit` estimates normexp parameters using a saddle-point approximation or other methods.

An overview of background correction functions is given in [04.Background](#).

Examples

```
## Not run:
# read in BeadChip data which do not have control data available
x <- read.ilmn(files="sample probe profile")
# estimated normexp parameters
normexp.fit.detection.p(x)
# normalization using inferred negative controls
y <- neqc(x)

## End(Not run)
```

| | |
|----------------|---|
| normexp.signal | <i>Expected Signal Given Observed Foreground Under Normal+Exp Model</i> |
|----------------|---|

Description

Adjust foreground intensities for observed background using Normal+Exp Model. This function is called by backgroundCorrect and is not normally called directly by the user.

Usage

```
normexp.signal(par, x)
```

Arguments

| | |
|-----|---|
| par | numeric vector containing the parameters of the Normal+Exp distribution, see normexp.fit for details. |
| x | numeric vector of (background corrected) intensities |

Details

In general the vector normmean is computed conditional on background at each spot.

Value

Numeric vector containing adjusted intensities.

Author(s)

Gordon Smyth

References

Ritchie, M. E., Silver, J., Oshlack, A., Silver, J., Holmes, M., Diyagama, D., Holloway, A., and Smyth, G. K. (2007). A comparison of background correction methods for two-colour microarrays. *Bioinformatics* 23, 2700-2707. <http://bioinformatics.oxfordjournals.org/content/23/20/2700>

Silver, JD, Ritchie, ME, and Smyth, GK (2009). Microarray background correction: maximum likelihood estimation for the normal-exponential convolution. *Biostatistics* 10, 352-363. <http://biostatistics.oxfordjournals.org/content/10/2/352>

See Also

[normexp.fit](#)

An overview of background correction functions is given in [04.Background](#).

Examples

```
# See normexp.fit
```

| | |
|---------------|----------------------------------|
| plotDensities | <i>Plot Expression Densities</i> |
|---------------|----------------------------------|

Description

Plot the density of expression values for multiple arrays on the same plot.

Usage

```
## S3 method for class 'RGList'
plotDensities(object, log=TRUE, group=NULL, col=NULL, main="RG Densities",
              bc.method="subtract", ...)
## S3 method for class 'MAList'
plotDensities(object, log=TRUE, group=NULL, col=NULL, main="RG Densities", ...)
## S3 method for class 'EListRaw'
plotDensities(object, log=TRUE, bc.method="subtract", ...)
## S3 method for class 'EList'
plotDensities(object, log=TRUE, ...)
## Default S3 method:
plotDensities(object, group=NULL, col=NULL, main=NULL, legend="topleft", ...)
```

Arguments

| | |
|-----------|--|
| object | an RGList, MAList, EListRaw or EList object containing expression data. Or any data object that can be coerced to a matrix. |
| log | logical, should densities be plotted on the log2 scale? |
| group | optional vector or factor classifying the arrays into groups. Should be same length as ncol(object). |
| col | optional vector of colors of the same length as the number of groups. |
| main | the main title for the plot. |
| bc.method | background subtraction method passed to backgroundCorrect . |
| legend | character string giving position to place legend. See legend for possible values. Can also be logical, with FALSE meaning no legend. |
| ... | other arguments are passed to plotDensities.default or density . |

Details

This function is useful to display and contrast the distribution of expression values on different arrays. It can for example be used to display the effects of between-array normalization. See the section on between-array normalization in the LIMMA User's Guide.

Value

A plot is created on the current graphics device.

Author(s)

Natalie Thorne and Gordon Smyth

See Also

An overview of diagnostic plots in LIMMA is given in [09.Diagnostics](#). There is a section using `plotDensities` in conjunction with between-array normalization in the [LIMMA User's Guide](#).

This function uses [density](#) and [matplot](#).

Examples

```
## Not run:
# Default is to plot red channels in red and green channels in green
plotDensities(MA)

# Alternatively colors
plotDensities(MA, col=c("red", "blue"))

# Color by group, with three groups:
plotDensities(MA, group=group, col=c("blue", "orange", "green"))

## End(Not run)
```

plotExonJunc

Differential splicing plot with junctions

Description

Plot differential usage results by exons and junctions for the specified gene and highlight the significantly spliced exons and junctions.

Usage

```
plotExonJunc(fit, coef=ncol(fit), geneid, genecolname=NULL, FDR=0.05, annotation=NULL)
```

Arguments

| | |
|-------------------|---|
| <code>fit</code> | MArrayLM fit object produced by <code>diffSplice</code> . Must have the Entrez gene ids for all the exons and junctions stored in <code>fit\$genes\$GeneID</code> , length information for all the exons and junctions stored in <code>fit\$genes\$Length</code> and the strand information stored in <code>fit\$genes\$Strand</code> . To distinguish between exons and junctions, <code>fit\$genes\$Length</code> has to be set to 1 for all the junctions. |
| <code>coef</code> | the coefficient (column) of fit for which differential splicing is assessed. |

| | |
|-------------|---|
| geneid | character string, ID of the gene to plot. |
| genecolname | column name of fit\$genes containing geneid. |
| FDR | numeric, highlight exons and junctions with false discovery rate less than this cutoff. Red indicates up-regulation whereas blue indicates down-regulation. The FDR of the individual exon/junction is calculated based on the exon-level t-statistics test for differences between each exon/junction and all other exons/junctions for the same gene. |
| annotation | data frame containing the full exon annotation of the corresponding species. Must have the Entrez gene ids for all the exons stored in the GeneID column, start and end positions for all the exons stored in the Start and End columns, respectively. |

Details

Plot differential usage results by exons and junctions for the specified gene. The significantly spliced individual exons are highlighted as red blocks if up-regulated and blue blocks if down-regulated. All other exons are displayed as black blocks. The significantly spliced individual junctions are highlighted as red lines if up-regulated and blue lines if down-regulated. All other junctions are displayed as black lines.

Since the diffSplice analysis is usually performed after filtering, the full annotation (e.g. the in-built annotation in featureCounts) is highly recommended for producing the plot. When annotation is provided, the filtered exons are displayed as grey blocks.

Value

A plot is created on the current graphics device.

Author(s)

Yunshun Chen and Gordon Smyth

See Also

[diffSplice](#), [topSplice](#)

Examples

```
## Not run:
# diffSplice analysis
v <- voom(dge, design)
fit <- lmFit(v, design)
ex <- diffSplice(fit, geneid="GeneID")

# Get full annotation from Rsubread
library(Rsubread)
annotation.full <- getInBuiltAnnotation("mm10")

# Make a plot
plotExonJunc(ex, geneid="Foxp1", genecolname="Symbol", annotation=annotation.full)
```

```
## End(Not run)
```

plotExons *Plot exons of differentially expressed gene*

Description

Plot exons of differentially expressed gene and mark the differentially expressed exons.

Usage

```
plotExons(fit, coef = ncol(fit), geneid = NULL, genecolname = "GeneID",
          exoncolname = NULL, rank = 1L, FDR = 0.05)
```

Arguments

| | |
|-------------|--|
| fit | MArrayLM fit object produced by eBayes. |
| coef | the coefficient (column) of fit for which differential expression is assessed. |
| geneid | character string, ID of the gene to plot. |
| genecolname | character string for the column name of fit\$genes containing gene IDs. Defaults to "GeneID" for Entrez Gene ID. |
| exoncolname | character string for the column name of fit\$genes containing exon IDs. |
| rank | integer, if geneid=NULL then this ranked gene will be plotted. |
| FDR | numeric, mark differentially expressed exons with false discovery rate less than this cutoff. |

Details

Plots log₂-fold-change by exon for the specified gene and highlight the differentially expressed exons. Show annotations such as GeneID, Symbol and Strand if available as title for the gene to plot. The significantly differentially expressed individual exons are highlighted as red dots for up-regulation and as blue dots for down-regulation. The size of the dots are weighted by its significance.

Value

A plot is created on the current graphics device.

Author(s)

Yifang Hu and Gordon Smyth

See Also

[lmFit](#), [eBayes](#), [plotSplice](#)

A summary of functions available in LIMMA for RNA-seq analysis is given in [11.RNAseq](#).

Examples

```
## Not run:
fit <- lmFit(y,design)
fit <- eBayes(fit)
plotExons(fit)
plotExons(fit, exoncolname = "Start", rank = 1)
plotExons(fit, geneid = "ps", genecolname = "Symbol", exoncolname = "Start")

## End(Not run)
```

plotFB

*FB-Plot***Description**

Creates foreground-background plots.

Usage

```
## S3 method for class 'RGList'
plotFB(x, array = 1, lim = "separate", pch = 16, cex = 0.3,
       xlab = "log2 Background", ylab = "log2 Foreground", main = colnames(x)[array], ...)
## S3 method for class 'EListRaw'
plotFB(x, array = 1, pch = 16, cex=0.3,
       xlab = "log2 Background", ylab = "log2 Foreground", main = colnames(x)[array], ...)
```

Arguments

| | |
|-------|--|
| x | an RGList or EListRaw object. |
| array | integer giving the array to be plotted. |
| lim | character string indicating whether the red and green plots should have "separate" or "common" x- and y- co-ordinate limits. |
| pch | vector or list of plotting characters. Defaults to integer code 16. |
| cex | numeric vector of plot symbol expansions. |
| xlab | character string, label for x-axis. |
| ylab | character string, label for y-axis. |
| main | character string, title for plot. |
| ... | any other arguments are passed to plot. |

Details

A foreground-background plot is a plot of log2-foreground vs log2-background for a particular array. For two-color arrays, this function produces a pair of plots, one for the green channel and one for the red.

See [points](#) for possible values for pch, col and cex.

Value

A plot is created on the current graphics device.

Author(s)

Gordon Smyth

See Also

An overview of diagnostic functions available in LIMMA is given in [09.Diagnostics](#).

plotlines

plotlines

Description

Time course style plot of expression data.

Usage

```
plotlines(x, first.column.origin=FALSE, xlab="Column", ylab="x", col="black", lwd=1, ...)
```

Arguments

| | |
|----------------------------------|--|
| <code>x</code> | numeric matrix or object containing expression data. |
| <code>first.column.origin</code> | logical, should the lines be started from zero? |
| <code>xlab</code> | x-axis label |
| <code>ylab</code> | y-axis label |
| <code>col</code> | vector of colors for lines |
| <code>lwd</code> | line width multiplier |
| <code>...</code> | any other arguments are passed to plot |

Details

Plots a line for each probe.

Value

A plot is created on the current graphics device.

Author(s)

Gordon Smyth

See Also

An overview of modeling functions and associated plots available in LIMMA is given in [06.Linear-Models](#).

plotMA *MA-Plot of Expression Data*

Description

Creates an MA-plot with color coding for control spots.

Usage

```
## Default S3 method:
plotMA(object, array = 1, xlab = "Average log-expression",
        ylab = "Expression log-ratio (this sample vs others)",
        main = colnames(object)[array], status=NULL, ...)
## S3 method for class 'EList'
plotMA(object, array = 1, xlab = "Average log-expression",
        ylab = "Expression log-ratio (this sample vs others)",
        main = colnames(object)[array], status=object$genes$Status,
        zero.weights = FALSE, ...)
## S3 method for class 'RGList'
plotMA(object, array = 1, xlab = "A", ylab = "M",
        main = colnames(object)[array], status=object$genes$Status,
        zero.weights = FALSE, ...)
## S3 method for class 'MAList'
plotMA(object, array = 1, xlab = "A", ylab = "M",
        main = colnames(object)[array], status=object$genes$Status,
        zero.weights = FALSE, ...)
## S3 method for class 'MArrayLM'
plotMA(object, coef = ncol(object), xlab = "Average log-expression",
        ylab = "log-fold-change", main = colnames(object)[coef],
        status=object$genes$Status, ...)
```

Arguments

| | |
|--------|---|
| object | an RGList, MAList, EList, ExpressionSet or MArrayLM object. Alternatively a numeric matrix. |
| array | integer giving the array to be plotted. |
| coef | integer giving the linear model coefficient to be plotted. |
| xlab | character string, label for x-axis |
| ylab | character string, label for y-axis |
| main | character string, title for plot |

| | |
|--------------|---|
| status | vector giving the control status of each spot on the array, of same length as the number of rows of object. If NULL, then all points are plotted in the default color, symbol and size. |
| zero.weights | logical, should spots with zero or negative weights be plotted? |
| ... | other arguments are passed to <code>plotWithHighlights</code> . |

Details

An MA-plot is a plot of log-intensity ratios (M-values) versus log-intensity averages (A-values). See Ritchie et al (2015) for a brief historical review.

For two color data objects, a within-array MA-plot is produced with the M and A values computed from the two channels for the specified array. This is the same as a mean-difference plot (`mdplot`) with the red and green log₂-intensities of the array providing the two columns.

For single channel data objects, a between-array MA-plot is produced. An artificial array is produced by averaging all the arrays other than the array specified. A mean-difference plot is then producing from the specified array and the artificial array. Note that this procedure reduces to an ordinary mean-difference plot when there are just two arrays total.

If object is an MArrayLM object, then the plot is an fitted model MA-plot in which the estimated coefficient is on the y-axis and the average A-value is on the x-axis.

The status vector can correspond to any grouping of the probes that is of interest. If object is a fitted model object, then status vector is often used to indicate statistical significance, so that differentially expressed points are highlighted. If object is a microarray data object, then status might distinguish control probes from regular probes so that different types of controls are highlighted.

The status can be included as the component `object$genes$Status` instead of being passed as an argument to `plotMA`.

See `plotWithHighlights` for how to set colors and graphics parameters for the highlighted and non-highlighted points.

Value

A plot is created on the current graphics device.

Note

The `plotMD` function provides the same functionality as `plotMA` with slightly different arguments.

Author(s)

Gordon Smyth

References

Ritchie, ME, Phipson, B, Wu, D, Hu, Y, Law, CW, Shi, W, and Smyth, GK (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* Volume 43, e47. <http://nar.oxfordjournals.org/content/43/7/e47>

See Also

The driver function for plotMA is [plotWithHighlights](#).

An overview of plot functions available in LIMMA is given in [09.Diagnostics](#).

Examples

```
A <- runif(1000,4,16)
y <- A + matrix(rnorm(1000*3,sd=0.2),1000,3)
status <- rep(c(0,-1,1),c(950,40,10))
y[,1] <- y[,1] + status
plotMA(y, array=1, status=status, values=c(-1,1), hl.col=c("blue","red"))

MA <- new("MAList")
MA$A <- runif(300,4,16)
MA$M <- rt(300,df=3)

# Spike-in values
MA$M[1:3] <- 0
MA$M[4:6] <- 3
MA$M[7:9] <- -3

status <- rep("Gene",300)
status[1:3] <- "M=0"
status[4:6] <- "M=3"
status[7:9] <- "M=-3"
values <- c("M=0","M=3","M=-3")
col <- c("blue","red","green")

plotMA(MA,main="MA-Plot with 12 spiked-in points",
       status=status, values=values, hl.col=col)

# Same as above but setting graphical parameters as attributes
attr(status,"values") <- values
attr(status,"col") <- col
plotMA(MA, main="MA-Plot with 12 spiked-in points", status=status)

# Same as above but passing status as part of object
MA$genes$Status <- status
plotMA(MA, main="MA-Plot with 12 spiked-in points")

# Change settings for background points
MA$genes$Status <- status
plotMA(MA, bg.pch=1, bg.cex=0.5)
```

plotMA3by2

Write MA-Plots to Files

Description

Write MA-plots to files in PNG format, six plots to a file in a 3 by 2 grid arrangement.

Usage

```
plotMA3by2(object, prefix="MA", path=NULL, main=colnames(object),
           zero.weights=FALSE, common.lim=TRUE, device="png", ...)
```

Arguments

| | |
|--------------|---|
| object | an MList, RGList, EListRaw or EList object, or a matrix containing log-intensities. |
| prefix | character string giving prefix to attach to file names |
| path | character string specifying directory for output files |
| main | character vector giving titles for plots |
| zero.weights | logical, should points with non-positive weights be plotted |
| common.lim | logical, should all plots on a page use the same axis limits |
| device | device driver for the plot. Choices are "png", "jpeg", "pdf", "postscript". |
| ... | any other arguments are passed to plotMA |

Details

This function writes a series of graphic files to disk. Each file contains six MA-plots in three rows and two columns. The layout is optimized for A4-sized paper.

The graph format can be "png" or "jpeg", which are screen-resolution formats, or "pdf" or "postscript", which are loss-less formats. "png" is not available on every R platform. Note that "pdf" or "postscript" may produce very large files.

Value

No value is returned, but one or more files are written to the working directory. The number of files is determined by the number of columns of object.

Author(s)

Gordon Smyth

See Also

[plotMA](#)

An overview of diagnostic functions available in LIMMA is given in [09.Diagnostics](#).

plotMD

*Mean-Difference Plot of Expression Data***Description**

Creates a mean-difference plot (aka MA plot) with color coding for highlighted points.

Usage

```
## Default S3 method:
plotMD(object, column = 1, xlab = "Average log-expression",
        ylab = "Expression log-ratio (this sample vs others)",
        main = colnames(object)[column], status=NULL, ...)
## S3 method for class 'EList'
plotMD(object, column = 1, array = NULL, xlab = "Average log-expression",
        ylab = "Expression log-ratio (this sample vs others)",
        main = colnames(object)[column], status=object$genes$Status,
        zero.weights = FALSE, ...)
## S3 method for class 'RGList'
plotMD(object, column = 1, array = NULL, xlab = "A", ylab = "M",
        main = colnames(object)[column], status=object$genes$Status,
        zero.weights = FALSE, ...)
## S3 method for class 'MAList'
plotMD(object, column = 1, array = NULL, xlab = "A", ylab = "M",
        main = colnames(object)[column], status=object$genes$Status,
        zero.weights = FALSE, ...)
## S3 method for class 'MArrayLM'
plotMD(object, column = ncol(object), coef = NULL, xlab = "Average log-expression",
        ylab = "log-fold-change", main = colnames(object)[column],
        status=object$genes$Status, ...)
```

Arguments

| | |
|--------|---|
| object | an RGList, MAList, EList, ExpressionSet or MArrayLM object. Alternatively a numeric matrix. |
| column | integer, column of object to be plotted. |
| array | alternative to column for microarray data objects. If specified, then column is ignored. |
| coef | alternative to column for fitted model objects. If specified, then column is ignored. |
| xlab | character string, label for x-axis. |
| ylab | character string, label for y-axis. |
| main | character string, title for plot. |
| status | vector giving the control status of each spot on the array, of same length as the number of rows of object. If NULL, then all points are plotted in the default color, symbol and size. |

zero.weights logical, should spots with zero or negative weights be plotted?
... other arguments are passed to `plotWithHighlights`.

Details

A mean-difference plot (MD-plot) is a plot of log-intensity ratios (differences) versus log-intensity averages (means). For two color data objects, a within-array MD-plot is produced with the M and A values computed from the two channels for the specified array. This is the same as a mean-difference plot (`mdplot`) with the red and green log₂-intensities of the array providing the two columns.

For single channel data objects, a between-array MD-plot is produced. An artificial array is produced by averaging all the arrays other than the array specified. A mean-difference plot is then producing from the specified array and the artificial array. Note that this procedure reduces to an ordinary mean-difference plot when there are just two arrays total.

If object is an MArrayLM object, then the plot is an fitted model MD-plot in which the estimated coefficient is on the y-axis and the average A-value is on the x-axis.

The status vector can correspond to any grouping of the probes that is of interest. If object is a fitted model object, then status vector is often used to indicate statistically significance, so that differentially expressed points are highlighted. If object is a microarray data object, then status might distinguish control probes from regular probes so that different types of controls are highlighted.

The status can be included as the component `object$genes$Status` instead of being passed as an argument to `plotMD`.

See `plotWithHighlights` for how to set colors and graphics parameters for the highlighted and non-highlighted points.

Value

A plot is created on the current graphics device.

Note

This function is an alternative to `plotMA`, which was one of the original functions of the `limma` package in 2002. The history of mean-difference plots and MA-plots is reviewed in Ritchie et al (2015).

Author(s)

Gordon Smyth

References

Ritchie, ME, Phipson, B, Wu, D, Hu, Y, Law, CW, Shi, W, and Smyth, GK (2015). `limma` powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* Volume 43, e47. <http://nar.oxfordjournals.org/content/43/7/e47>

See Also

The driver function for plotMD is [plotWithHighlights](#). See also [mdplot](#) for a very basic mean-difference plot function.

An overview of plot functions available in LIMMA is given in [09.Diagnostics](#).

Examples

```
A <- runif(1000,4,16)
y <- A + matrix(rnorm(1000*3,sd=0.2),1000,3)
status <- rep(c(0,-1,1),c(950,40,10))
y[,1] <- y[,1] + status
plotMD(y, column=1, status=status, values=c(-1,1), hl.col=c("blue","red"))

MA <- new("MAMList")
MA$A <- runif(300,4,16)
MA$M <- rt(300,df=3)

# Spike-in values
MA$M[1:3] <- 0
MA$M[4:6] <- 3
MA$M[7:9] <- -3

status <- rep("Gene",300)
status[1:3] <- "M=0"
status[4:6] <- "M=3"
status[7:9] <- "M=-3"
values <- c("M=0","M=3","M=-3")
hl.col <- c("blue","red","green3")

plotMD(MA,main="MA-Plot with 12 spiked-in points",
       status=status, values=values, hl.col=hl.col)

# Same as above but setting graphical parameters as attributes
attr(status,"values") <- values
attr(status,"col") <- hl.col
plotMD(MA, main="Mean-Difference Plot with 12 spiked-in points", status=status)

# Same as above but passing status as part of object
MA$genes$Status <- status
plotMD(MA, main="Mean-Difference Plot with 12 spiked-in points")

# Change settings for background points
MA$genes$Status <- status
plotMD(MA, bg.pch=1, bg.cex=0.5)
```

Description

Plot samples on a two-dimensional scatterplot so that distances on the plot approximate the typical log₂ fold changes between the samples.

Usage

```
## Default S3 method:
plotMDS(x, top = 500, labels = NULL, pch = NULL, cex = 1,
        dim.plot = c(1,2), gene.selection = "pairwise",
        xlab = NULL, ylab = NULL, plot = TRUE, var.explained = TRUE, ...)
## S3 method for class 'MDS'
plotMDS(x, labels = NULL, pch = NULL, cex = 1, dim.plot = NULL,
        xlab = NULL, ylab = NULL, var.explained = TRUE, ...)
```

Arguments

| | |
|----------------|---|
| x | any data object that can be coerced to a matrix of log-expression values, for example an ExpressionSet or an EList. Rows represent genes or genomic features while columns represent samples. |
| top | number of top genes used to calculate pairwise distances. |
| labels | character vector of sample names or labels. Defaults to colnames(x). |
| pch | plotting symbol or symbols. See points for possible values. Ignored if labels is non-NULL. |
| cex | numeric vector of plot symbol expansions. |
| dim.plot | integer vector of length two specifying which principal components should be plotted. |
| gene.selection | character, "pairwise" to choose the top genes separately for each pairwise comparison between the samples or "common" to select the same genes for all comparisons. |
| xlab | title for the x-axis. |
| ylab | title for the y-axis. |
| plot | logical. If TRUE then a plot is created on the current graphics device. |
| var.explained | logical. If TRUE then the percentage variation explained is included in the axis labels. |
| ... | any other arguments are passed to plot, and also to text (if pch is NULL). |

Details

This function uses multidimensional scaling (MDS) to produce a principal coordinate (PCoA) or principal component (PCA) plot showing the relationships between the expression profiles represented by the columns of x. If gene.selection = "common", or if the top is equal to or greater than the number of rows of x, then a PCA plot is constructed from the top genes with largest standard deviations across the samples.

If gene.section = "pairwise" and top is less than nrow(x) then a PCoA plot is produced and distances on the plot represent the *leading log₂-fold-changes*. The leading log-fold-change between

a pair of samples is defined as the root-mean-square average of the top largest log₂-fold-changes between those two samples. The PCA and PCoA plots produced by `gene.selection="common"` and `gene.selection="pairwise"`, respectively, use similar distance measures but the PCA plot uses the same genes throughout whereas the PCoA plot potentially selects different genes to distinguish each pair of samples. The pairwise choice is the default. It potentially gives better resolution than a PCA plot if different molecular pathways are relevant for distinguishing different pairs of samples.

If `pch=NULL`, then each sample is represented by a text label, defaulting to the column names of `x`. If `pch` is not `NULL`, then plotting symbols are used.

See [text](#) for possible values for `col` and `cex`.

Value

If `plot=TRUE` or if `x` is an object of class "MDS", then a plot is created on the current graphics device. An object of class "MDS" is also invisibly returned. This is a list containing the following components:

| | |
|--------------------------------------|--|
| <code>eigen.values</code> | eigen values |
| <code>eigen.vectors</code> | eigen vectors |
| <code>var.explained</code> | proportion of variance explained by each dimension |
| <code>distance.matrix.squared</code> | numeric matrix of squared pairwise distances between columns of <code>x</code> |
| <code>dim.plot</code> | dimensions plotted |
| <code>x</code> | x-coordinates of plotted points |
| <code>y</code> | y-coordinates of plotted points |
| <code>gene.selection</code> | gene selection method |

Author(s)

Di Wu and Gordon Smyth

References

Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, and Smyth GK (2015). *limma* powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* 43, e47. <http://nar.oxfordjournals.org/content/43/7/e47>

See Also

An overview of diagnostic functions available in LIMMA is given in [09.Diagnostics](#).

Examples

```
# Simulate gene expression data for 1000 probes and 6 microarrays.
# Samples are in two groups
# First 50 probes are differentially expressed in second group
sd <- 0.3*sqrt(4/rchisq(1000,df=4))
```

```

ExprMatrix <- matrix(rnorm(1000*6,sd=sd),1000,6)
rownames(ExprMatrix) <- paste("Gene",1:1000)
ExprMatrix[1:50,4:6] <- ExprMatrix[1:50,4:6] + 2
# without labels, indexes of samples are plotted.
mds <- plotMDS(ExprMatrix, col=c(rep("black",3), rep("red",3)) )
# or labels can be provided, here group indicators:
plotMDS(mds, col=c(rep("black",3), rep("red",3)), labels= c(rep("Grp1",3), rep("Grp2",3)))

```

plotPrintTipLoess *MA Plots by Print-Tip Group*

Description

Creates a coplot giving MA-plots with loess curves by print-tip groups.

Usage

```
plotPrintTipLoess(object, layout, array=1, span=0.4, ...)
```

Arguments

| | |
|--------|--|
| object | MAList or RGList object or list with components M containing log-ratios and A containing average intensities |
| layout | a list specifying the number of tip rows and columns and the number of spot rows and columns printed by each tip. Defaults to MA\$printer if that is non-null. |
| array | integer giving the array to be plotted. Corresponds to columns of M and A. |
| span | span of window for lowess curve |
| ... | other arguments passed to panel.smooth |

Details

Note that spot quality weights in object are not used for computing the loess curves for this plot even though such weights would be used for loess normalization using `normalizeWithinArrays`.

Value

A plot is created on the current graphics device. If there are missing values in the data, then the vector of row numbers for spots with missing values is invisibly returned, as for `coplot`.

Author(s)

Gordon Smyth

See Also

An overview of diagnostic functions available in LIMMA is given in [09.Diagnostics](#).

plotRLDF

*Plot of regularized linear discriminant functions for microarray data***Description**

Plot regularized linear discriminant functions for classifying samples based on expression data.

Usage

```
plotRLDF(y, design = NULL, z = NULL, nprobes = 100, plot = TRUE,
         labels.y = NULL, labels.z = NULL, pch.y = NULL, pch.z = NULL,
         col.y = "black", col.z = "black",
         show.dimensions = c(1,2), ndim = max(show.dimensions),
         var.prior = NULL, df.prior = NULL, trend = FALSE, robust = FALSE, ...)
```

Arguments

| | |
|-----------------|--|
| y | the training dataset. Can be any data object which can be coerced to a matrix, such as ExpressionSet or EList. |
| design | design matrix defining the training groups to be distinguished. The first column is assumed to represent the intercept. Defaults to <code>model.matrix(~factor(labels.y))</code> . |
| z | the dataset to be classified. Can be any data object which can be coerced to a matrix, such as ExpressionSet or EList. Rows must correspond to rows of y. |
| nprobes | number of probes to be used for the calculations. The probes will be selected by moderated F statistic. |
| plot | logical, should a plot be created? |
| labels.y | character vector of sample names or labels in y. Defaults to <code>colnames(y)</code> or failing that to <code>1:n</code> . |
| labels.z | character vector of sample names or labels in z. Defaults to <code>colnames(z)</code> or failing that to <code>letters[1:n]</code> . |
| pch.y | plotting symbol or symbols for y. See points for possible values. Takes precedence over <code>labels.y</code> if both are specified. |
| pch.z | plotting symbol or symbols for y. See points for possible values. Takes precedence over <code>labels.z</code> if both are specified. |
| col.y | colors for the plotting <code>labels.y</code> . |
| col.z | colors for the plotting <code>labels.z</code> . |
| show.dimensions | integer vector of length two indicating which two discriminant functions to plot. Functions are in decreasing order of discriminatory power. |
| ndim | number of discriminant functions to compute |
| var.prior | prior variances, for regularizing the within-group covariance matrix. By default is estimated by <code>squeezeVar</code> . |

| | |
|-----------------------|---|
| <code>df.prior</code> | prior degrees of freedom for regularizing the within-group covariance matrix. By default is estimated by <code>squeezeVar</code> . |
| <code>trend</code> | logical, should a trend be estimated for <code>var.prior</code> ? See <code>eBayes</code> for details. Only used if <code>var.prior</code> or <code>df.prior</code> are <code>NULL</code> . |
| <code>robust</code> | logical, should <code>var.prior</code> and <code>df.prior</code> be estimated robustly? See <code>eBayes</code> for details. Only used if <code>var.prior</code> or <code>df.prior</code> are <code>NULL</code> . |
| <code>...</code> | any other arguments are passed to <code>plot</code> . |

Details

The function builds discriminant functions from the training data (`y`) and applies them to the test data (`z`). The method is a variation on classical linear discriminant functions (LDFs), in that the within-group covariance matrix is regularized to ensure that it is invertible, with eigenvalues bounded away from zero. The within-group covariance matrix is squeezed towards a diagonal matrix with empirical Bayes posterior variances as diagonal elements.

The calculations are based on a filtered list of probes. The `nprobes` probes with largest moderated F statistics are used to discriminate.

The `ndim` argument allows all required LDFs to be computed even though only two are plotted.

Value

If `plot=TRUE` a plot is created on the current graphics device. A list containing the following components is (invisibly) returned:

| | |
|------------------------------|---|
| <code>training</code> | numeric matrix with <code>ncol(y)</code> rows and <code>ndim</code> columns containing discriminant functions evaluated for the training data. |
| <code>predicting</code> | numeric matrix with <code>ncol(z)</code> rows and <code>ndim</code> columns containing discriminant functions evaluated on the classification data. |
| <code>top</code> | integer vector of length <code>nprobes</code> giving indices of probes used. |
| <code>metagenes</code> | numeric matrix with <code>nprobes</code> rows and <code>ndim</code> columns containing probe weights defining each discriminant function. |
| <code>singular.values</code> | <code>singular.values</code> showing the predictive power of each discriminant function. |
| <code>rank</code> | maximum number of discriminant functions with <code>singular.values</code> greater than zero. |
| <code>var.prior</code> | numeric vector of prior variances. |
| <code>df.prior</code> | numeric vector of prior degrees of freedom. |

Note

The default values for `df.prior` and `var.prior` were changed in `limma` 3.27.10. Previously these were preset values. Now the default is to estimate them using `squeezeVar`.

Author(s)

Gordon Smyth, Di Wu and Yifang Hu

See Also

lda in package MASS

Examples

```
# Simulate gene expression data for 1000 probes and 6 microarrays.
# Samples are in two groups
# First 50 probes are differentially expressed in second group
sd <- 0.3*sqrt(4/rchisq(1000,df=4))
y <- matrix(rnorm(1000*6,sd=sd),1000,6)
rownames(y) <- paste("Gene",1:1000)
y[1:50,4:6] <- y[1:50,4:6] + 2

z <- matrix(rnorm(1000*6,sd=sd),1000,6)
rownames(z) <- paste("Gene",1:1000)
z[1:50,4:6] <- z[1:50,4:6] + 1.8
z[1:50,1:3] <- z[1:50,1:3] - 0.2

design <- cbind(Grp1=1,Grp2vs1=c(0,0,0,1,1,1))
options(digit=3)

# Samples 1-6 are training set, samples a-f are test set:
plotRLDF(y, design, z=z, col.y="black", col.z="red")
legend("top", pch=16, col=c("black","red"), legend=c("Training","Predicted"))
```

plotSA

Sigma vs A plot for microarray linear model

Description

Plot residual standard deviation versus average log expression for a fitted microarray linear model.

Usage

```
plotSA(fit, xlab = "Average log-expression", ylab = "sqrt(sigma)", zero.weights = FALSE,
      pch = 16, cex = 0.3, col = c("black","red"), ...)
```

Arguments

| | |
|--------------|---|
| fit | an MArrayLM object. |
| xlab | label for x-axis |
| ylab | label for y-axis |
| zero.weights | logical, should genes with all zero weights be plotted? |
| pch | vector of codes for plotting characters. |
| cex | numeric, vector of expansion factors for plotting characters. |
| col | plotting colors for regular and outlier variances respectively. |
| ... | any other arguments are passed to plot |

Details

This plot is used to check the mean-variance relationship of the expression data, after fitting a linear model. A scatterplot of residual-variances vs average log-expression is created. The plot is especially useful for examining the mean-variance trend estimated by [eBayes](#) or [treat](#) with `trend=TRUE`. It can be considered as a routine diagnostic plot in the limma-trend pipeline.

If robust empirical Bayes was used to create `fit`, then outlier variances are highlighted in the color given by `col[2]`.

The y-axis is square-root `fit$sigma`, where `sigma` is the estimated residual standard deviation. The y-axis therefore corresponds to quarter-root variances. The y-axis was changed from log2-variance to quarter-root variance in limma version 3.31.21. The quarter-root scale matches the similar plot produced by the `voom` function and gives a better plot when some of the variances are close to zero.

See [points](#) for possible values for `pch` and `cex`.

Value

A plot is created on the current graphics device.

Author(s)

Gordon Smyth

See Also

[eBayes](#)

An overview of diagnostic functions available in LIMMA is given in [09.Diagnostics](#).

plotSplice

Differential splicing plot

Description

Plot relative log-fold changes by exons for the specified gene and highlight the significantly spliced exons.

Usage

```
plotSplice(fit, coef=ncol(fit), geneid=NULL, genecolname=NULL, rank=1L, FDR = 0.05)
```

Arguments

| | |
|--------------------------|---|
| <code>fit</code> | MArrayLM fit object produced by <code>diffSplice</code> . |
| <code>coef</code> | the coefficient (column) of <code>fit</code> for which differentially splicing is assessed. |
| <code>geneid</code> | character string, ID of the gene to plot. |
| <code>genecolname</code> | column name of <code>fit\$genes</code> containing gene IDs. Defaults to <code>fit\$genecolname</code> . |
| <code>rank</code> | integer, if <code>geneid=NULL</code> then this ranked gene will be plotted. |

FDR numeric, highlight exons as red dots with false discovery rate less than this cut-off. The FDR of the individual exon is calculated based on the exon-level t-statistics test for differences between each exon and all other exons for the same gene.

Details

Plot relative log₂-fold-changes by exon for the specified gene. The relative logFC is the difference between the exon's logFC and the overall logFC for the gene, as computed by `diffSplice`. The significantly spliced individual exons are highlighted as red dots. The size of the red dots are weighted by its significance.

Value

A plot is created on the current graphics device.

Author(s)

Gordon Smyth and Yifang Hu

See Also

[diffSplice](#), [topSplice](#)

A summary of functions available in LIMMA for RNA-seq analysis is given in [11.RNAseq](#).

Examples

```
# See diffSplice
```

`plotWithHighlights` *Scatterplot With Highlighting of Special Points*

Description

Creates scatterplot, with optional size and color coding for points of special interest. This is the engine for `plotMD` and `plotMA`.

Usage

```
plotWithHighlights(x, y, status = NULL, values = NULL,  
                  hl.pch = 16, hl.col = NULL, hl.cex = 1, legend = "topright",  
                  bg.pch = 16, bg.col = "black", bg.cex = 0.3,  
                  pch = NULL, col = NULL, cex = NULL, ...)
```

Arguments

| | |
|---------------------|---|
| <code>x</code> | numeric vector. |
| <code>y</code> | numeric vector of same length as <code>x</code> . |
| <code>status</code> | character vector giving the control status of each point, of same length as <code>x</code> and <code>y</code> , or else a <code>TestResults</code> object with one column and number of rows matching the length of <code>x</code> . If <code>NULL</code> , then all points are plotted in the background color, symbol and size. |
| <code>values</code> | character vector giving values of <code>status</code> to be highlighted on the plot. Set automatically if <code>status</code> contains <code>TestResults</code> . Defaults to unique values of <code>status</code> in decreasing order of frequency, with the most frequent value set as the background value. Ignored if there is no <code>status</code> vector. |
| <code>hl.pch</code> | vector of plotting characters for highlighted points, either of unit length or of same length as <code>values</code> . Ignored if there is no <code>status</code> vector. |
| <code>hl.col</code> | vector of colors for highlighted points, either of unit length or of same length as <code>values</code> . Set automatically if <code>status</code> contains <code>TestResults</code> . Defaults to <code>1+1:length(values)</code> . Ignored if there is no <code>status</code> vector. |
| <code>hl.cex</code> | numeric vector of plot symbol expansions for highlighted points, either of unit length or of same length as <code>values</code> . Ignored if there is no <code>status</code> vector. |
| <code>legend</code> | character string giving position to place legend. See legend for possible values. Can also be logical, with <code>FALSE</code> meaning no legend. Ignored if there is no <code>status</code> vector. |
| <code>bg.pch</code> | plotting character for background (non-highlighted) points. |
| <code>bg.col</code> | color for background (non-highlighted) points. |
| <code>bg.cex</code> | plot symbol expansion for background (non-highlighted) points. |
| <code>pch</code> | synonym for <code>hl.pch</code> allowed for backward compatibility. |
| <code>col</code> | synonym for <code>hl.col</code> allowed for backward compatibility. |
| <code>cex</code> | synonym for <code>hl.cex</code> allowed for backward compatibility. |
| <code>...</code> | other arguments are passed to <code>plot</code> . |

Details

This function produces a scatterplot in which the highlighted points are, by default, larger and colored compared to background points.

The `status` vector establishes the status of each point and `values` indicates which values of `status` should be highlighted. If `values=NULL`, then the most common value of `status` is assumed to correspond to background points and all other values are highlighted.

The arguments `hl.pch`, `hl.col` and `hl.cex` give graphics settings for highlighted points. By default, highlighted points are larger than background points and a different color is used for each distinct highlighted value.

The arguments `bg.pch`, `bg.col` and `bg.cex` give the graphics settings for non-highlighted (background) points. The same settings are used for all background points.

The arguments `values`, `pch`, `col` and `cex` can be included as attributes to `status` instead of being passed as arguments to `plotWithHighlights`. This is for compatibility with [controlStatus](#).

See [points](#) for possible values for the graphics parameters.

Value

A plot is created on the current graphics device.

Author(s)

Gordon Smyth

References

Ritchie, ME, Phipson, B, Wu, D, Hu, Y, Law, CW, Shi, W, and Smyth, GK (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* 43, e47. <http://nar.oxfordjournals.org/content/43/7/e47>

See Also

[plotMD](#), [plotMA](#), [mdplot](#)

An overview of diagnostic plots available in LIMMA is given in [09.Diagnostics](#).

Examples

```
x <- runif(1000, min=4, max=16)
status <- rep(c(0,-1,1), c(950,40,10))
y <- status + rnorm(1000, sd=0.2)
plotWithHighlights(x, y, status=status)
```

poolVar

Pool Sample Variances with Unequal Variances

Description

Compute the Satterthwaite (1946) approximation to the distribution of a weighted sum of sample variances.

Usage

```
poolVar(var, df=n-1, multiplier=1/n, n)
```

Arguments

| | |
|------------|---|
| var | numeric vector of independent sample variances |
| df | numeric vector of degrees of freedom for the sample variances |
| multiplier | numeric vector giving multipliers for the sample variances |
| n | numeric vector of sample sizes |

Details

The sample variances `var` are assumed to follow scaled chi-square distributions. A scaled chi-square approximation is found for the distribution of `sum(multiplier * var)` by equating first and second moments. On output the sum to be approximated is equal to `multiplier * var` which follows approximately a scaled chisquare distribution on `df` degrees of freedom. The approximation was proposed by Satterthwaite (1946).

If there are only two groups and the degrees of freedom are one less than the sample sizes then this gives the denominator of Welch's t-test for unequal variances.

Value

A list with components

| | |
|-------------------------|-------------------------------------|
| <code>var</code> | effective pooled sample variance |
| <code>df</code> | effective pooled degrees of freedom |
| <code>multiplier</code> | pooled multiplier |

Author(s)

Gordon Smyth

References

Welch, B. L. (1938). The significance of the difference between two means when the population variances are unequal. *Biometrika* **29**, 350-362.

Satterthwaite, F. E. (1946). An approximate distribution of estimates of variance components. *Biometrics Bulletin* **2**, 110-114.

Welch, B. L. (1947). The generalization of 'Student's' problem when several different population variances are involved. *Biometrika* **34**, 28-35.

Welch, B. L. (1949). Further note on Mrs. Aspin's tables and on certain approximations to the tabulated function. *Biometrika* **36**, 293-296.

Examples

```
# Welch's t-test with unequal variances
x <- rnorm(10,mean=1,sd=2)
y <- rnorm(20,mean=2,sd=1)
s2 <- c(var(x),var(y))
n <- c(10,20)
out <- poolVar(var=s2,n=n)
tstat <- (mean(x)-mean(y)) / sqrt(out$var*out$multiplier)
pvalue <- 2*pt(-abs(tstat),df=out$df)
# Equivalent to t.test(x,y)
```

predFCm *Predictive log fold change for microarrays*

Description

Calculate the predictive log fold change for a particular coefficient from a fit object.

Usage

```
predFCm(fit, coef=2, var.indep.of.fc=TRUE, all.de=TRUE, prop.true.null.method="lfdr")
```

Arguments

| | |
|-----------------------|---|
| fit | an MArrayLM fitted model object produced by lmFit and eBayes |
| coef | integer vector indicating which columns in the fit object are to be shrunk |
| var.indep.of.fc | assume the genewise variances are independent of genewise fold changes? |
| all.de | assume all genes are have a non-zero true fold change (TRUE)? If FALSE, then the proportion of truly non-differentially (non-DE) genes expressed will be estimated. |
| prop.true.null.method | method used to estimate proportion of truly non-DE genes. See propTrueNull for possible values. |

Details

The predictive log fold changes are calculated as the posterior mean log fold changes in the empirical Bayes hierarchical model. We call them predictive log fold changes because they are the best prediction of what the log fold change will be for each gene in a comparable future experiment.

The log fold changes are shrunk towards zero depending on how variable they are. The `var.indep.of.fc` argument specifies whether the prior belief is that the log fold changes are independent of the variability of the genes or whether the log fold changes increase with increasing variability of the genes.

If `all.de=TRUE`, then all genes are assumed to have a non-zero log fold change, even if quite small. If `all.de=FALSE`, then some genes are assumed to have log fold changes exactly zero. The proportion of non-DE genes is estimated and taken into account in the calculation.

Value

numeric vector of predictive (shrunk) log fold changes

Author(s)

Belinda Phipson and Gordon Smyth

References

Phipson, B. (2013). *Empirical Bayes modelling of expression profiles and their associations*. PhD Thesis. University of Melbourne, Australia. <http://hdl.handle.net/11343/38162>

See Also

[lmFit](#), [eBayes](#), [contrasts.fit](#)

Examples

```
# Simulate gene expression data,
# 6 microarrays with 1000 genes on each array
set.seed(2004)
y <- matrix(rnorm(6000),ncol=4)

# two experimental groups and one control group with two replicates each
group <- factor(c("A","A","B","B"))
design <- model.matrix(~group)

# fit a linear model
fit <- lmFit(y,design)
fit <- eBayes(fit)

# output predictive log fold changes for first 5 genes
pfc <- predFCm(fit,coef=2)
```

printHead

Print Leading Rows of Large Objects

Description

Print the leading rows of a large vector, matrix or data.frame. This function is used by show methods for data classes defined in LIMMA.

Usage

```
printHead(x)
```

Arguments

x any object

Details

If x is a vector with more than 20 elements, then printHead(x) prints only the first 5 elements. If x is a matrix or data.frame with more than 10 rows, then printHead(x) prints only the first 5 rows. Any other type of object is printed normally.

Author(s)

Gordon Smyth

See AlsoAn overview of classes defined in LIMMA is given in [02.Classes](#)

PrintLayout*Print Layout - class*

Description

A list-based class for storing information about the process used to print spots on a microarray.

PrintLayout objects can be created using [getLayout](#). The printer component of an RGList or MAList object is of this class.

Slots/List Components

Objects of this class contains no slots but should contain the following list components:

`ngrid.r`: number of grid rows on the arrays
`ngrid.c`: number of grid columns on the arrays
`nspot.r`: number of rows of spots in each grid
`nspot.c`: number of columns of spots in each grid
`ndups`: number of duplicates of each DNA clone, i.e., number of times print-head dips into each well of DNA
`spacing`: number of spots between duplicate spots. Only applicable if `ndups>1`. `spacing=1` for side-by-side spots by rows,
`npins`: actual number of pins or tips on the print-head
`start`: character string giving position of the spot printed first in each grid. Choices are "topleft" or "topright" and p

Author(s)

Gordon Smyth

See Also[02.Classes](#) gives an overview of all the classes defined by this package.**Examples**

```
# Settings for Swirl and ApoAI example data sets in User's Guide
printer <- list(ngrid.r=4, ngrid.c=4, nspot.r=22, nspot.c=24,
               ndups=1, spacing=1, npins=16, start="topleft")

# Typical settings at the Australian Genome Research Facility

# Full pin set, duplicates side-by-side on same row
printer <- list(ngrid.r=12, ngrid.c=4, nspot.r=20, nspot.c=20,
```

```

        ndups=2, spacing=1, npins=48, start="topright")

# Half pin set, duplicates in top and lower half of slide
printer <- list(ngrid.r=12, ngrid.c=4, nspot.r=20, nspot.c=20,
              ndups=2, spacing=9600, npins=24, start="topright")

```

printorder

Identify Order in which Spots were Printed

Description

Identify order in which spots were printed and the 384-well plate from which they were printed.

Usage

```
printorder(layout, ndups=1, spacing="columns", npins, start="topleft")
```

Arguments

| | |
|---------|--|
| layout | list with the components <code>ngrid.r</code> , <code>ngrid.c</code> , <code>nspot.r</code> and <code>nspot.c</code> , or an <code>RGList</code> or <code>MAList</code> object from which the printer layout may be extracted. |
| ndups | number of duplicate spots, i.e., number of times print-head dips into each well |
| spacing | character string indicating layout of duplicate spots. Choices are "columns", "rows" or "topbottom". |
| npins | actual number of pins or tips on the print-head |
| start | character string giving position of the spot printed first in each grid. Choices are "topleft" or "topright" and partial matches are accepted. |

Details

In most cases the printer-head contains the `layout$ngrid.r` times `layout$ngrid.c` pins or tips and the array is printed using `layout$nspot.r` times `layout$nspot.c` dips of the head. The plate holding the DNA to be printed is assumed to have 384 wells in 16 rows and 24 columns.

`ndups` indicates the number of spots printed from each well. The replicate spots from multiple dips into the same wells are assumed to be side-by-side by columns (`spacing="columns"`), by rows (`spacing="rows"`) or in the top and bottom halves of the array (`spacing="topbottom"`).

In some cases a smaller number of physical pins is used and the total number of grids is built up by effectively printing two or more sub-arrays on the same slide. In this case the number of grids should be a multiple of the number of pins.

Printing is assumed to proceed by rows within in each grid starting either from the top-left or the top-right.

Value

List with components

| | |
|---------------|--|
| printorder | numeric vector giving printorder of each spot, i.e., which dip of the print-head was used to print it |
| plate | numeric vector giving plate number from which each spot was printed |
| plate.r | numeric vector giving plate-row number of the well from which each spot was printed |
| plate.c | numeric vector giving plate-column number of the well from which each spot was printed |
| plateposition | character vector summarizing plate number and plate position of the well from which each spot was printed with letters for plate rows and number for columns. For example 02B13 is second row, 13th column, of the second plate. |

Author(s)

Gordon Smyth

See Also

[normalizeForPrintorder.](#)

An overview of LIMMA functions for reading data is given in [03.ReadingData](#).

Examples

```
printorder(list(ngrid.r=2,ngrid.c=2,nspot.r=12,nspot.c=8))
```

printtipWeights *Sub-array Quality Weights*

Description

Estimates relative quality weights for each sub-array in a multi-array experiment.

Usage

```
printtipWeights(object, design = NULL, weights = NULL, method = "genebygene", layout,  
                  maxiter = 50, tol = 1e-10, trace=FALSE)
```

Arguments

| | |
|---------|---|
| object | object of class <code>numeric</code> , <code>matrix</code> , <code>MAList</code> , <code>marrayNorm</code> , or <code>ExpressionSet</code> containing log-ratios or log-values of expression for a series of spotted microarrays. |
| design | the design matrix of the microarray experiment, with rows corresponding to arrays and columns to coefficients to be estimated. Defaults to the unit vector meaning that the arrays are treated as replicates. |
| weights | optional numeric matrix containing prior weights for each spot. |
| method | character string specifying the estimating algorithm to be used. Choices are "genebygene" and "reml". |
| layout | list specifying the dimensions of the spot matrix and the grid matrix. For details see PrintLayout-class . |
| maxiter | maximum number of iterations allowed. |
| tol | convergence tolerance. |
| trace | logical variable. If true then output diagnostic information at each iteration of "reml" algorithm. |

Details

The relative reliability of each sub-array (print-tip group) is estimated by measuring how well the expression values for that sub-array follow the linear model.

The method described in Ritchie et al (2006) and implemented in the `arrayWeights` function is adapted for this purpose. A heteroscedastic model is fitted to the expression values for each gene by calling the function `lm.wfit`. The dispersion model is fitted to the squared residuals from the mean fit, and is set up to have sub-array specific coefficients, which are updated in either full REML scoring iterations, or using an efficient gene-by-gene update algorithm. The final estimates of the sub-array variances are converted to weights.

The data object `object` is interpreted as for `lmFit`. In particular, the arguments `design`, `weights` and `layout` will be extracted from the data object if available and do not normally need to be set explicitly in the call; if any of these are set in the call then they will over-ride the slots or components in the data object.

Value

A matrix of sub-array weights.

Author(s)

Matthew Ritchie and Gordon Smyth

References

Ritchie ME, Diyagama D, Neilson J, van Laar R, Dobrovic A, Holloway A, Smyth GK (2006). Empirical array quality weights in the analysis of microarray data. *BMC Bioinformatics* 7, 261. doi:10.1186/147121057261

See Also

An overview of linear model functions in limma is given by [06.LinearModels](#).

Examples

```
## Not run:
# This example is designed for work on a subset of the data
# from ApoAI case study in Limma User's Guide

RG <- backgroundCorrect(RG, method="normexp")
MA <- normalizeWithinArrays(RG)
targets <- data.frame(Cy3=I(rep("Pool",6)),Cy5=I(c("WT", "WT", "WT", "KO", "KO", "KO")))
design <- modelMatrix(targets, ref="Pool")
subarrayw <- printtipWeights(MA, design, layout=mouse.setup)
fit <- lmFit(MA, design, weights=subarrayw)
fit2 <- contrasts.fit(fit, contrasts=c(-1,1))
fit2 <- eBayes(fit2)
# Use of sub-array weights increases the significance of the top genes
topTable(fit2)
# Create an image plot of sub-array weights from each array
zlim <- c(min(subarrayw), max(subarrayw))
par(mfrow=c(3,2), mai=c(0.1,0.1,0.3,0.1))
for(i in 1:6)
  imageplot(subarrayw[,i], layout=mouse.setup, zlim=zlim, main=paste("Array", i))

## End(Not run)
```

propexpr

Estimate Proportion of Expressed Probes

Description

Estimate the proportion of microarray probes which are expressed in each array.

Usage

```
propexpr(x, neg.x=NULL, status=x$genes$Status, labels=c("negative", "regular"))
```

Arguments

| | |
|--------|--|
| x | matrix or similar object containing raw intensities for a set of arrays. |
| neg.x | matrix or similar object containing raw intensities for negative control probes for the same arrays. If NULL, then negative controls must be provided in x. |
| status | character vector specifying control type of each probe. Only used if neg.x is NULL. |
| labels | character vector giving the status values for negative control probes and regular (non-control) probes respectively. If of length 1, then all probes other than the negative controls are assumed to be regular. Only used if neg.x is NULL. |

Details

This function estimates the overall proportion of probes on each microarray that correspond to expressed genes using the method of Shi et al (2010). The function is especially useful for Illumina BeadChips arrays, although it can in principle be applied to any platform with good quality negative controls.

The negative controls can be supplied either as rows of `x` or as a separate matrix. If supplied as rows of `x`, then the negative controls are identified by the status vector. `x` might also include other types of control probes, but these will be ignored in the calculation.

Illumina BeadChip arrays contain 750~1600 negative control probes. If `read.idat` is used to read Illumina expression IDAT files, then the control probes will be populated as rows of the output `EListRaw` object, and the vector `x$genes$Status` will be set to identify control probes.

Alternatively, expression values can be exported from Illumina's GenomeStudio software as tab-delimited text files. In this case, the control probes are usually written to a separate file from the regular probes.

Value

Numeric vector giving the proportions of expressed probes in each array.

Author(s)

Wei Shi and Gordon Smyth

References

Shi W, de Graaf C, Kinkel S, Achtman A, Baldwin T, Schofield L, Scott H, Hilton D, Smyth GK (2010). Estimating the proportion of microarray probes expressed in an RNA sample. *Nucleic Acids Research* 38(7), 2168-2176. doi:10.1093/nar/gkp1204

See Also

Description to the control probes in Illumina BeadChips can be found in [read.ilmn](#).

Examples

```
## Not run:
# Read Illumina binary IDAT files
x <- read.idat(idat, bgx)
propexpr(x)

# Read text files exported from GenomeStudio
x <- read.ilmn(files = "sample probe profile.txt",
              ctrlfiles = "control probe profile.txt")
propexpr(x)

## End(Not run)
```

propTrueNull *Estimate Proportion of True Null Hypotheses*

Description

Estimate the proportion of true null hypotheses from a vector of p-values.

Usage

```
propTrueNull(p, method="lfdr", nbins=20, ...)  
convest(p, niter=100, plot=FALSE, report=FALSE, file="", tol=1e-6)
```

Arguments

| | |
|--------|---|
| p | numeric vector of p-values. |
| method | estimation method. Choices are "lfdr", "mean", "hist" or "convest". |
| nbins | number of histogram bins (if method="hist"). |
| niter | number of iterations to be used in fitting the convex, decreasing density for the p-values. |
| plot | logical, should updated plots of fitted convex decreasing p-value density be produced at each iteration? |
| report | logical, should the estimated proportion be printed at each iteration? |
| file | name of file to which to write the report. Defaults to standard output. |
| tol | accuracy of the bisectional search for finding a new convex combination of the current iterate and the mixing density |
| ... | other arguments are passed to convest if method="convest". |

Details

The proportion of true null hypotheses in a collection of hypothesis tests is often denoted π_0 . This function estimates π_0 from a vector of p-values.

method="lfdr" implements the method of Phipson (2013) based on averaging local false discovery rates across the p-values.

method="mean" is a very simple method based on averaging the p-values. It gives a slightly smaller estimate than $2*\text{mean}(p)$.

method="hist" implements the histogram method of Mosig et al (2001) and Nettleton et al (2006).

method="convest" calls convest, which implements the method of Langaas et al (2005) based on a convex decreasing density estimate.

Value

Numeric value in the interval [0,1] representing the estimated proportion of true null hypotheses.

Author(s)

Belinda Phipson and Gordon Smyth for propTrueNull. Egil Ferkingstad, Mette Langaas and Marcus Davy for convest.

References

Langaas, M, Ferkingstad, E, and Lindqvist, B (2005). Estimating the proportion of true null hypotheses, with application to DNA microarray data. *Journal of the Royal Statistical Society Series B* 67, 555-572.

Mosig MO, Lipkin E, Khutoreskaya G, Tchourzyna E, Soller M, Friedmann A (2001). A whole genome scan for quantitative trait loci affecting milk protein percentage in Israeli-Holstein cattle, by means of selective milk DNA pooling in a daughter design, using an adjusted false discovery rate criterion. *Genetics* 157, 1683-1698.

Nettleton D, Hwang JTG, Caldo RA, Wise RP (2006). Estimating the number of true null hypotheses from a histogram of p values. *Journal of Agricultural, Biological, and Environmental Statistics* 11, 337-356.

Phipson, B (2013). Empirical Bayes Modelling of Expression Profiles and Their Associations. PhD Thesis, University of Melbourne, Australia. <http://hdl.handle.net/11343/38162>

Ritchie, ME, Phipson, B, Wu, D, Hu, Y, Law, CW, Shi, W, and Smyth, GK (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* 43, e47. doi:10.1093/nar/gkv007

See Also

See [08.Tests](#) for other functions for producing or interpreting p-values.

Examples

```
# Test statistics
z <- rnorm(200)

# First 40 are have non-zero means
z[1:40] <- z[1:40]+2

# True pi0
160/200

# Two-sided p-values
p <- 2*pnorm(-abs(z))

# Estimate pi0
propTrueNull(p, method="lfdr")
propTrueNull(p, method="hist")
```

| | |
|-----------------|-------------------------------|
| protectMetachar | <i>Protect Metacharacters</i> |
|-----------------|-------------------------------|

Description

Add backslashes before any metacharacters found in a string.

Usage

```
protectMetachar(x)
```

Arguments

| | |
|---|------------------|
| x | character vector |
|---|------------------|

Details

This function is used to protect strings containing metacharacters so that the metacharacters can be treated as ordinary characters in string matching functions operations.

Value

A character vector of the same length as x in which two backslashes have been inserted before any metacharacter.

Author(s)

Gordon Smyth

See Also

An overview of LIMMA functions for reading data is given in [03.ReadingData](#).

Examples

```
# without protectMetachar, this would be no match  
grep(protectMetachar("Ch1 (mean)"), "Ch1 (mean)")
```

qqt

*Student's t or Fisher's F Quantile-Quantile Plot***Description**

Plots the quantiles of a data sample against the theoretical quantiles of a Student's t distribution.

Usage

```
qqt(y, df = Inf, ylim = range(y), main = "Student's t Q-Q Plot",
    xlab = "Theoretical Quantiles", ylab = "Sample Quantiles", plot.it = TRUE, ...)
qqf(y, df1, df2, ylim=range(y), main= "F Distribution Q-Q Plot",
    xlab = "Theoretical Quantiles", ylab = "Sample Quantiles", plot.it = TRUE, ...)
```

Arguments

| | |
|---------|---|
| y | a numeric vector or array containing the data sample |
| df | degrees of freedom for the t-distribution. The default df=Inf represents the normal distribution. |
| df1 | numerator degrees of freedom for the F-distribution. |
| df2 | denominator degrees of freedom for the F-distribution. |
| ylim | plotting range for y |
| main | main title for the plot |
| xlab | x-axis title for the plot |
| ylab | y-axis title for the plot |
| plot.it | whether or not to produce a plot |
| ... | other arguments to be passed to plot |

Details

This function is analogous to qqnorm for normal probability plots. In fact qqt(y, df=Inf) is identical to qqnorm(y) in all respects except the default title on the plot.

Value

A list is invisibly returned containing the values plotted in the QQ-plot:

| | |
|---|---|
| x | theoretical quantiles of the t-distribution or F-distribution |
| y | the data sample, same as input y |

Author(s)

Gordon Smyth

See Also[qqnorm](#)**Examples**

```
# See also the lmFit examples

y <- rt(50,df=4)
qqt(y,df=4)
abline(0,1)
```

QualityWeights

Spot Quality Weights for Spotted Microarrays

Description

Functions to calculate quality weights for individual spots based on the image analysis output file for a spotted microarray.

Usage

```
wtarea(ideal = c(160,170))
wtflags(weight = 0, cutoff = 0)
wtIgnore.Filter
```

Arguments

| | |
|--------|---|
| ideal | numeric vector giving the ideal range of areas for good quality spots (in pixels). The minimum and maximum values are used to specify the range of ideal values. All values should be positive. |
| weight | non-negative weight to be given to flagged spots. |
| cutoff | cutoff value for Flags below which spots will be downweighted. |

Details

These functions can be passed as an argument to `read.maimages` to construct quality weights as the microarray data is read in.

`wtarea` downweights unusually small or large spots and is designed for SPOT output. It gives weight 1 to spots that have areas in the ideal range, given in pixels, and linearly downweights spots that are smaller or larger than this range.

`wtflags` is designed for GenePix output and gives the specified weight to spots with Flags value less than the cutoff value. Choose `cutoff=0` to downweight all flagged spots. Choose `cutoff=-50` to downweight bad or absent spots or `cutoff=-75` to downweight only spots which have been manually flagged as bad.

`wtIgnore.Filter` is designed for QuantArray output and sets the weights equal to the column Ignore Filter produced by QuantArray. These weights are 0 for spots to be ignored and 1 otherwise.

Value

A function that takes a dataframe or matrix as argument and produces a numeric vector of weights between 0 and 1.

Author(s)

Gordon Smyth

See Also

An overview of LIMMA functions for reading data is given in [03.ReadingData](#).

Examples

```
# Read in spot output files from current directory and give full weight to 165
# pixel spots. Note: for this example to run you must set fnames to the names
# of actual spot output files (data not provided).
## Not run:
RG <- read.maimages(fnames,source="spot",wt.fun=wtarea(165))
# Spot will be downweighted according to weights found in RG
MA <- normalizeWithinArrays(RG,layout)

## End(Not run)
```

rankSumTestWithCorrelation

Two Sample Wilcoxon-Mann-Whitney Rank Sum Test Allowing For Correlation

Description

A extension of the well-known rank-based test, but allowing for correlations between cases.

Usage

```
rankSumTestWithCorrelation(index, statistics, correlation=0, df=Inf)
```

Arguments

| | |
|-------------|---|
| index | any index vector such that statistics[index] contains the values of the statistic for the test group. |
| statistics | numeric vector giving values of the test statistic. |
| correlation | numeric scalar, average correlation between cases in the test group. Cases in the second group are assumed independent of each other and other the first group. |
| df | degrees of freedom which the correlation has been estimated. |

Details

This function implements a correlation-adjusted version of the Wilcoxon-Mann-Whitney test proposed by Wu and Smyth (2012). It tests whether the mean rank of statistics in the test group is greater or less than the mean rank of the remaining statistic values.

When the correlation (or variance inflation factor) is zero, the function performs the usual two-sample Wilcoxon-Mann-Whitney rank sum test. The Wilcoxon-Mann-Whitney test is implemented following the formulas given in Zar (1999) Section 8.10, including corrections for ties and for continuity.

The test allows for the possibility that cases in the test group may be more highly correlated on average than cases not in the group. When the correlation is non-zero, the variance of the rank-sum statistic is computed using a formula derived from equation (4.5) of Barry et al (2008). When the correlation is positive, the variance is increased and test will become more conservative.

Value

Numeric vector of length 2 containing the `left.tail` and `right.tail` p-values.

Author(s)

Gordon Smyth and Di Wu

References

Barry, W.T., Nobel, A.B., and Wright, F.A. (2008). A statistical framework for testing functional categories in microarray data. *Annals of Applied Statistics* 2, 286-315.

Wu, D, and Smyth, GK (2012). Camera: a competitive gene set test accounting for inter-gene correlation. *Nucleic Acids Research* 40, e133. doi:10.1093/nar/gks461

Zar, JH (1999). *Biostatistical Analysis 4th Edition*. Prentice-Hall International, Upper Saddle River, New Jersey.

See Also

`wilcox.test` performs the usual Wilcoxon-Mann-Whitney test assuming independence.

An overview of tests in limma is given in [08.Tests](#).

Examples

```
stat <- rnorm(100)
index <- 1:10
stat[index] <- stat[1:10]+1

rankSumTestWithCorrelation(index, stat)
rankSumTestWithCorrelation(index, stat, correlation=0.1)

group <- rep(1,100)
group[index] <- 2
group <- factor(group)
wilcox.test(stat ~ group)
```

| | |
|--------------|---|
| read.columns | <i>Read specified columns from a file</i> |
|--------------|---|

Description

Reads specified columns from a file in table format and creates a data frame from it, with cases corresponding to lines and variables to fields in the file.

Usage

```
read.columns(file, required.col=NULL, text.to.search="", sep="\t", quote="\\"", skip=0,
             fill=TRUE, blank.lines.skip=TRUE, comment.char="", allowEscapes=FALSE, ...)
```

Arguments

| | |
|------------------|--|
| file | the name of the file which the data are to be read from. |
| required.col | character vector of names of the required columns |
| text.to.search | character string. If any column names can be found in this string, those columns will also be read. |
| sep | the field separator character |
| quote | character string of characters to be treated as quote marks |
| skip | the number of lines of the data file to skip before beginning to read data. |
| fill | logical. If TRUE then in case the rows have unequal length, blank fields are implicitly added. |
| blank.lines.skip | logical: if TRUE blank lines in the input are ignored. |
| comment.char | character: a character vector of length one containing a single character or an empty string. |
| allowEscapes | logical. Should C-style escapes such as ‘\n’ be processed or read verbatim (the default)? |
| ... | other arguments are passed to read.table, excluding the following which are reserved and cannot be set by the user: header, col.names, check.names and colClasses. |

Details

This function is an interface to read.table in the base package. It uses required.col and text.to.search to set up the colClasses argument of read.table.

Note the following arguments of read.table are used by read.columns and therefore cannot be set by the user: header, col.names, check.names and colClasses.

This function is used by [read.maimages](#).

Value

A data frame (data.frame) containing a representation of the data in the file.

Author(s)

Gordon Smyth

See Also[read.maimages](#), [read.table](#).An overview of LIMMA functions for reading data is given in [03.ReadingData](#).

| | |
|-----------|--|
| read.idat | <i>Read Illumina expression data from IDAT files</i> |
|-----------|--|

Description

Read Illumina BeadArray data from IDAT and manifest (.bgx) files for gene expression platforms.

Usage

```
read.idat(idatfiles, bgxfile, path = NULL, bgxpath = path,
          dateinfo = FALSE, annotation = "Symbol", tolerance = 0, verbose = TRUE)
```

Arguments

| | |
|------------|---|
| idatfiles | character vector specifying the IDAT files to be read in. Gzipped files are not accepted. |
| bgxfile | character string specifying bead manifest file (.bgx) to be read in. May be gzipped. |
| path | character string giving the directory containing the IDAT files. The default is the current working directory. |
| bgxpath | character string giving the directory containing the bgx manifest file. Defaults to the same directory as for IDAT files. |
| dateinfo | logical. Should date and software version information be read in? |
| annotation | character vector of annotation columns to be read from the manifest file. |
| tolerance | integer. The number of probe ID discrepancies allowed between the manifest and any of the IDAT files. |
| verbose | logical. Should progress messages are sent to standard output? |

Details

Illumina's BeadScan/iScan software outputs probe intensities in IDAT format (encrypted XML files) and uses probe information stored in a platform specific manifest file (.bgx). These files can be processed using the low-level functions `readIDAT` and `readBGX` from the `illuminaio` package (Smith et al, 2013).

The `read.idat` function provides a convenient way to read these files into R and to store them in an `EListRaw`-class object. The function serves a similar purpose to [read.ilmn](#), which reads text

files exported by Illumina's GenomeStudio software, but it reads the IDAT files directly without any need to convert them first to text.

The function reads information on control probes as well for regular probes. Probe types are indicated in the Status column of the genes component of the EListRaw object.

The annotation argument specifies probe annotation columns to be extracted from the manifest file. The manifest typically contains the following columns: Species, Source, Search_Key, Transcript, ILMN_Gene, Source_Reference_ID, RefSeq_ID, Unigene_ID, Entrez_Gene_ID, GI, Accession, Symbol, Protein_Product, Probe_Id, Array_Address_Id, Probe_Type, Probe_Start, Probe_Sequence, Chromosome, Probe_Chr_Orientation, Probe_Coordinates, Cytoband, Definition, Ontology_Component, Ontology_Process, Ontology_Function, Synonyms, Obsolete_Probe_Id. Note that the Probe_Id and Array_Address_Id columns are always read and do not need to be included in the annotation argument.

If more than tolerance probes in the manifest cannot be found in an IDAT file then the function will return an error.

Value

An EListRaw object with the following components:

| | |
|-----------------|--|
| E | numeric matrix of raw intensities. |
| other\$NumBeads | numeric matrix of same dimensions as E giving number of beads used for each intensity value. |
| other\$STDEV | numeric matrix of same dimensions as E giving bead-level standard deviation or standard error for each intensity value. |
| genes | data.frame of probe annotation. This includes the Probe_Id and Array_Address_Id columns extracted from the manifest file, plus a Status column identifying control probes, plus any other columns specified by annotation. |
| targets | data.frame of sample information. This includes the IDAT file names plus other columns if dateinfo=TRUE. |

Author(s)

Matt Ritchie and Gordon Smyth

References

Smith ML, Baggerly KA, Bengtsson H, Ritchie ME, Hansen KD (2013). illuminaio: An open source IDAT parsing tool. *F1000 Research* 2, 264. doi:10.12688/f1000research.2264.v1

See Also

[read.ilmn](#) imports gene expression data as a text file exported from GenomeStudio.

[neqc](#) performs normexp by control background correction, log transformation and quantile between-array normalization for Illumina expression data.

[propexpr](#) estimates the proportion of expressed probes in a microarray.

[detectionPValues](#) computes detection p-values from the negative controls.

Examples

```
## Not run:
idatfiles <- dir(pattern="idat")
bgxfile <- dir(pattern="bgx")
x <- read.idat(idatfiles, bgxfile)
x$other$Detection <- detectionPValues(x)
propexpr(x)
y <- neqc(x)

## End(Not run)
```

read.ilmn

*Read Illumina Expression Data***Description**

Read Illumina summary probe profile files and summary control probe profile files

Usage

```
read.ilmn(files=NULL, ctrlfiles=NULL, path=NULL, ctrlpath=NULL, probeid="Probe",
          annotation=c("TargetID", "SYMBOL"), expr="AVG_Signal",
          other.columns="Detection", sep="\t", quote="", verbose=TRUE, ...)
```

Arguments

| | |
|---------------|---|
| files | character vector giving the names of the summary probe profile files. |
| ctrlfiles | character vector giving the names of the summary control probe profile files. |
| path | character string giving the directory containing the summary probe profile files. Default is the current working directory. |
| ctrlpath | character string giving the directory containing the summary control probe profile files. Default is the same directory as for the probe profile files. |
| probeid | character string giving the name of the probe identifier column. |
| annotation | character vector giving possible column names for probe annotation. |
| expr | character string giving a keyword identifying the expression intensity columns. Any input column with column name containing this key will be read as containing intensity values. |
| other.columns | character vector giving keywords sufficient to identify any extra data columns that should be read in, such as "Detection", "Avg_NBEADS", "BEAD_STDEV" etc. The default of Detection is usually sufficient to identify the columns containing detection p-values. |
| sep | the field separator character. |
| quote | character string of characters to be treated as quote marks. |
| verbose | logical, TRUE to report names of profile files being read. |
| ... | any other parameters are passed on to read.columns . |

Details

Illumina BeadStudio outputs probe intensities (regular probe intensities) and control probe intensities to summary probe profile files (containing regular probes) and summary control probe profile files, respectively. If both `files` and `ctrlfiles` are not `NULL`, this function will combine the data read from the two file types and save them to an `EListRaw-class` object. If one of them is `NULL`, then only the required data are read in.

Probe types are indicated in the `Status` column of `genes`, a component of the returned `EListRaw-class` object. There are totally seven types of control probes including `negative`, `biotin`, `labeling`, `cy3_hyb`, `housekeeping`, `high_stringency_hyb` or `low_stringency_hyb`. Regular probes have the probe type `regular`. The `Status` column will not be created if `ctrlfiles` is `NULL`.

To read in columns other than `probeid`, `annotation` and `expr`, users needs to specify keywords in other `.columns`. One keyword corresponds to one type of columns. Examples of keywords are "Detection", "Avg_NBEADS", "BEAD_STDEV" etc.

Value

An `EListRaw-class` object with the following components:

| | |
|--------------------|--|
| <code>E</code> | numeric matrix of intensities. |
| <code>genes</code> | data.frame of probe annotation. Contains any columns specified by <code>annotation</code> that are found in the input files. |
| <code>other</code> | a list of matrices corresponding to any other <code>.columns</code> found in the input files. |

Author(s)

Wei Shi and Gordon K Smyth

See Also

`read.ilmn.targets` reads in Illumina expression data using the file information extracted from a target data frame which is often created by the `readTargets` function.

`neqc` performs `normexp` by control background correction, log transformation and quantile between-array normalization for Illumina expression data.

`normexp.fit.control` estimates the parameters of the normal+exponential convolution model with the help of negative control probes.

`propexpr` estimates the proportion of expressed probes in a microarray.

Examples

```
## Not run:
x <- read.ilmn(files="sample probe profile.txt",
              ctrlfiles="control probe profile.txt")

## End(Not run)
# See neqc and beadCountWeights for other examples using read.ilmn
```

| | |
|-------------------|---|
| read.ilmn.targets | <i>Read Illumina Data from a Target Dataframe</i> |
|-------------------|---|

Description

Read Illumina data from a target dataframe

Usage

```
read.ilmn.targets(targets, ...)
```

Arguments

| | |
|---------|---|
| targets | data frame including names of profile files. |
| ... | any other parameters are passed on to read.ilmn . |

Details

targets is often created by calling the function [readTargets](#). Rows in targets are arrays and columns contain related array or RNA sample information.

At least one of the two columns called files and/or ctrlfiles should be present in targets, which includes names of summary probe profile files and names of summary control probe profile files respectively. This function calls [read.ilmn](#) to read in the data.

Value

An [EListRaw-class](#) object. See return value of the function [read.ilmn](#) for details.

Author(s)

Wei Shi

See Also

[read.ilmn](#)

read.maimages *Read RGList or EListRaw from Image Analysis Output Files*

Description

Reads an RGList from a set of two-color microarray image analysis output files, or an EListRaw from a set of one-color files.

Usage

```
read.maimages(files=NULL, source="generic", path=NULL, ext=NULL, names=NULL,
              columns=NULL, other.columns=NULL, annotation=NULL, green.only=FALSE,
              wt.fun=NULL, verbose=TRUE, sep="\t", quote=NULL, ...)
read.imagene(files, path=NULL, ext=NULL, names=NULL, columns=NULL, other.columns=NULL,
             wt.fun=NULL, verbose=TRUE, sep="\t", quote="", ...)
```

Arguments

| | |
|---------------|---|
| files | character vector giving the names of the files containing image analysis output or, for Imagene data, a character matrix of names of files. Alternatively, it can be a data.frame containing a column called FileName. If omitted, then all files with extension ext in the specified directory will be read in alphabetical order. |
| source | character string specifying the image analysis program which produced the output files. Choices are "generic", "agilent", "agilent.median", "agilent.mean", "arrayvision", "arrayvision.ARM", "arrayvision.MTM", "bluefuse", "genepix", "genepix.custom", "genepix.median", "imagene", "imagene9", "quantarray", "scanarrayexpress", "smd.old", "smd", "spot" or "spot.close.open". |
| path | character string giving the directory containing the files. The default is the current working directory. |
| ext | character string giving optional extension to be added to each file name |
| names | character vector of unique names to be associated with each array as column name. Can be supplied as files\$Label if files is a data.frame. Defaults to removeExt(files). |
| columns | list, or named character vector. For two color data, this should have fields R, G, Rb and Gb giving the column names to be used for red and green foreground and background or, in the case of Imagene data, a list with fields f and b. For single channel data, the fields are usually E and Eb. This argument is optional if source is specified, otherwise it is required. |
| other.columns | character vector of names of other columns to be read containing spot-specific information |
| annotation | character vector of names of columns containing annotation information about the probes |
| green.only | logical, for use with source, should the green (Cy3) channel only be read, or are both red and green required? |

| | |
|----------------------|---|
| <code>wt.fun</code> | function to calculate spot quality weights |
| <code>verbose</code> | logical, TRUE to report each time a file is read |
| <code>sep</code> | the field separator character |
| <code>quote</code> | character string of characters to be treated as quote marks |
| <code>...</code> | any other arguments are passed to <code>read.table</code> |

Details

These are the main data input functions for the LIMMA package. `read.maimages` reads either single channel or two-color microarray intensity data from text files. `read.imagene` is specifically for two-color ImaGene intensity data created by ImaGene versions 1 through 8, and is called by `read.maimages` to read such data.

`read.maimages` is designed to read data from any microarray platform except for Illumina Bead-Chips, which are read by `read.ilmn`, and Affymetrix GeneChip data, which is best read and pre-processed by specialist packages designed for that platform.

`read.maimages` extracts the foreground and background intensities from a series of files, produced by an image analysis program, and assembles them into the components of one list. The image analysis programs Agilent Feature Extraction, ArrayVision, BlueFuse, GenePix, ImaGene, QuantArray (Version 3 or later), Stanford Microarray Database (SMD) and SPOT are supported explicitly. Almost all these programs write the intensity data for each microarray to one file. The exception is ImaGene, early versions of which wrote the red and green channels of each microarray to different files. Data from some other image analysis programs not mentioned above can be read if the appropriate column names containing the foreground and background intensities are specified using the `columns` argument. (Reading custom columns will work provided the column names are unique and there are no rows in the file after the last line of data. Header lines are ok.)

For Agilent files, two possible foreground estimators are supported: `source="agilent.median"` use median foreground while `source="agilent.mean"` uses mean foreground. Background estimates are always medians. The use of `source="agilent"` defaults to `"agilent.median"`. Note that this behavior is new from 9 March 2012. Previously, in `limma` 3.11.16 or earlier, `"agilent"` had the same meaning as `"agilent.mean"`.

For GenePix files, two possible foreground estimators are supported as well as custom background: `source="genepix.median"` uses the median foreground estimates while `source="genepix.mean"` uses mean foreground estimates. The use of `source="genepix"` defaults to `"genepix.mean"`. Background estimates are always medians unless `source="genepix.custom"` is specified. GenePix 6.0 and later supply some custom background options, notably morphological background. If the GPR files have been written using a custom background, then `source="genepix.custom"` will cause it to be read and used.

For SPOT files, two possible background estimators are supported: `source="spot"` uses background intensities estimated from the morphological opening algorithm. If `source="spot.close.open"` then background intensities are estimated from morphological closing followed by opening.

ArrayVision reports spot intensities in a number of different ways. `read.maimages` caters for ArrayVision's Artifact-removed (ARM) density values using `source="arrayvision.ARM"` or for Median-based Trimmed Mean (MTM) density values with `"arrayvision.MTM"`. ArrayVision users may find it useful to read the top two lines of their data file to check which version of density values they have.

SMD data should consist of raw data files from the database, in tab-delimited text form. There are two possible sets of column names depending on whether the data was entered into the database before or after September 2003. `source="smd.old"` indicates that column headings in use prior to September 2003 should be used.

Intensity data from ImaGene versions 1 to 8 (`source="imagine"`) is different from other image analysis programs in that the read and green channels were written to separate files. `read.maimages` handles the special behaviour of the early ImaGene versions by requiring that the argument `files` should be a matrix with two columns instead of a vector. The first column should contain the names of the files containing green channel (`cy3`) data and the second column should contain names of files containing red channel (`cy5`) data. Alternately, files can be entered as a vector of even length instead of a matrix. In that case, each consecutive pair of file names is assumed to contain the green (`cy3`) and red (`cy5`) intensities respectively from the same array. The function `read.imagine` is called by `read.maimages` when `source="imagine"`, so `read.imagine` does not need to be called directly by users.

ImaGene version~9 (`source="imagine9"`) reverts to the same behavior as the other image analysis programs. For ImaGene~9, `files` is a vector of length equal to the number of microarrays, same as for other image analysis programs.

Spot quality weights may be extracted from the image analysis files using a weight function `wt.fun`. `wt.fun` may be any user-supplied function which accepts a `data.frame` argument and returns a vector of non-negative weights. The columns of the `data.frame` are as in the image analysis output files. There is one restriction, which is that the column names should be referred to in full form in the weight function, i.e., do not rely on name expansion for partial matches when referring to the names of the columns. See [QualityWeights](#) for suggested weight functions.

The argument `other.columns` allows arbitrary columns of the image analysis output files to be preserved in the data object. These become matrices in the component `other` component. For ImaGene data, the other column headings should be prefixed with "R " or "G " as appropriate.

Value

For one-color data, an [EListRaw](#) object. For two-color data, an [RGList](#) object containing the components

| | |
|---------|---|
| R | matrix containing the red channel foreground intensities for each spot for each array. |
| Rb | matrix containing the red channel background intensities for each spot for each array. |
| G | matrix containing the green channel foreground intensities for each spot for each array. |
| Gb | matrix containing the green channel background intensities for each spot for each array. |
| weights | spot quality weights, if <code>wt.fun</code> is given |
| other | list containing matrices corresponding to <code>other.columns</code> if given |
| genes | data frame containing annotation information about the probes, for example gene names and IDs and spatial positions on the array, currently set only if <code>source</code> is "agilent", "genepix" or <code>source="imagine"</code> or if the annotation argument is set |

| | |
|---------|---|
| targets | data frame with column FileName giving the names of the files read. If files was a data.frame on input, then the whole data.frame is stored here on output. |
| source | character string giving the image analysis program name |
| printer | list of class <code>PrintLayout</code> , currently set only if source="image" |

Warnings

All image analysis files being read are assumed to contain data for the same genelist in the same order. No checking is done to confirm that this is true. Probe annotation information is read from the first file only.

Author(s)

Gordon Smyth, with speed improvements suggested by Marcus Davy

References

Ritchie, ME, Phipson, B, Wu, D, Hu, Y, Law, CW, Shi, W, and Smyth, GK (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* 43, e47. <http://nar.oxfordjournals.org/content/43/7/e47>

Web pages for the image analysis software packages mentioned here are listed at <http://www.statsci.org/micrarra/image.html>

See Also

read.maimages uses `read.columns` for efficient reading of text files. As far as possible, it has similar behavior to `read.table` in the base package.

`read.ilmn` reads probe or gene summary profile files from Illumina BeadChips.

An overview of LIMMA functions for reading data is given in [03.ReadingData](#).

Examples

```
# Read all .gpr files from current working directory
# and give weight 0.1 to spots with negative flags

## Not run: files <- dir(pattern="*\\.gpr$")
RG <- read.maimages(files,"genepix",wt.fun=wtflags(0.1))
## End(Not run)

# Read all .spot files from current working director and down-weight
# spots smaller or larger than 150 pixels

## Not run: files <- dir(pattern="*\\.spot$")
RG <- read.maimages(files,"spot",wt.fun=wtarea(150))
## End(Not run)
```

| | |
|---------|------------------------|
| readGAL | <i>Read a GAL file</i> |
|---------|------------------------|

Description

Read a GenePix Array List (GAL) file into a dataframe.

Usage

```
readGAL(galfile=NULL, path=NULL, header=TRUE, sep="\t", quote="", skip=NULL, as.is=TRUE, ...)
```

Arguments

| | |
|---------|---|
| galfile | character string giving the name of the GAL file. If NULL then a file with extension .gal is found in the directory specified by path. |
| path | character string giving the directory containing the files. If NULL then assumed to be the current working directory. |
| header | logical variable, if TRUE then the first line after skip is assumed to contain column headings. If FALSE then a value should be specified for skip. |
| sep | the field separator character |
| quote | the set of quoting characters |
| skip | number of lines of the GAL file to skip before reading data. If NULL then this number is determined by searching the file for column headings. |
| as.is | logical variable, if TRUE then read in character columns as vectors rather than factors. |
| ... | any other arguments are passed to read.table |

Details

A GAL file is a list of genes IDs and associated information produced by an Axon microarray scanner. Apart from header information, the file must contain data columns labeled Block, Column, Row and ID. A Name column is usually included as well. Other columns are optional. See the Axon URL below for a detailed description of the GAL file format.

This function reads in the data columns with a minimum of user information. In most cases the function can be used without specifying any of the arguments.

Value

A data frame with columns

| | |
|--------|--|
| Block | numeric vector containing the print tip indices |
| Column | numeric vector containing the spot columns |
| Row | numeric vector containing the spot rows |
| ID | character vector, for factor if as.is=FALSE, containing gene library identifiers |
| Name | character vector, for factor if as.is=FALSE, containing gene names |

The data frame will be sorted so that Column is the fastest moving index, then Row, then Block.

Author(s)

Gordon Smyth

References

http://www.cryer.co.uk/file-types/a/atf/genepix_file_formats.htm

See Also

read.Galfile in the marray package.

An overview of LIMMA functions for reading data is given in [03.ReadingData](#).

Examples

```
# readGAL()
# will read in the first GAL file (with suffix ".gal")
# found in the current working directory
```

readHeader

Read Header Information from Microarray Raw Data File

Description

Read the header information from a microarray raw data file, as output from an image analysis software program such as GenePix. These functions are used internally by read.maimages and are not usually called directly by users.

Usage

```
readGenericHeader(file, columns, sep="\t")
readGPRHeader(file)
readSMDHeader(file)
```

Arguments

| | |
|---------|---|
| file | character string giving file name. If it does not contain an absolute path, the file name is relative to the current working directory. |
| columns | character vector specifying data column headings expected to be in file |
| sep | the character string separating column names |

Details

Raw data files exported by image analysis programs include a number of header lines which contain information about the scanning process. This function extracts that information and locates the line where the intensity data begins. readGPRHeader is for GenePix output and readSMDHeader is for files from the Stanford Microarray Database (SMD). readGenericHeader finds the line in the file on which the data begins by searching for specified column headings.

Value

A list with components corresponds to lines of header information. A key component is NHeaderRecords which gives the number of lines in the file before the intensity data begins. All other components are character vectors.

Author(s)

Gordon Smyth

References

See http://www.cryer.co.uk/file-types/a/atf/genepix_file_formats.htm for GenePix formats.

See <http://smd.princeton.edu> for the SMD.

See Also

[read.maimages](#)

An overview of LIMMA functions to read data is given in [03.ReadingData](#).

readImaGeneHeader

Read ImaGene Header Information

Description

Read the header information from an ImaGene image analysis output file. This function is used internally by `read.maimages` and is not usually called directly by users.

Usage

```
readImaGeneHeader(file)
```

Arguments

`file` character string giving file name or path

Details

The raw data files exported by the microarray image analysis software ImaGene include a number of header lines which contain information about the printing and scanning processes. This function extracts that information and locates the line where the intensity data begins.

Value

A list containing information read from the header of the ImaGene file. Each Begin-End environment found in the file header will become a recursive list in the output object, with components corresponding to fields in the file. See the ImaGene documentation for further information. The output object will also contain a component NHeaderRecords giving the number of lines in the file before the intensity data begins.

Author(s)

Gordon Smyth

See Also[read.imagene](#)An overview of LIMMA functions to read data is given in [03.ReadingData](#).**Examples**

```
## Not run:
h <- readImaGeneHeader("myImaGenefile.txt")
names(h)
h$headerRecords
h[["Field Dimensions"]]

## End(Not run)
```

`readSpotTypes`*Read Spot Types File*

Description

Read a table giving regular expressions to identify different types of spots in the gene-dataframe.

Usage

```
readSpotTypes(file="SpotTypes.txt", path=NULL, sep="\t", check.names=FALSE, ...)
```

Arguments

| | |
|--------------------------|---|
| <code>file</code> | character string giving the name of the file specifying the spot types. |
| <code>path</code> | character string giving the directory containing the file. Can be omitted if the file is in the current working irectory. |
| <code>sep</code> | the field separator character |
| <code>check.names</code> | logical, if FALSE column names will not be converted to valid variable names, for example spaces in column names will not be left as is |
| <code>...</code> | any other arguments are passed to <code>read.table</code> |

Details

The file is a text file with rows corresponding to types of spots and the following columns: `SpotType` gives the name for the spot type, `ID` is a regular expression matching the ID column, `Name` is a regular expression matching the Name column, and `Color` is the R name for the color to be associated with this type.

Value

A data frame with columns

| | |
|----------|---|
| SpotType | character vector giving names of the spot types |
| ID | character vector giving regular expressions |
| Name | character vector giving regular expressions |
| Color | character vector giving names of colors |

Author(s)

Gordon Smyth following idea of James Wettenhall

See Also

An overview of LIMMA functions for reading data is given in [03.ReadingData](#).

readTargets

Read Targets File

Description

Read targets file for a microarray experiment into a dataframe.

Usage

```
readTargets(file="Targets.txt", path=NULL, sep="\t", row.names=NULL, quote="",...)
```

Arguments

| | |
|-----------|---|
| file | character string giving the name of the targets file. |
| path | character string giving the directory containing the file. Can be omitted if the file is in the current working irectory. |
| sep | field separator character |
| row.names | character string giving the name of a column from which to obtain row names |
| quote | the set of quoting characters |
| ... | other arguments are passed to read.table |

Details

The targets file is a text file containing information about the RNA samples used as targets in the microarray experiment. Rows correspond to arrays and columns to covariates associated with the targets. For a two-color experiment, the targets file will normally include columns labelled Cy3 and Cy5 or similar specifying which RNA samples are hybridized to each channel of each array. Other columns may contain any other covariate information associated with the arrays or targets used in the experiment.

If row.names is non-null and there is a column by that name with unique values, then those values will be used as row names for the dataframe. If row.names is null, then the column Label will be used if such exists or, failing that, the column FileName.

See the Limma User's Guide for examples of this function.

Value

A dataframe. Character columns are not converted into factors.

Author(s)

Gordon Smyth

See Also

An overview of LIMMA functions for reading data is given in [03.ReadingData](#).

| | |
|-------------------|----------------------------|
| removeBatchEffect | <i>Remove Batch Effect</i> |
|-------------------|----------------------------|

Description

Remove batch effects from expression data.

Usage

```
removeBatchEffect(x, batch = NULL, batch2 = NULL, covariates = NULL,  
                 design = matrix(1,ncol(x),1), group = NULL, ...)
```

Arguments

| | |
|------------|---|
| x | numeric matrix, or any data object that can be processed by getEAWP containing log-expression values for a series of samples. Rows correspond to probes and columns to samples. |
| batch | factor or vector indicating batches. |
| batch2 | factor or vector indicating a second series of batches. |
| covariates | matrix or vector of numeric covariates to be adjusted for. |

| | |
|--------|--|
| design | design matrix relating to experimental conditions to be preserved, usually the design matrix with all experimental factors other than the batch effects. Ignored if group is not NULL. |
| group | factor defining the experimental conditions to be preserved. An alternative way to specify the design matrix design. |
| ... | other arguments are passed to <code>lmFit</code> . |

Details

This function is useful for removing unwanted batch effects, associated with hybridization time or other technical variables, ready for plotting or unsupervised analyses such as PCA, MDS or heatmaps. The design matrix or group factor is used to define comparisons between the samples, for example treatment effects, that should not be removed. The function fits a linear model to the data, including both batches and regular treatments, then removes the component due to the batch effects.

In most applications, only the first batch argument will be needed. This case covers the situation where the data has been collected in a series of separate batches.

The `batch2` argument is used when there is a second series of batch effects, independent of the first series. For example, `batch` might correspond to time of data collection while `batch2` might correspond to operator or some other change in operating characteristics. If `batch2` is included, then the effects of `batch` and `batch2` are assumed to be additive.

The `covariates` argument allows correction for one or more continuous numeric effects, similar to the analysis of covariance method in statistics. If `covariates` contains more than one column, then the columns are assumed to have additive effects. Setting `covariates` to be a design matrix constructed from batch effects and technical effects allows very general batch effects to be accounted for.

The data object `x` can be of any class for which `lmFit` works. If `x` contains weights, then these will be used in estimating the batch effects.

Value

A numeric matrix of log-expression values with batch and covariate effects removed.

Note

This function is intended for plotting and data exploration purposes. This function is not intended to be used to prepare data for linear modeling by `lmFit`. For linear modeling, it is better to include the batch factors in the linear model so that `lmFit` can correctly assess the standard errors of the linear model parameters.

Author(s)

Gordon Smyth and Carolyn de Graaf

See Also

[05.Normalization](#)

Examples

```
ngenes <- 10
nsamples <- 8
y <- matrix(rnorm(ngenes*nsamples),ngenes,nsamples)
group <- factor(c("A","A","A","A","B","B","B","B"))
batch <- factor(c(1,1,2,2,1,1,2,2))
colnames(y) <- paste(group,batch,sep=".")
y[,batch==2] <- y[,batch==2] + 5
y[,group=="B"] <- y[,group=="B"] + 1
y.corrected <- removeBatchEffect(y, batch=batch, group=group)
oldpar <- par(mfrow=c(1,2))
plotMDS(y,main="Original")
plotMDS(y.corrected,main="Batch corrected")
par(oldpar)
devAskNewPage(FALSE)
```

`removeExt`*Remove Common Extension from File Names*

Description

Finds and removes any common extension from a vector of file names.

Usage

```
removeExt(x, sep=".")
```

Arguments

| | |
|------------------|---|
| <code>x</code> | character vector |
| <code>sep</code> | character string that separates the body of each character string from the extension. |

Details

This function is used for simplifying file names, or any vector of character strings, when the strings all finish with the same suffix or extension. If the same extension is not shared by every element of `x`, then it is not removed from any element.

Note that `sep` is interpreted as a literal character string: it is not a regular expression.

Value

A character vector of the same length as `x` in which any common extension has been stripped off.

Author(s)

Gordon Smyth

See Also

An overview of LIMMA functions for reading data is given in [03.ReadingData](#).

Examples

```
x <- c("slide1.spot", "slide2.spot", "slide3.spot")
removeExt(x)
```

```
x <- c("Harry - a name from Harry Potter", "Hermione - a name from Harry Potter")
removeExt(x, sep=" - ")
```

residuals.MArrayLM *Extract Residuals from MArrayLM Fit*

Description

This method extracts the residuals from all the probewise linear model fits and returns them in a matrix.

Usage

```
## S3 method for class 'MArrayLM'
residuals(object, y, ...)
```

Arguments

| | |
|--------|---|
| object | a fitted model object inheriting from class MarrayLM. |
| y | a data object containing the response data used to compute the fit. This can be of any class for which <code>.matrix</code> is defined, including <code>MAList</code> , <code>ExpressionSet</code> , <code>marrayNorm</code> etc. |
| ... | other arguments are not used |

Value

Numeric matrix of residuals.

See Also

[residuals.](#)

RGList-class

*Red, Green Intensity List - class***Description**

A list-based S4 class for storing red and green channel foreground and background intensities for a batch of spotted microarrays. RGList objects are normally created by [read.maimages](#).

Slots/List Components

RGList objects can be created by `new("RGList",RG)` where RG is a list. Objects of this class contains no slots (other than `.Data`), but objects should contain the following list components:

- R numeric matrix containing the red (cy5) foreground intensities. Rows correspond to spots and columns to arrays.
- G numeric matrix containing the green (cy3) foreground intensities. Rows correspond to spots and columns to arrays.

Optional components include

- Rb numeric matrix containing the red (cy5) background intensities
- Gb numeric matrix containing the green (cy3) background intensities
- weights numeric matrix of same dimension as R containing relative spot quality weights. Elements should be non-negative.
- other list containing other matrices, all of the same dimensions as R and G.
- genes data.frame containing probe information. Should have one row for each spot. May have any number of columns.
- targets data.frame containing information on the target RNA samples. Rows correspond to arrays. May have any number of columns.
- printer list containing information on the process used to print the spots on the arrays. See [PrintLayout](#).

Valid RGList objects may contain other optional components, but all probe or array information should be contained in the above components.

Methods

This class inherits directly from class `list` so any operation appropriate for lists will work on objects of this class. In addition, RGList objects can be [subsetting](#), [combined](#) and [merged](#). RGList objects will return dimensions and hence functions such as `dim`, `nrow` and `ncol` are defined. RGLists also inherit a `show` method from the virtual class `LargeDataObject`, which means that RGLists will print in a compact way.

RGList objects can be converted to `exprSet2` objects by `as(RG, "exprSet2")`.

Other functions in LIMMA which operate on RGList objects include [normalizeBetweenArrays](#), [normalizeForPrintorder](#), [normalizeWithinArrays](#).

Author(s)

Gordon Smyth

See Also

[02.Classes](#) gives an overview of all the classes defined by this package.
[marrayRaw](#) is the corresponding class in the `marray` package.

roast

*Rotation Gene Set Tests***Description**

Rotation gene set testing for linear models.

Usage

```
## Default S3 method:
roast(y, index = NULL, design = NULL, contrast = ncol(design), geneid = NULL,
      set.statistic = "mean", gene.weights = NULL, var.prior = NULL, df.prior = NULL,
      nrot = 1999, approx.zscore = TRUE, legacy = FALSE, ...)

## Default S3 method:
mroast(y, index = NULL, design = NULL, contrast = ncol(design), geneid = NULL,
       set.statistic = "mean", gene.weights = NULL, var.prior = NULL, df.prior = NULL,
       nrot = 1999, approx.zscore = TRUE, legacy = FALSE, adjust.method = "BH",
       midp = TRUE, sort = "directional", ...)

## Default S3 method:
fry(y, index = NULL, design = NULL, contrast = ncol(design), geneid = NULL,
   gene.weights = NULL, standardize = "posterior.sd", sort = "directional", ...)
```

Arguments

| | |
|----------------------------|--|
| <code>y</code> | numeric matrix giving log-expression or log-ratio values for a series of microarrays, or any object that can be coerced to a matrix including <code>ExpressionSet</code> , <code>MAList</code> , <code>EList</code> or <code>PLMSet</code> objects. Rows correspond to probes and columns to samples. NA or infinite values are not allowed. If either <code>var.prior</code> or <code>df.prior</code> are NULL, then <code>y</code> should contain values for all genes on the arrays. If both prior parameters are given, then only <code>y</code> values for the test set are required. |
| <code>index</code> | index vector specifying which rows (probes) of <code>y</code> are in the test set. Can be a vector of integer indices, or a logical vector of length <code>nrow(y)</code> , or a vector of gene IDs corresponding to entries in <code>geneid</code> . Alternatively it can be a <code>data.frame</code> with the first column containing the index vector and the second column containing directional gene contribution weights. For <code>mroast</code> or <code>fry</code> , <code>index</code> is a list of index vectors or a list of <code>data.frames</code> . |
| <code>design</code> | design matrix |
| <code>contrast</code> | contrast for which the test is required. Can be an integer specifying a column of <code>design</code> , or the name of a column of <code>design</code> , or a numeric contrast vector of length equal to the number of columns of <code>design</code> . |
| <code>geneid</code> | gene identifiers corresponding to the rows of <code>y</code> . Can be either a vector of length <code>nrow(y)</code> or the name of the column of <code>y\$genes</code> containing the gene identifiers. Defaults to <code>rownames(y)</code> . |
| <code>set.statistic</code> | summary set statistic. Possibilities are "mean", "floodmean", "mean50" or "msq". |

| | |
|----------------------------|--|
| <code>gene.weights</code> | numeric vector of directional (positive or negative) contribution weights specifying the size and direction of the contribution of each probe to the gene set statistics. For <code>mroast</code> or <code>fry</code> , this vector must have length equal to <code>nrow(y)</code> . For <code>roast</code> , can be of length <code>nrow(y)</code> or of length equal to the number of genes in the test set. |
| <code>var.prior</code> | prior value for residual variances. If not provided, this is estimated from all the data using <code>squeezeVar</code> . |
| <code>df.prior</code> | prior degrees of freedom for residual variances. If not provided, this is estimated using <code>squeezeVar</code> . |
| <code>nrot</code> | number of rotations used to compute the p-values. Low values like 999 are suitable for testing but higher values such as 9999 or more are recommended for publication purposes. |
| <code>approx.zscore</code> | logical, if TRUE then a fast approximation is used to convert t-statistics into z-scores prior to computing set statistics. If FALSE, z-scores will be exact. |
| <code>legacy</code> | logical. See Note below for usage. |
| <code>adjust.method</code> | method used to adjust the p-values for multiple testing. See p.adjust for possible values. |
| <code>midp</code> | logical, should mid-p-values be used in instead of ordinary p-values when adjusting for multiple testing? |
| <code>sort</code> | character, whether to sort output table by directional p-value ("directional"), non-directional p-value ("mixed"), or not at all ("none"). |
| <code>standardize</code> | how to standardize for unequal probewise variances. Possibilities are "residual.sd", "posterior.sd" or "none". |
| <code>...</code> | any argument that would be suitable for lmFit or eBayes can be included. |

Details

These functions implement rotation gene set tests proposed by Wu et al (2010). They perform *self-contained* gene set tests in the sense defined by Goeman and Buhlmann (2007). For *competitive* gene set tests, see [camera](#). For a gene set enrichment analysis (GSEA) style analysis using a database of gene sets, see [romer](#).

`roast` and `mroast` test whether any of the genes in the set are differentially expressed. They can be used for any microarray experiment that can be represented by a linear model. The design matrix for the experiment is specified as for the `lmFit` function, and the contrast of interest is specified as for the `contrasts.fit` function. This allows users to focus on differential expression for any coefficient or contrast in a linear model. If `contrast` is not specified, then the last coefficient in the linear model will be tested.

The argument `index` is often made using [ids2indices](#) but does not have to be. Each set to be tested is represented by a vector of row numbers or a vector of gene IDs. Gene IDs should correspond to either the rownames of `y` or the entries of `geneid`.

All three functions support directional contribution gene weights, which can be entered either through the `gene.weights` argument or via `index`. Directional gene weights allow each gene to be flagged as to its direction and magnitude of change based on prior experimentation. A typical use is to make the `gene.weights` 1 or -1 depending on whether the gene is up or down-regulated in the pathway under consideration. Probes with directional weights of opposite signs are expected to

have expression changes in opposite directions. Gene with larger gene weights in absolute size will have more weight in the set statistic calculation.

Gene weights can be either genome-wide or set-specific. Genome-wide weights can be entered via the `gene.weights` argument. Set specific weights can be input by including the gene weights as part of the set's entry in `index`. If any of the components of `index` are `data.frames`, then the second column will be assumed to be gene contribution weights for that set. All three functions (`roast`, `mroast` and `fry`) support set-specific gene contribution weights as part of an `index data.frame`.

Set-specific directional gene weights are used to represent *expression signatures* assembled from previous experiments, from gene annotation or from prior hypotheses. In the output from `roast`, `mroast` or `fry`, a significant "Up" p-value means that the differential expression results found in `y` are positively correlated with the expression signature coded by the gene weights. Conversely, a significant "Down" p-value means that the differential expression log-fold-changes are negatively correlated with the expression signature.

Note that the contribution weights set by `gene.weights` are different in nature and purpose to the precision weights set by the `weights` argument of `lmFit`. `gene.weights` control the contribution of each gene to the formation of the gene set statistics and are directional, i.e., can be positive or negative. `weights` indicate the precision of the expression measurements and should be positive. The `weights` are used to construct genewise test statistics whereas `gene.weights` are used to combine the genewise test statistics.

The arguments `df.prior` and `var.prior` have the same meaning as in the output of the `eBayes` function. If these arguments are not supplied, then they are estimated exactly as is done by `eBayes`.

The gene set statistics "mean", "floormean", "mean50" and `msq` are defined by Wu et al (2010). The different gene set statistics have different sensitivities when only some of the genes in a set are differentially expressed. If `set.statistic="mean"` then the set will be statistically significantly only when the majority of the genes are differentially expressed. "floormean" and "mean50" will detect as few as 25% differentially expressed in a set. "msq" is sensitive to even smaller proportions of differentially expressed genes, if the effects are reasonably large. Overall, the "msq" statistic gives the best power for rejecting the null hypothesis of no differentially expressed genes, but the significance can be driven by a small number of genes. In many genomic applications it is appropriate to limit results to gene sets for which most of the genes response in a concordance direction, so the relatively conservative "mean" statistic is the default choice.

The output gives p-values three possible alternative hypotheses, "Up" to test whether the genes in the set tend to be up-regulated, with positive t-statistics, "Down" to test whether the genes in the set tend to be down-regulated, with negative t-statistics, and "Mixed" to test whether the genes in the set tend to be differentially expressed, without regard for direction.

`roast` estimates p-values by simulation, specifically by random rotations of the orthogonalized residuals (Langsrud, 2005), so p-values will vary slightly from run to run. The p-value is computed as $(b+1)/(nrot+1)$ where `b` is the number of rotations giving a more extreme statistic than that observed (Phipson and Smyth, 2010). This means that the smallest possible mixed or two-sided p-values are $1/(nrot+1)$. The function uses a symmetry argument to double the effective number of rotations for the one-sided tests, so the smallest possible "Up" or "Down" p-value is $1/(2*nrot+1)$.

The number of rotations `nrot` can (and should) be increased to get more precise p-values from `roast` or `mroast`. The default `nrot` is set fairly low to facilitate quick testing and experimentation but the smallest possible two-sided p-value is $1/(nrot+1)$. To get definitive p-values for publication, at least `nrot=9999` or higher is recommended.

mroast does roast tests for multiple sets, including adjustment for multiple testing. By default, mroast reports ordinary p-values but uses mid-p-values (Routledge, 1994) at the multiple testing stage. Mid-p-values are probably a good choice when using false discovery rates (`adjust.method="BH"`) but not when controlling the family-wise type I error rate (`adjust.method="holm"`).

To improve the performance of the gene set statistics, roast and mroast transform the genewise moderated t-statistics to normality using `zscoreT`. By default, an approximate closed-form transformation is used (`approx.zscore=TRUE`), which is very much faster than the exact transformation and performs just as well. In Bioconductor 2.10, the transformation used has been changed from Hill's (1970) approximation to Bailey's (1980) formula because the latter is faster and gives more even accuracy; see `zscoreT` for more details.

fry is a fast alternative designed to approximate what mroast with `set.stat="mean"` would give for a very large (infinite) number of rotations. In the special case that `df.prior` is very large and `set.statistic="mean"`, fry gives the same directional p-values that mroast would give if an infinite number of rotations could be performed. In other circumstances, when genes have different variances, fry uses a standardization strategy to approximate the mroast results. Using fry is recommended when performing tests for a large number of sets because it is fast and because it returns higher resolution p-values that are not limited by the number of rotations performed. Note, the close approximation of fry to mroast is only for the directional p-values. The fry mixed p-values are computed by a different method and will not necessarily be very close to those from mroast.

Value

roast produces an object of class "Roast". This consists of a list with the following components:

| | |
|------------------------|--|
| <code>p.value</code> | data.frame with columns <code>Active.Prop</code> and <code>P.Value</code> , giving the proportion of genes in the set contributing materially to significance and estimated p-values, respectively. Rows correspond to the alternative hypotheses <code>Down</code> , <code>Up</code> , <code>UpOrDown</code> (two-sided) and <code>Mixed</code> . |
| <code>var.prior</code> | prior value for residual variances. |
| <code>df.prior</code> | prior degrees of freedom for residual variances. |

mroast produces a data.frame with a row for each set and the following columns:

| | |
|---------------------------|---|
| <code>NGenes</code> | number of genes in set |
| <code>PropDown</code> | proportion of genes in set with $z < -\sqrt{2}$ |
| <code>PropUp</code> | proportion of genes in set with $z > \sqrt{2}$ |
| <code>Direction</code> | direction of change, "Up" or "Down" |
| <code>PValue</code> | two-sided directional p-value |
| <code>FDR</code> | two-sided directional false discovery rate |
| <code>PValue.Mixed</code> | non-directional p-value |
| <code>FDR.Mixed</code> | non-directional false discovery rate |

fry produces the same output format as mroast but without the columns `PropDown` and `PropUp`.

Note

For Bioconductor 3.10, `roast` and `mroast` have been revised to use much less memory by conducting the rotations in chunks and to be about twice as fast by updating the normalizing transformation used when `approx.zscore=TRUE`. For a limited time, users wishing to reproduce Bioconductor 3.9 results exactly can set `legacy=TRUE` to turn these revisions off.

`approx.score=TRUE` become the default in Bioconductor 3.0 (October 2014).

The default set statistic was changed from "msq" to "mean" in Bioconductor 2.7 (October 2010).

Author(s)

Gordon Smyth and Di Wu

References

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Langsrud O (2005). Rotation tests. *Statistics and Computing* 15, 53-60.

Phipson B, Smyth GK (2010). Permutation P-values should never be zero: calculating exact P-values when permutations are randomly drawn. *Statistical Applications in Genetics and Molecular Biology*, Volume 9, Issue 1, Article 39. doi:10.2202/15446115.1585. See also the Preprint Version <https://gksmyth.github.io/pubs/PermPValuesPreprint.pdf> with corrections.

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Wu D, Lim E, Vaillant F, Asselin-Labat M-L, Visvader JE, Smyth GK (2010). ROAST: rotation gene set tests for complex microarray experiments. *Bioinformatics* 26, 2176-2182. doi:10.1093/bioinformatics/btq401

See Also

See [10.GeneSetTests](#) for a description of other functions used for gene set testing.

Examples

```
y <- matrix(rnorm(100*4,sd=0.3),100,4)
design <- cbind(Intercept=1,Group=c(0,0,1,1))

# First set of 5 genes are all up-regulated
index1 <- 1:5
y[index1,3:4] <- y[index1,3:4]+3
roast(y,index1,design,contrast=2)

# Second set of 5 genes contains none that are DE
index2 <- 6:10
mroast(y,list(set1=index1,set2=index2),design,contrast=2)
fry(y,list(set1=index1,set2=index2),design,contrast=2)

# Third set of 6 genes contains three down-regulated genes and three up-regulated genes
index3 <- 11:16
```

```

y[index3[1:3],3:4] <- y[index3[1:3],3:4]-3
y[index3[4:6],3:4] <- y[index3[4:6],3:4]+3

# Without gene weights
# Mixed p-value is significant for set3 but not the directional p-values
mroast(y,list(set1=index1,set2=index2,set3=index3),design,contrast=2)
fry(y,list(set1=index1,set2=index2,set3=index3),design,contrast=2)

# With gene weights
# Set3 is significantly up (i.e., positively correlated with the weights)
index3 <- data.frame(Gene=11:16,Weight=c(-1,-1,-1,1,1,1))
mroast(y,list(set1=index1,set2=index2,set3=index3),design,contrast=2)
fry(y,list(set1=index1,set2=index2,set3=index3),design,contrast=2)

```

romer

*Rotation Gene Set Enrichment Analysis***Description**

Gene set enrichment analysis for linear models using rotation tests (ROtation testing using MEan Ranks).

Usage

```

## Default S3 method:
romer(y, index, design = NULL, contrast = ncol(design),
      array.weights = NULL, block = NULL, correlation,
      set.statistic = "mean", nrot = 9999, shrink.resid = TRUE, ...)

```

Arguments

| | |
|----------------------------|---|
| <code>y</code> | numeric matrix giving log-expression values. |
| <code>index</code> | list of indices specifying the rows of <code>y</code> in the gene sets. The list can be made using ids2indices . |
| <code>design</code> | design matrix. |
| <code>contrast</code> | contrast for which the test is required. Can be an integer specifying a column of design, or else a contrast vector of length equal to the number of columns of design. |
| <code>array.weights</code> | optional numeric vector of array weights. |
| <code>block</code> | optional vector of blocks. |
| <code>correlation</code> | correlation between blocks. |
| <code>set.statistic</code> | statistic used to summarize the gene ranks for each set. Possible values are "mean", "floormean" or "mean50". |
| <code>nrot</code> | number of rotations used to estimate the p-values. |
| <code>shrink.resid</code> | logical, should the residuals be shrunk to remove systematic effects before rotation. |
| <code>...</code> | other arguments not currently used. |

Details

This function implements the ROMER procedure described by Majewski et al (2010) and Ritchie et al (2015). `romer` tests a hypothesis similar to that of Gene Set Enrichment Analysis (GSEA) (Subramanian et al, 2005) but is designed for use with linear models. Like GSEA, it is designed for use with a database of gene sets. Like GSEA, it is a competitive test in that the different gene sets are pitted against one another. Instead of permutation, it uses rotation, a parametric resampling method suitable for linear models (Langsrud, 2005; Wu et al, 2010). `romer` can be used with any linear model with some level of replication.

In the output, p-values are given for each set for three possible alternative hypotheses. The alternative "up" means the genes in the set tend to be up-regulated, with positive t-statistics. The alternative "down" means the genes in the set tend to be down-regulated, with negative t-statistics. The alternative "mixed" test whether the genes in the set tend to be differentially expressed, without regard for direction. In this case, the test will be significant if the set contains mostly large test statistics, even if some are positive and some are negative. The first two alternatives are appropriate if you have a prior expectation that all the genes in the set will react in the same direction. The "mixed" alternative is appropriate if you know only that the genes are involved in the relevant pathways, without knowing the direction of effect for each gene.

Note that `romer` estimates p-values by simulation, specifically by random rotations of the orthogonalized residuals (called effects in R). This means that the p-values will vary slightly from run to run. To get more precise p-values, increase the number of rotations `nrot`. By default, the orthogonalized residual corresponding to the contrast being tested is shrunk have the same expected squared size as a null residual.

The argument `set.statistic` controls the way that t-statistics are summarized to form a summary test statistic for each set. In all cases, genes are ranked by moderated t-statistic. If `set.statistic="mean"`, the mean-rank of the genes in each set is the summary statistic. If `set.statistic="floormean"` then negative t-statistics are put to zero before ranking for the up test, and vice versa for the down test. This improves the power for detecting genes with a subset of responding genes. If `set.statistic="mean50"`, the mean of the top 50% ranks in each set is the summary statistic. This statistic performs well in practice but is slightly slower to compute. See Wu et al (2010) for discussion of these set statistics.

Value

Numeric matrix giving p-values and the number of matched genes in each gene set. Rows correspond to gene sets. There are four columns giving the number of genes in the set and p-values for the alternative hypotheses mixed, up or down.

Author(s)

Yifang Hu and Gordon Smyth

References

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Ritchie, ME, Phipson, B, Wu, D, Hu, Y, Law, CW, Shi, W, and Smyth, GK (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* 43, e47. doi:10.1093/nar/gkv007

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Wu, D, Lim, E, Francois Vaillant, F, Asselin-Labat, M-L, Visvader, JE, and Smyth, GK (2010). ROAST: rotation gene set tests for complex microarray experiments. *Bioinformatics* 26, 2176-2182. doi:10.1093/bioinformatics/btq401

See Also

[topRomer](#), [ids2indices](#), [roast](#), [camera](#), [wilcoxGST](#)

There is a topic page on [10.GeneSetTests](#).

Examples

```
y <- matrix(rnorm(100*4),100,4)
design <- cbind(Intercept=1,Group=c(0,0,1,1))
index <- 1:5
y[index,3:4] <- y[index,3:4]+3

index1 <- 1:5
index2 <- 6:10
r <- romer(y=y,index=list(set1=index1,set2=index2),design=design,contrast=2,nrot=99)
r
topRomer(r,alt="up")
topRomer(r,alt="down")
```

sampleInfoFromGEO

Read Sample Characteristics From GEO Series Matrix File

Description

Read sample annotation from a GEO Series Matrix File into data.frames.

Usage

```
sampleInfoFromGEO(file, remove.constant.columns = TRUE)
```

Arguments

`file` file name or path of GEO series matrix file.
`remove.constant.columns` logical, if TRUE then columns that have the same entry for every sample are removed.

Details

This function parses a GEO series matrix file. Sample characteristics associated with expression channels 1 and 2 are separated into separate character matrices. The function particularly allows for the fact that not every sample characteristic will have an entry for every sample.

Value

A list with three components:

SampleInfo character matrix of sample annotation.

CharacteristicsCh1

 character matrix of sample characteristics associated with expression channel 1.

CharacteristicsCh2

 character matrix of sample characteristics associated with expression channel 2.

Each sample corresponds to one row.

Author(s)

Gordon Smyth

See Also

An overview of LIMMA functions for reading data is given in [03.ReadingData](#).

Examples

```
# This example downloads a series matrix file of about 33MB
## Not run:
url <- "https://ftp.ncbi.nlm.nih.gov/geo/series/GSE40nnn/GSE40115/matrix/GSE40115-GPL15931_series_matrix.txt.gz"
download.file(url, "GSE40115.txt.gz")
a <- sampleInfoFromGEO("GSE40115.txt.gz")
colnames(a$SampleInfo)
colnames(a$CharacteristicsCh1)
colnames(a$CharacteristicsCh2)

## End(Not run)
```

selectModel

Select Appropriate Linear Model

Description

Select the best fitting linear model for each gene by minimizing an information criterion.

Usage

```
selectModel(y, designlist, criterion="aic", df.prior=0, s2.prior=NULL, s2.true=NULL, ...)
```

Arguments

| | |
|------------|---|
| y | a matrix-like data object, containing log-ratios or log-values of expression for a series of microarrays. Any object class which can be coerced to matrix is acceptable including numeric, matrix, MAList, marrayNorm, ExpressionSet or PLMset. |
| designlist | list of design matrices |
| criterion | information criterion to be used for model selection, "aic", "bic" or "mallowscp". |
| df.prior | prior degrees of freedom for residual variances. See squeezeVar |
| s2.prior | prior value for residual variances, to be used if df.prior>0. |
| s2.true | numeric vector of true variances, to be used if criterion="mallowscp". |
| ... | other optional arguments to be passed to lmFit |

Details

This function chooses, for each probe, the best fitting model out of a set of alternative models represented by a list of design matrices. Selection is by Akaike's Information Criterion (AIC), Bayesian Information Criterion (BIC) or by Mallows's Cp.

The criteria have been generalized slightly to accommodate an information prior on the variances represented by s2.prior and df.prior or by s2.post. Suitable values for these parameters can be estimated using [squeezeVar](#).

Value

List with components

| | |
|------|--|
| IC | matrix of information criterion scores, rows for probes and columns for models |
| pref | factor indicating the model with best (lowest) information criterion score |

Author(s)

Alicia Oshlack and Gordon Smyth

See Also

An overview of linear model functions in limma is given by [06.LinearModels](#).

Examples

```
nprobes <- 100
narrays <- 5
y <- matrix(rnorm(nprobes*narrays), nprobes, narrays)
A <- c(0,0,1,1,1)
B <- c(0,1,0,1,1)
designlist <- list(
  None=cbind(Int=c(1,1,1,1,1)),
  A=cbind(Int=1,A=A),
  B=cbind(Int=1,B=B),
  Both=cbind(Int=1,AB=A*B),
```

```

Add=cbind(Int=1,A=A,B=B),
Full=cbind(Int=1,A=A,B=B,AB=A*B)
)
out <- selectModel(y,designlist)
table(out$pref)

```

squeezeVar

*Squeeze Sample Variances***Description**

Squeeze a set of sample variances together by computing empirical Bayes posterior means.

Usage

```

squeezeVar(var, df, covariate = NULL, robust = FALSE, winsor.tail.p = c(0.05,0.1),
           legacy = NULL)

```

Arguments

| | |
|---------------|---|
| var | numeric vector of independent sample variances. |
| df | numeric vector of degrees of freedom for the sample variances. Can be a unit vector or of same length as var. |
| covariate | numeric covariate of same length as var for estimating a trended prior variance. If NULL, then the prior variance var.prior is constant. |
| robust | logical, should the estimation of df.prior and var.prior be robustified against outlier sample variances? |
| winsor.tail.p | numeric vector of length 1 or 2, giving left and right tail proportions of x to Winsorize when robust=TRUE. |
| legacy | logical. If FALSE then the new function fitFDistUnequalDF1 will be called internally, otherwise the legacy functions fitFDist or fitFDistRobustly will be used. If NULL, then fitFDistUnequalDF1 will be used whenever the degrees of freedom df are not all equal. |

Details

This function implements empirical Bayes algorithms proposed by Smyth (2004) and Phipson et al (2016).

A conjugate Bayesian hierarchical model is assumed for a set of sample variances. The hyperparameters are estimated by fitting a scaled F-distribution to the sample variances. The function returns the posterior variances and the estimated hyperparameters.

Specifically, the sample variances var are assumed to follow scaled chi-squared distributions, conditional on the true variances, and an scaled inverse chi-squared prior is assumed for the true variances. The scale and degrees of freedom of this prior distribution are estimated from the values of var.

The effect of this function is to squeeze the variances towards a common value, or to a global trend if a covariate is provided. The squeezed variances have a smaller expected mean square error to the true variances than do the sample variances themselves.

The amount of squeezing is controlled by the `prior.df`. Both the global trend and the prior df are estimated internally but fitting an F-distribution to the sample variances, using either `fitFDist()` or `fitFDistRobustly()` or `fitFDistUnequalDF1()`.

If `covariate` is non-null, then the scale parameter of the prior distribution is assumed to depend on the covariate. If the covariate is average log-expression, then the effect is an intensity-dependent trend similar to that in Sartor et al (2006).

`robust=TRUE` implements the robust empirical Bayes procedure of Phipson et al (2016), which allows some of the `var` values to be outliers.

The `legacy` argument was added in `limma` version 3.61.8 (August 2024). If `legacy=FALSE`, then the new function `fitFDistUnequalDF1()` provides improved estimation of the global trend and prior df hyperparameters, especially when the df values are unequal. `legacy=TRUE` provides legacy behavior for backward compatibility.

Value

A list with components

| | |
|------------------------|---|
| <code>var.post</code> | numeric vector of posterior variances. Of same length as <code>var</code> . |
| <code>var.prior</code> | location or scale of prior distribution. A vector of same length as <code>var</code> if <code>covariate</code> is non-NULL, otherwise a single value. |
| <code>df.prior</code> | degrees of freedom of prior distribution. A vector of same length as <code>var</code> if <code>robust=TRUE</code> , otherwise a single value. |

Note

This function is called by `eBayes`, but beware a possible confusion with the output from that function. The values `var.prior` and `var.post` output by `squeezeVar` correspond to the quantities `s2.prior` and `s2.post` output by `eBayes`, whereas `var.prior` output by `eBayes` relates to a different parameter.

Author(s)

Gordon Smyth

References

- Phipson B, Lee S, Majewski IJ, Alexander WS, and Smyth GK (2016). Robust hyperparameter estimation protects against hypervariable genes and improves power to detect differential expression. *Annals of Applied Statistics* 10, 946-963. doi:10.1214/16AOAS920
- Sartor MA, Tomlinson CR, Wesselkamper SC, Sivaganesan S, Leikauf GD, Medvedovic M (2006). Intensity-based hierarchical Bayes method improves testing for differentially expressed genes in microarray experiments. *BMC bioinformatics* 7, 538.
- Smyth GK (2004). Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Statistical Applications in Genetics and Molecular Biology* Volume 3,

Issue 1, Article 3. doi:10.2202/15446115.1027. See also the Preprint Version <https://gksmyth.github.io/pubs/ebayes.pdf> incorporating corrections to 30 June 2009.

See Also

This function is called by [eBayes](#).

This function calls [fitFDist](#), [fitFDistRobustly](#) or [fitFDistUnequalDF1](#).

An overview of linear model functions in limma is given by [06.LinearModels](#).

Examples

```
s2 <- rchisq(20,df=5)/5
squeezeVar(s2, df=5)
```

strsplit2

Split Composite Names

Description

Split a vector of composite names into a matrix of simple names.

Usage

```
strsplit2(x, split, ...)
```

Arguments

| | |
|-------|--|
| x | character vector |
| split | character to split each element of vector on, see strsplit |
| ... | other arguments are passed to strsplit |

Details

This function is the same as [strsplit](#) except that the output value is a matrix instead of a list. The first column of the matrix contains the first component from each element of *x*, the second column contains the second components etc. The number of columns is equal to the maximum number of components for any element of *x*.

The motivation for this function in the limma package is handle input columns which are composites of two or more annotation fields.

Value

A list containing components

| | |
|------------|---|
| Name | character vector of the same length as <i>x</i> contain first splits of each element |
| Annotation | character vector of the same length as <i>x</i> contain second splits of each element |

Author(s)

Gordon Smyth

See Also[strsplit](#).An overview of LIMMA functions for reading data is given in [03.ReadingData](#).**Examples**

```
x <- c("AA196000;actinin, alpha 3",
      "AA464163;acyl-Coenzyme A dehydrogenase, very long chain",
      "3E7;W15277;No Annotation")
strsplit2(x,split=";")
```

| | |
|------------|--|
| subsetting | <i>Subset RGList, MAList, EListRaw, EList, MArrayLM or TestResults Objects</i> |
|------------|--|

Description

Return an RGList, MAList, EListRaw, EList, MArrayLM or TestResults object with only selected rows and columns of the original object.

Usage

```
## S3 method for class 'EList'
object[i, j, ...]
subsetListOfArrays(object, i, j, IJ, IX, I, JX)
```

Arguments

| | |
|--------|---|
| object | object of class RGList, MAList, EListRaw, EList, MArrayLM or TestResults. |
| i, j | elements to extract. i subsets the probes or spots while j subsets the arrays. |
| IJ | character vector giving names of components that should be subsetted by i and j. |
| IX | character vector giving names of 2-dimensional components that should be subsetted by i only. |
| I | character vector giving names of vector components that should be subsetted by i. |
| JX | character vector giving names of 2-dimensional components whose row dimension corresponds to j. |
| ... | other arguments are not currently used. |

Details

All these objects can be subsetted as if they were matrices. i, j may take any values acceptable for the matrix components of `object`. Either or both can be missing. See the [Extract](#) help entry for more details on subsetting matrices.

`object[]` will return the whole object unchanged. A single index `object[i]` will be taken to subset rows, so `object[i]` and `object[i,]` are equivalent.

`subsetListOfArrays` is used internally as a utility function by the subsetting operations. It is not intended to be called directly by users. Values must be supplied for all arguments other than i and j .

Value

An object the same as `object` but containing data from the specified subset of rows and columns only.

Note the output object is of the same class as `object` will have two dimensions attached even if i or j select a single row or column, i.e., subsetting for these objects does not drop dimensions. Subsetting is exactly analogous to subsetting of matrices in R with `drop=FALSE`.

Author(s)

Gordon Smyth

See Also

[Extract](#) in the base package.

[02.Classes](#) for a summary of the different data classes.

Examples

```
M <- A <- matrix(11:14,4,2)
rownames(M) <- rownames(A) <- c("a","b","c","d")
colnames(M) <- colnames(A) <- c("A","B")
MA <- new("MAList",list(M=M,A=A))
MA[1:2,]
MA[c("a","b"),]
MA[1:2,2]
MA[,2]
```

Description

Briefly summarize microarray data objects.

Usage

```
## S3 method for class 'RGList'
summary(object, ...)
```

Arguments

object an object of class RGList, MAList, EListRaw, EList or MArrayLM
 ... other arguments are not used

Details

The data objects are summarized as if they were lists, i.e., brief information about the length and type of the components is given.

Value

A table.

Author(s)

Gordon Smyth

See Also

[summary](#) in the base package.

[02.Classes](#) gives an overview of data classes used in LIMMA.

| | |
|------------|--|
| targetsA2C | <i>Convert Two-Color Targets Dataframe from One-Row-Per-Array to One-Row-Per-Channel</i> |
|------------|--|

Description

Convert a two-color targets dataframe with one row per array to one with one row per channel.

Usage

```
targetsA2C(targets, channel.codes = c(1,2), channel.columns = list(Target=c("Cy3","Cy5")),
            grep = FALSE)
```

Arguments

| | |
|-----------------|---|
| targets | data.frame with one row per array giving information about target samples associated covariates. |
| channel.codes | numeric or character vector of length 2 giving codes for the channels |
| channel.columns | named list of character vectors of length 2. Each entry gives a pair of names of columns in targets which contain channel-specific information. This pair of columns should be assembled into one column in the output. |
| grep | logical, if TRUE then the channel column names are found by greping, i.e., the actual column names need only contain the names given by channel.columns as substrings |

Details

The targets dataframe holds information about the RNA samples used as targets in the microarray experiment. It is often read from a file using `readTargets`. This function is used to convert the dataframe from an array-orientated format with one row for each array and two columns for the two channels into a channel-orientated format with one row for each individual channel observations. In statistical terms, the first format treats the arrays as cases and treats the channels as repeated measurements. The second format treats the individual channel observations as cases. The second format may be more appropriate if the data is to be analyzed in terms of individual log-intensities.

Value

data.frame with twice as many rows as targets. Any pair of columns named by channel.columns will now be one column.

Author(s)

Gordon Smyth

References

Smyth, GK, and Altman, NS (2013). Separate-channel analysis of two-channel microarrays: recovering inter-spot information. *BMC Bioinformatics* 14, 165. doi:10.1186/1471210514165

See Also

targetsA2C is used by the coerce method from RGList to ExpressionSet in the convert package. An overview of methods for single channel analysis in limma is given by [07.SingleChannel](#).

Examples

```
targets <- data.frame(FileName=c("file1.gpr", "file2.gpr"), Cy3=c("WT", "KO"), Cy5=c("KO", "WT"))
targetsA2C(targets)
```

| | |
|-------------------|---------------------------------------|
| TestResults-class | <i>Matrix of Test Results - class</i> |
|-------------------|---------------------------------------|

Description

A matrix-based class for storing the results of simultaneous tests. TestResults objects are usually created by [decideTests](#).

Usage

```
## S3 method for class 'TestResults'
summary(object, ...)
## S3 method for class 'TestResults'
labels(object, ...)
## S3 method for class 'TestResults'
levels(x)
```

Arguments

| | |
|-----------|------------------------------|
| object, x | object of class TestResults |
| ... | other arguments are not used |

Slots/List Components

A TestResults object is essentially a numeric matrix with elements equal to 0, 1 or -1. Zero represents acceptance of the null hypothesis, 1 indicates rejection in favor of the right tail alternative and -1 indicates rejection in favor of the left tail alternative.

TestResults objects can be created by `new("TestResults", results)` where `results` is a matrix. Objects of this class contain no slots (other than `.Data`), although the attributes `dim` and `dimnames` may be treated as slots.

Methods

This class inherits directly from class `matrix` so any operation appropriate for matrices will work on objects of this class. [show](#) and [summary](#) methods are also implemented.

Functions in LIMMA which operate on TestResults objects include [heatDiagram](#), [vennCounts](#), [vennDiagram](#), [write.fit](#).

Author(s)

Gordon Smyth

See Also

[02.Classes](#) gives an overview of all the classes defined by this package. [08.Tests](#) gives an overview of multiple testing.

Examples

```
## Not run:
# Assume a data object y and a design matrix
fit <- lmFit(y, design)
fit <- eBayes(fit)
results <- decideTests(fit)
summary(results)

## End(Not run)
```

tmixture

Estimate Scale Factor in Mixture of t-Distributions

Description

These functions estimate the unscaled standard deviation of the true (unobserved) log fold changes for differentially expressed genes. They are used internally by the eBayes function and are not intended to be called directly by users.

Usage

```
tmixture.vector(tstat, stdev.unscaled, df, proportion, v0.lim = NULL)
tmixture.matrix(tstat, stdev.unscaled, df, proportion, v0.lim = NULL)
```

Arguments

| | |
|----------------|---|
| tstat | numeric vector or matrix of t-statistics. <code>tmixture.vector</code> assumes a vector while <code>tmixture.matrix</code> assumes a matrix. |
| stdev.unscaled | numeric vector or matrix, conformal with <code>tstat</code> , containing the unscaled standard deviations of the coefficients used to compute the t-statistics. |
| df | numeric vector giving the degrees of freedom associated with <code>tstat</code> . |
| proportion | assumed proportion of genes that are differentially expressed. |
| v0.lim | numeric vector of length 2 giving the lower and upper limits for the estimated unscaled standard deviations. |

Details

The values in each column of `tstat` are assumed to follow a mixture of an ordinary t-distribution, with mixing proportion $1 - \text{proportion}$, and $(\sqrt{v_0} + \sqrt{v_1}) / \sqrt{v_1}$ times a t-distribution, with mixing proportion `proportion`. Here v_1 is `stdev.unscaled^2` and v_0 is the value to be estimated.

Value

Numeric vector, of length equal to the number of columns of `tstat`, containing estimated v_0 values.

Author(s)

Gordon Smyth

See Also[eBayes](#)

topGO

*Table of Top GO Terms or Top KEGG Pathways***Description**

Extract top GO terms from goana output or top KEGG pathways from kegg output.

Usage

```
topGO(results, ontology = c("BP", "CC", "MF"), sort = NULL, number = 20L,
       truncate.term = NULL, p.value = 1)
topKEGG(results, sort = NULL, number = 20L, truncate.path = NULL, p.value = 1)
```

Arguments

| | |
|---------------|---|
| results | data frame produced by goana or kegga . |
| ontology | character vector of ontologies to be included in output. Elements should be one or more of "BP", "CC" or "MF". |
| sort | character vector of names of gene lists for which results are required. Should be one or more of the column names of results. Defaults to all gene lists. |
| number | maximum number of top GO terms or top KEGG pathways to list. For all terms or all pathways, set number=Inf. |
| truncate.term | truncate the name of the GO term at this number of characters. |
| truncate.path | truncate the name of the KEGG pathway at this number of characters. |
| p.value | p.value cutoff. Only GO terms or pathways with lower p-values are included in the output. |

Details

topGO organizes the output from [goana](#) into top-tables of the most significant GO terms. topKEGG similarly extracts the most significant KEGG pathways from [kegga](#) output. In either case, rows are sorted by the minimum p-value of any of the result columns specified by sort.

Value

Same as results but with rows subsetted by Ontology and sorted by p-value.

Author(s)

Gordon Smyth and Yifang Hu

See Also

[goana](#), [kegga](#)

See [10.GeneSetTests](#) for a description of other functions used for gene set testing.

Examples

```
# See goana examples
```

topRomer

Top Gene Set Testing Results from Romer

Description

Extract a matrix of the top gene set testing results from the [romer](#) output.

Usage

```
topRomer(x, n=10, alternative="up")
```

Arguments

| | |
|-------------|---|
| x | matrix which is the output from romer . |
| n | number of top gene set testing results to be extracted. |
| alternative | character which can be one of the three possible alternative p values: "up", "down" or "mixed". |

Details

This function takes the results from [romer](#) and returns a number of top gene set testing results that are sorted by the p values.

Value

matrix, which is sorted by the "up", "down" or "mixed" p values, with the rows corresponding to estimated p-values for the top number of gene sets and the columns corresponding to the number of genes for each gene set and the alternative hypotheses mixed, up, down.

Author(s)

Gordon Smyth and Yifang Hu

See Also[romer](#)There is a topic page on [10.GeneSetTests](#).**Examples**

See romer for examples

| | |
|-----------|---|
| topSplice | <i>Top table of differentially spliced genes or exons</i> |
|-----------|---|

Description

Top table ranking the most differentially spliced genes or exons.

Usage

```
topSplice(fit, coef = ncol(fit), test = "F", number = 10, FDR=1, sort.by = "p")
```

Arguments

| | |
|---------|--|
| fit | MArrayLM fit object produced by diffSplice. |
| coef | the coefficient (column) of fit for which differentially splicing is assessed. |
| test | character string specifying which statistical test to apply. Possible values are "simes", "F" or "t". "F" gives F-tests for each gene. "t" gives t-tests for each exon. "simes" gives genewise p-values derived from the t-tests after Simes adjustment for each gene. |
| number | integer, maximum number of rows to output. |
| FDR | numeric, only show exons or genes with false discovery rate less than this cutoff. |
| sort.by | character string specifying which column to sort results by. Possible values for "p", "logFC", "NExons" or "none". "logFC" is only available if test="t" and "NExons" is only available if test="simes" or test="F". |

Details

Ranks genes or exons by evidence of differential exon-usage arising from differential splicing. Choosing test="F" or test="simes" gives gene-level tests, where the null hypothesis is that all exons are used equally. Choosing test="t" gives exon-level tests of usage for each exon vs all other exons for the same gene.

The F-statistic option chosen by test="F" conducts an ANOVA-like F-tests of differential usage for each gene. Simes method chosen by test="simes" processes the exon-level p-values for each gene to give an overall call of differential splicing for that gene. The F-tests are likely to be powerful for genes in which several exons are differentially splices. The Simes p-values is likely to be more powerful when only a minority of the exons for a gene are differentially spliced.

The F-test option gives the best error rate control. Simes method and the exon-level t-tests are both slightly liberal in terms of error rate control.

Value

A data.frame with any annotation columns found in `fit` plus the following columns

| | |
|---------|---|
| logFC | log2-fold change of exon vs other exons for the same gene (if level="exon") |
| t | moderated t-statistic (if level="exon") |
| F | moderated F-statistic (if level="gene") |
| P.Value | p-value |
| FDR | false discovery rate |

Author(s)

Gordon Smyth

See Also

[diffSplice](#), [plotSplice](#)

A summary of functions available in LIMMA for RNA-seq analysis is given in [11.RNAseq](#).

Examples

```
# See diffSplice
```

topTable

Table of Top Genes from Linear Model Fit

Description

Extract a table of the top-ranked genes from a linear model fit.

Usage

```
topTable(fit, coef = NULL, number = 10, genelist = fit$genes,
         adjust.method = "BH", sort.by = "B", resort.by = NULL,
         p.value = 1, fc = NULL, lfc = NULL, confint = FALSE)
topTableF(fit, number = 10, genelist = fit$genes,
          adjust.method="BH", sort.by="F",
          p.value = 1, fc = NULL, lfc = NULL)
topTreat(fit, coef = 1, sort.by = "p", resort.by = NULL, ...)
```

Arguments

| | |
|---------------|--|
| fit | list containing a linear model fit produced by <code>lmFit</code> , <code>lm.series</code> , <code>gls.series</code> or <code>mr1m</code> . For <code>topTable</code> , <code>fit</code> should be an object of class <code>MArrayLM</code> as produced by <code>lmFit</code> and <code>eBayes</code> . |
| coef | column number or column name specifying which coefficient or contrast of the linear model is of interest. For <code>topTable</code> , can also be a vector of column subscripts, in which case the gene ranking is by F-statistic for that set of contrasts. |
| number | maximum number of genes to list |
| genelist | data frame or character vector containing gene information. For <code>topTable</code> only, this defaults to <code>fit\$genes</code> . |
| adjust.method | method used to adjust the p-values for multiple testing. Options, in increasing conservatism, include "none", "BH", "BY" and "holm". See p.adjust for the complete list of options. A NULL value will result in the default adjustment method, which is "BH". |
| sort.by | character string specifying which statistic to rank the genes by. Possible values for <code>topTable</code> are "logFC", "AveExpr", "t", "P", "p", "B" or "none". (Permitted synonyms are "M" for "logFC", "A" or "Amean" for "AveExpr", "T" for "t" and "p" for "P".) Possible values for <code>topTableF</code> are "F" or "none". <code>topTreat</code> accepts the same values as <code>topTable</code> except for "B". |
| resort.by | character string specifying statistic to sort the selected genes by in the output data.frame. Possibilities are the same as for <code>sort.by</code> . |
| p.value | cutoff value for adjusted p-values. Only genes with lower p-values are listed. |
| fc | optional minimum fold-change required. |
| lfc | optional minimum log ₂ -fold-change required, equal to $\log_2(\text{fc})$. <code>fc</code> and <code>lfc</code> are alternative ways to specify a fold-change cutoff and, if both are specified, then <code>fc</code> take precedence. If specified, then the results from <code>topTable</code> , <code>topTableF</code> or <code>topTreat</code> will include only genes with (at least one) absolute log-fold-change greater than <code>lfc</code> . This argument is not normally used with <code>topTreat</code> , which handles fold-change thresholds differently via the <code>treat</code> function. |
| confint | logical, should confidence 95% intervals be output for logFC? Alternatively, can be a numeric value between zero and one specifying the confidence level required. |
| ... | other <code>topTreat</code> arguments are passed to <code>topTable</code> . |

Details

These functions summarize the linear model fit object produced by `lmFit`, `lm.series`, `gls.series` or `mr1m` by selecting the top-ranked genes for any given contrast, or for a set of contrasts. `topTable` assumes that the linear model fit has already been processed by `eBayes`. `topTreat` assumes that the fit has been processed by `treat`.

If `coef` has a single value, then the moderated t-statistics and p-values for that coefficient or contrast are used. If `coef` takes two or more values, the moderated F-statistics for that set of coefficients or contrasts are used. If `coef` is left NULL, then all the coefficients or contrasts in the fitted model are used, except that any coefficient named (Intercept) will be removed.

The p-values for the coefficient/contrast of interest are adjusted for multiple testing by a call to `p.adjust`. The "BH" method, which controls the expected false discovery rate (FDR) below the specified value, is the default adjustment method because it is the most likely to be appropriate for microarray studies. Note that the adjusted p-values from this method are bounds on the FDR rather than p-values in the usual sense. Because they relate to FDRs rather than rejection probabilities, they are sometimes called q-values. See `help("p.adjust")` for more information.

Note, if there is no good evidence for differential expression in the experiment, that it is quite possible for all the adjusted p-values to be large, even for all of them to be equal to one. It is quite possible for all the adjusted p-values to be equal to one if the smallest p-value is no smaller than $1/n_{\text{genes}}$ where n_{genes} is the number of genes with non-missing p-values.

The `sort.by` argument specifies the criterion used to select the top genes. The choices are: "logFC" to sort by the (absolute) coefficient representing the log-fold-change; "A" to sort by average expression level (over all arrays) in descending order; "T" or "t" for absolute t-statistic; "P" or "p" for p-values; or "B" for the lods or B-statistic.

Normally the genes appear in order of selection in the output table. If a different order is wanted, then the `resort.by` argument may be useful. For example, `topTable(fit, sort.by="B", resort.by="logFC")` selects the top genes according to log-odds of differential expression and then orders the selected genes by log-ratio in decreasing order. Or `topTable(fit, sort.by="logFC", resort.by="logFC")` would select the genes by absolute log-fold-change and then sort them from most positive to most negative.

TopTable output for all probes in original (unsorted) order can be obtained by `topTable(fit, sort="none", n=Inf)`. However `write.fit` or `write` may be preferable if the intention is to write the results to a file. A related method is `as.data.frame(fit)` which coerces an MArrayLM object to a data.frame.

By default number probes are listed. Alternatively, by specifying `p.value` and `number=Inf`, all genes with adjusted p-values below a specified value can be listed.

The arguments `fc` and `lfc` give the ability to filter genes by log-fold change, but see the Note below. This argument is not available for `topTreat` because `treat` already handles fold-change thresholding in a more sophisticated way.

The function `topTableF` is scheduled for removal in a future version of limma. It is equivalent to `topTable` with `coef=NULL`.

Value

A dataframe with a row for the number top genes and the following columns:

| | |
|-----------------------|---|
| <code>genelist</code> | one or more columns of probe annotation, if <code>genelist</code> was included as input |
| <code>logFC</code> | estimate of the log ₂ -fold-change corresponding to the effect or contrast (for <code>topTableF</code> there may be several columns of log-fold-changes) |
| <code>CI.L</code> | left limit of confidence interval for logFC (if <code>confint=TRUE</code> or <code>confint</code> is numeric) |
| <code>CI.R</code> | right limit of confidence interval for logFC (if <code>confint=TRUE</code> or <code>confint</code> is numeric) |
| <code>AveExpr</code> | average log ₂ -expression for the probe over all arrays and channels, same as <code>Amean</code> in the MarrayLM object |
| <code>t</code> | moderated t-statistic (omitted for <code>topTableF</code>) |

| | |
|-------------|---|
| F | moderated F-statistic (omitted for topTable unless more than one coef is specified) |
| P.Value | raw p-value |
| adj.P.Value | adjusted p-value or q-value |
| B | log-odds that the gene is differentially expressed (omitted for topTreat) |

If `fit` had unique rownames, then the `row.names` of the above data.frame are the same in sorted order. Otherwise, the `row.names` of the data.frame indicate the row number in `fit`. If `fit` had duplicated row names, then these are preserved in the ID column of the data.frame, or in `ID0` if `genelist` already contained an ID column.

Note

Although `topTable` enables users to set both p-value and fold-change cutoffs, the use of fold-change cutoffs is not generally recommended. If the fold changes and p-values are not highly correlated, then the use of a fold change cutoff can increase the false discovery rate above the nominal level. Users wanting to use fold change thresholding are usually recommended to use `treat` and `topTreat` instead.

In general, the adjusted p-values returned by `adjust.method="BH"` remain valid as FDR bounds only when the genes remain sorted by p-value. Resorting the table by log-fold-change can increase the false discovery rate above the nominal level for genes at the top of resorted table.

Author(s)

Gordon Smyth

See Also

An overview of linear model and testing functions is given in [06.LinearModels](#). See also [p.adjust](#) in the stats package.

Examples

```
# See lmFit examples
```

`tricubeMovingAverage` *Moving Average Smoother With Tricube Weights*

Description

Apply a moving average smoother with tricube distance weights to a numeric vector.

Usage

```
tricubeMovingAverage(x, span=0.5, power=3)
```

Arguments

| | |
|-------|---|
| x | numeric vector |
| span | the smoother span. This gives the proportion of x values that contribute to each moving average. Larger values give more smoothness. Should be positive but not greater than 1. |
| power | a positive exponent used to compute the tricube weights. power=3 gives the usual tricube weights. Smaller values give more even weighting. Should be greater than 0. |

Details

This function smooths a vector (considered as a time series) using a moving average with tricube weights. Specifically, the function computes running weighted means of w consecutive values of x , where the window width w is equal to $2*h+1$ with $h = 2*\text{floor}(\text{span}*\text{length}(x)/2)$. The window width w is always odd so that each window has one of the original x values at its center. Each weighted mean uses a set of tricube weights so that values near the ends of the window receive less weight.

The smoother returns a vector of the same length as input. At the start and end of the vector, the series is considered to be extended by missing values, and the weighted average is computed only over the observed values. In other words, the window width is reduced to $h+1$ at the boundaries with asymmetric weights.

The result of this function is similar to a least squares loess curve of degree zero, with a couple of differences. First, a continuity correction is applied when computing the distance to neighbouring points, so that exactly w points are included with positive weights in each average. Second, the span halves at the end points so that the smoother is more sensitive to trends at the ends.

The `filter` function in the `stats` package is called to do the low-level calculations.

This function is used by `barcodeplot` to compute enrichment worms.

Value

Numeric vector of same length as x containing smoothed values.

Author(s)

Gordon Smyth

See Also

[filter](#), [barcodeplot](#), [loessByCol](#)

Examples

```
x <- rbinom(100,size=1,prob=0.5)
plot(1:100, tricubeMovingAverage(x))
```

| | |
|-----------------|----------------------------------|
| trigammaInverse | <i>Inverse Trigamma Function</i> |
|-----------------|----------------------------------|

Description

The inverse of the trigamma function.

Usage

```
trigammaInverse(x)
```

Arguments

x numeric vector or array

Details

The function uses Newton's method with a clever starting value to ensure monotonic convergence.

Value

Numeric vector or array y satisfying $\text{trigamma}(y) = x$.

Note

This function does not accept a data.frame as argument although the base package function `trigamma` does.

Author(s)

Gordon Smyth

See Also

This function is the inverse of [trigamma](#) in the base package.

This function is called by [fitFDist](#).

Examples

```
y <- trigammaInverse(5)
trigamma(y)
```

| | |
|----------------|--|
| trimWhiteSpace | <i>Trim Leading and Trailing White Space</i> |
|----------------|--|

Description

Trims leading and trailing white space from character strings.

Usage

```
trimWhiteSpace(x)
```

Arguments

x character vector

Value

A character vector of the same length as x in which leading and trailing white space has been stripped off each value.

Author(s)

Tim Beissbarth and Gordon Smyth

See Also

An overview of LIMMA functions for reading data is given in [03.ReadingData](#).

Examples

```
x <- c("a ", " b ")
trimWhiteSpace(x)
```

| | |
|----------------|---|
| uniquegenelist | <i>Eliminate Duplicate Names from the Gene List</i> |
|----------------|---|

Description

Eliminate duplicate names from the gene list. The new list is shorter than the full list by a factor of ndups.

Usage

```
uniquegenelist(genelist, ndups=2, spacing=1)
```

Arguments

| | |
|----------|---|
| genelist | vector of gene names |
| ndups | number of duplicate spots. The number of rows of genelist must be divisible by ndups. |
| spacing | the spacing between duplicate names in genelist |

Value

A vector of length $\text{length}(\text{genelist})/\text{ndups}$ containing each gene name once only.

Author(s)

Gordon Smyth

See Also

[unwrapdups](#)

Examples

```
genelist <- c("A", "A", "B", "B", "C", "C", "D", "D")
uniquegenelist(genelist, ndups=2)
genelist <- c("A", "B", "A", "B", "C", "D", "C", "D")
uniquegenelist(genelist, ndups=2, spacing=2)
```

unwrapdups

Unwrap Duplicate Spot Values from Rows into Columns

Description

Reshape a matrix so that a set of consecutive rows becomes a single row in the output.

Usage

```
unwrapdups(M, ndups=2, spacing=1)
```

Arguments

| | |
|---------|---|
| M | a matrix. |
| ndups | number of duplicate spots. The number of rows of M must be divisible by ndups. |
| spacing | the spacing between the rows of M corresponding to duplicate spots, spacing=1 for consecutive spots |

Details

This function is used on matrices corresponding to a series of microarray experiments. Rows corresponding to duplicate spots are re-arranged so that all values corresponding to a single gene are on the same row. This facilitates fitting models or computing statistics for each gene.

Value

A matrix containing the same values as M but with fewer rows and more columns by a factor of ndups. Each set of ndups rows in M is strung out to a single row so that duplicate values originally in consecutive rows in the same column are in consecutive columns in the output.

Author(s)

Gordon Smyth

Examples

```
M <- matrix(1:12,6,2)
unwrapdups(M,ndups=2)
unwrapdups(M,ndups=3)
unwrapdups(M,ndups=2,spacing=3)
```

 venn

Venn Diagrams

Description

Compute classification counts and draw a Venn diagram.

Usage

```
vennCounts(x, include="both")
vennDiagram(object, include="both", names=NULL, mar=rep(1,4), cex=c(1.5,1,0.7), lwd=1,
            circle.col=NULL, counts.col=NULL, show.include=NULL, ...)
```

Arguments

| | |
|---------|---|
| x | a TestResults matrix. This is numeric matrix of 0's, 1's and -1's indicating significance of a test or membership of a set. Each row corresponds to a gene and each column to a contrast or set. Usually created by decideTests . |
| object | either a TestResults matrix or a VennCounts object produced by vennCounts. |
| include | character vector specifying whether all differentially expressed genes should be counted, or whether the counts should be restricted to genes changing in a certain direction. Choices are "both" for all differentially expressed genes, "up" for up-regulated genes only or "down" for down-regulated genes only. If include=c("up", "down") then both the up and down counts will be shown. This argument is ignored if object if object is already a vennCounts object. |
| names | character vector giving names for the sets or contrasts |
| mar | numeric vector of length 4 specifying the width of the margins around the plot. This argument is passed to par. |
| cex | numerical vector of length 3 giving scaling factors for large, medium and small text on the plot. |

| | |
|---------------------------|---|
| <code>lwd</code> | numerical value giving the amount by which the circles should be scaled on the plot. See <code>par</code> . |
| <code>circle.col</code> | vector of colors for the circles. See <code>par</code> for possible values. |
| <code>counts.col</code> | vector of colors for the counts. Of same length as <code>include</code> . See <code>par</code> for possible values. |
| <code>show.include</code> | logical value whether the value of <code>include</code> should be printed on the plot. Defaults to <code>FALSE</code> if <code>include</code> is a single value and <code>TRUE</code> otherwise |
| <code>...</code> | any other arguments are passed to <code>plot</code> |

Details

Each column of `x` corresponds to a contrast or set, and the entries of `x` indicate membership of each row in each set or alternatively the significance of each row for each contrast. In the latter case, the entries can be negative as well as positive to indicate the direction of change.

`vennCounts` can collate intersection counts for any number of sets. `vennDiagram` can plot up to five sets.

Value

`vennCounts` produces an object of class "VennCounts". This contains only one slot, which is numerical matrix with $2^{\text{ncol}\{x\}}$ rows and $\text{ncol}(x)+1$ columns. Each row corresponds to a particular combination of set memberships. The first $\text{ncol}\{x\}$ columns of output contain 1 or 0 indicating membership or not in each set. The last column called "Counts" gives the number of rows of `x` corresponding to that combination of memberships.

`vennDiagram` produces no output but causes a plot to be produced on the current graphical device.

Author(s)

Gordon Smyth, James Wettenhall, Francois Pepin, Steffen Moeller and Yifang Hu

See Also

An overview of linear model functions in `limma` is given by [06.LinearModels](#).

Examples

```
Y <- matrix(rnorm(100*6),100,6)
Y[1:10,3:4] <- Y[1:10,3:4]+3
Y[1:20,5:6] <- Y[1:20,5:6]+3
design <- cbind(1,c(0,0,1,1,0,0),c(0,0,0,0,1,1))
fit <- eBayes(lmFit(Y,design))
results <- decideTests(fit)
a <- vennCounts(results)
print(a)
mfrow.old <- par()$mfrow
par(mfrow=c(1,2))
vennDiagram(a)
vennDiagram(results,
  include=c("up", "down"),
```

```
counts.col=c("red", "blue"),
circle.col = c("red", "blue", "green3"))
par(mfrow=mfrow.old)
```

volcanoplot

Volcano Plot

Description

Creates a volcano plot for a specified coefficient of a linear model.

Usage

```
volcanoplot(fit, coef = 1, style = "p-value",
            highlight = 0, names = fit$genes$ID, hl.col = "blue",
            xlab = "Log2 Fold Change", ylab = NULL, pch=16, cex=0.35, ...)
```

Arguments

| | |
|-----------|---|
| fit | an MArrayLM fitted linear model object. |
| coef | index indicating which coefficient of the linear model is to be plotted. |
| style | character string indicating which significance statistic to plot on the y-axis. Possibilities are "p-value" or "B-statistic". |
| highlight | number of top genes to be highlighted by name. |
| names | character vector of length nrow(fit) giving gene names. Only used if highlight > 0. |
| hl.col | color for the gene names. Only used if highlight > 0. |
| xlab | character string giving label for x-axis |
| ylab | character string giving label for y-axis |
| pch | vector or list of plotting characters. |
| cex | numeric vector of plot symbol expansions. |
| ... | any other arguments are passed to plot |

Details

A volcano plot displays log fold changes on the x-axis versus a measure of statistical significance on the y-axis. Here the significance measure can be $-\log(\text{p-value})$ or the B-statistics, which give the posterior log-odds of differential expression.

The plot is optionally annotated with the names of the most significant genes.

Value

No value is returned but a plot is created on the current graphics device.

Author(s)

Gordon Smyth

See Also

An overview of presentation plots following the fitting of a linear model in LIMMA is given in [06.LinearModels](#).

Examples

```
# See lmFit examples
```

| | |
|------|--|
| voom | <i>Transform RNA-Seq Counts for Linear Modeling With Precision Weights</i> |
|------|--|

Description

Transform count data to log₂ counts-per-million (logCPM), estimate the mean-variance relationship and use it to compute observation-level precision weights. The logCPM and associated precision weights are then ready for linear modeling.

Usage

```
voom(counts, design = NULL, lib.size = NULL, normalize.method = "none",
      block = NULL, correlation = NULL, weights = NULL,
      span = 0.5, adaptive.span = FALSE, plot = FALSE, save.plot = FALSE)
```

Arguments

| | |
|------------------|---|
| counts | a numeric matrix containing raw counts, or an ExpressionSet containing raw counts, or a DGEList object. Counts must be non-negative and NAs are not permitted. |
| design | design matrix with rows corresponding to samples and columns to coefficients to be estimated. Defaults to <code>model.matrix(~group, data=counts\$samples)</code> if counts is a DGEList, otherwise defaults to the unit vector meaning that all samples are treated as replicates. |
| lib.size | numeric vector containing the library sizes for each sample. Defaults to the columnwise count totals if counts is a matrix or to <code>normLibSizes(counts)</code> if counts is a DGEList. |
| normalize.method | the microarray-style normalization method to be applied to the logCPM values. Choices are as for the method argument of <code>normalizeBetweenArrays</code> when the data is single-channel. |
| block | vector or factor specifying a blocking variable on the samples. Has length equal to the number of samples (<code>ncol(counts)</code>). |

| | |
|----------------------------|--|
| <code>correlation</code> | the intrablock correlation. Normally a single numeric value between -1 and 1, but a vector of genewise correlations is also allowed. |
| <code>weights</code> | prior weights. Can be a numeric matrix of individual weights of same dimensions as the counts, or a numeric vector of sample weights with length equal to <code>ncol(counts)</code> , or a numeric vector of gene weights with length equal to <code>nrow(counts)</code> . |
| <code>span</code> | width of the smoothing window used for the lowess mean-variance trend. Expressed as a proportion between 0 and 1. |
| <code>adaptive.span</code> | logical. If TRUE, then an optimal value for span will be chosen depending on the number of genes. |
| <code>plot</code> | logical, should a plot of the mean-variance trend be displayed? |
| <code>save.plot</code> | logical, should the coordinates and line of the plot be saved in the output? |

Details

This function processes sequence count data from technologies such as RNA-seq or ChIP-seq to make it ready for linear modeling in `limma`.

`voom` is an acronym for "mean-variance modeling at the observational level". The idea is to estimate the mean-variance relationship in the data, then use this to compute an appropriate precision weight for each observation. Count data always show marked mean-variance relationships. Raw counts show increasing variance with increasing count size, while log-counts typically show a decreasing mean-variance trend. This function estimates the mean-variance trend for log-counts, then assigns a weight to each observation based on its predicted variance. The weights are then used in the linear modeling process to adjust for heteroscedasticity. The mean-variance trend is estimated from gene-level data but is extrapolated back to individual observations to obtain a precision weight (inverse variance) for each observation.

`voom` performs the following specific calculations. First, the counts are converted to logCPM values, adding 0.5 to all the counts to avoid taking the logarithm of zero. The logCPM calculation uses normalized library sizes if `counts` is a `DGEList` or simply the column sums if `counts` is a matrix. A microarray-style normalization method can also be optionally applied to the matrix of logCPM values. The `lmFit` function is used to fit row-wise linear models. The `lowess` function is then used to fit a trend to the square-root residual standard deviations as a function of an average log-count measure. The trend line is then used to predict the variance of each logCPM value as a function of its fitted value on the count scale, and the inverse variances become the estimated precision weights.

The optional arguments `block`, `correlation` and `weights` are passed to `lmFit` in the above calling sequence, so they influence the row-wise standard deviations to which the mean-variance trend is fitted. The arguments `block` and `correlation` have the same meaning as for `lmFit`. Most users will not need to specify the `weights` argument but, if it is included, then the output `weights` are taken to modify the input prior weights in a multiplicative fashion.

For good results, the counts matrix should be filtered to remove rows with very low counts before running `voom()`. The `filterByExpr` function in the `edgeR` package can be used for that purpose.

If `counts` is a `DGEList` object from the `edgeR` package, then `voom` will use the normalization factors found in the object when computing the logCPM values. In other words, the logCPM values are computed from the effective library sizes rather than the raw library sizes. If the `DGEList` object has been scale-normalized in `edgeR`, then it is usual to leave `normalize.method="none"` in `voom`, i.e., the logCPM values should not usually be re-normalized in the `voom` call.

The voom method is similar in purpose to the limma-trend method, which uses `eBayes` or `treat` with `trend=TRUE`. The voom method incorporates the mean-variance trend into the precision weights, whereas limma-trend incorporates the trend into the empirical Bayes moderation. The voom method takes into account the sequencing depths (library sizes) of the individual columns of counts and applies the mean-variance trend on an individual observation basis. limma-trend, on the other hand, assumes that the library sizes are not wildly different and applies the mean-variance trend on a genewise basis. As noted by Law et al (2014), voom should be more powerful than limma-trend if the library sizes are very different but, otherwise, the two methods should give similar results.

If `adaptive.span` is TRUE, then `span` is set to `chooseLowessSpan(nrow(counts), small.n=50, min.span=0.3, power=1/3)`.

Note that `edgeR::voomLmFit` is a further-developed version voom with more functionality and convenience. `voomLmFit` is now recommended over voom, particularly if an intrablock correlation needs to be estimated or if the counts are sparse with a high proportion of zeros.

Value

An `EList` object with the following components:

| | |
|------------------------|--|
| <code>E</code> | numeric matrix of normalized expression values on the log2 scale |
| <code>weights</code> | numeric matrix of inverse variance weights |
| <code>design</code> | design matrix |
| <code>lib.size</code> | numeric vector of total normalized library sizes |
| <code>genes</code> | data-frame of gene annotation extracted from counts |
| <code>span</code> | if <code>adaptive.span</code> , the chosen value for span is returned |
| <code>voom.xy</code> | if <code>save.plot</code> , list containing x and y coordinates for points in mean-variance plot |
| <code>voom.line</code> | if <code>save.plot</code> , list containing coordinates of loess line in the mean-variance plot |

Note

voom is designed to accept counts. Usually these will be sequence read counts, but counts of species abundance or other biological quantities might also be appropriate. Estimated counts are also acceptable provided that the column sums are representative of the total library size (total number of reads) for that sample. voom can analyze scaled counts provided that the column sums remain proportional to the total library sizes. voom is designed to take account of sample-specific library sizes and hence voom should not be used to analyze quantities that have been normalized for library size such as RPKM, transcripts per million (TPM) or counts per million (CPM). Such quantities prevent voom from inferring the correct library sizes and hence the correct precision with which each value was measured.

Author(s)

Charity Law and Gordon Smyth

References

Law CW (2013). *Precision weights for gene expression analysis*. PhD Thesis. University of Melbourne, Australia. <http://hdl.handle.net/11343/38150>

Law CW, Chen Y, Shi W, Smyth GK (2014). Voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biology* 15, R29. doi:10.1186/gb2014152r29. See also the Preprint Version at <https://gksmyth.github.io/pubs/VoomPreprint.pdf> incorporating some notational corrections.

Law CW, Alhamdoosh M, Su S, Smyth GK, Ritchie ME (2016). RNA-seq analysis is easy as 1-2-3 with limma, Glimma and edgeR. *F1000Research* 5, 1408. <https://f1000research.com/articles/5-1408>

Law CW, Alhamdoosh M, Su S, Dong X, Tian L, Smyth GK, Ritchie ME (2018). RNA-seq analysis is easy as 1-2-3 with limma, Glimma and edgeR. *Bioconductor Workflow Package*. <https://www.bioconductor.org/packages/RNAseq123/>

See Also

[lmFit](#) and [eBayes](#) are downstream of voom.

[voomWithQualityWeights](#) and `edgeR::voomLmFit` are further developed versions of voom with extra capabilities. Either can be used as a replacement for voom. [voomWithQualityWeights](#) estimates sample weights. `voomLmFit` estimates both sample weights and intrablock correlation and also improves variance estimation for sparse data.

[vooma](#) is analogous to voom but for continuous log-expression data instead of RNA-seq counts.

A summary of limma functions for RNA-seq analysis is given in [11.RNAseq](#).

Examples

```
## Not run:
keep <- filterByExpr(counts, design)
v <- voom(counts[keep,], design, plot=TRUE)
fit <- lmFit(v, design)
fit <- eBayes(fit, robust=TRUE)
## End(Not run)
```

vooma

*Convert Variance Trend to Observation Precision Weights for
Microarray-style Data*

Description

Estimate the variance trend for microarray data and use it to compute appropriate observational-level weights. The variance trend optionally depends on a second predictor as well as on average log-expression.

Usage

```
vooma(y, design = NULL, block = NULL, correlation,
      predictor = NULL, span = NULL, legacy.span = FALSE, plot = FALSE, save.plot = FALSE)
```

```
voomaByGroup(y, group, design = NULL, block = NULL, correlation,
             span = NULL, legacy.span = FALSE, plot = FALSE, col = NULL, lwd = 1,
             pch = 16, cex = 0.3, alpha = 0.5, legend = "topright")
```

Arguments

| | |
|--------------------------|--|
| <code>y</code> | a numeric matrix, EList object, or any object containing log-expression data that can be coerced to a matrix. Rows correspond to genes and columns to samples. |
| <code>design</code> | design matrix with rows corresponding to samples and columns to coefficients to be estimated. Defaults to the unit vector meaning that samples are treated as replicates. |
| <code>block</code> | vector or factor specifying a blocking variable on the arrays. Has length equal to the number of arrays. |
| <code>correlation</code> | intra-block correlation |
| <code>predictor</code> | precision predictor. Numeric matrix of the same dimensions as <code>y</code> that predicts the precision of each log-expression value. Is used as a second covariate together with the log-intensities to predict the variances and produce the final precision weights. |
| <code>span</code> | width of the smoothing window, as a proportion of the data set. Defaults to a value that depends the number of genes (<code>nrow(y)</code>). Equal to 1 if the number of genes is less than or equal to 50, then decreases slowly to 0.3 if the number of genes is very large. |
| <code>legacy.span</code> | logical. If TRUE, then the original default setting will be used for span, which is slightly smaller than the new default. |
| <code>plot</code> | logical. If TRUE, a plot of the mean-variance trend is displayed. |
| <code>save.plot</code> | logical, should the coordinates and line of the plot be saved in the output? |
| <code>group</code> | categorical vector or factor giving group membership of columns of <code>y</code> . |
| <code>col</code> | vector of colors for plotting group trends |
| <code>lwd</code> | line width for plotting group trends |
| <code>pch</code> | plotting character. Default is integer code 16, which gives a solid circle. If a vector, then should be of length <code>nrow(y)</code> . |
| <code>cex</code> | numeric vector of plot symbol expansions. If a vector, then should be of length equal to number of groups. |
| <code>alpha</code> | transparency of points, on scale from 0 for fully transparent to 1 for fully opaque. |
| <code>legend</code> | character string giving position to place legend. |

Details

vooma is an acronym for "mean-variance modelling at the observational level for arrays". It is analogous to voom but for continuous log-expression values rather than for sequencing counts.

vooma estimates the mean-variance relationship in the data and uses it to compute appropriate precision weights for each observation. The mean-variance trend is estimated from gene-level data but is extrapolated back to individual observations to obtain a precision weight (inverse variance) for each observation. The weights can then be used by other functions such as `lmFit` to adjust for heteroscedasticity.

If `span=NULL`, then an optimal span value is estimated depending on `nrow(y)`. The span is chosen by `chooseLowessSpan` with `n=nrow(y)`, `small.n=50`, `min.span=0.3` and `power=1.3`. If `legacy.span=TRUE`, then the `chooseLowessSpan` arguments are reset to `small.n=10`, `min.span=0.3` and `power=0.5` to match the settings used by vooma in limma version 3.59.1 and earlier.

The variance trend can be modeled using a second optional predictor as well as in terms of log-expression. If `predictor` is not `NULL`, then the variance trend is modeled as a function of both the mean log-expression and the predictor using a multiple linear regression with the two predictors. In this case, the predictor is assumed to be some prior predictor of the precision or standard deviation of each log-expression value. Any predictor that is correlated with the precision of each observation should give good results.

`voomaByGroup` estimates precision weights separately for different groups of samples. In other words, it allows for different mean-variance curves in different groups. `voomaByGroup` has a quite simple implementation and simply subsets the design matrix for each group. This subsetting is equivalent to interacting the design factors with the groups and might not work well with complex design matrices. It will work fine if the design matrix corresponds to the same groups as defined by the `group` argument. It can work well for large datasets, for example it has been used by Ravindra et al (2023) to account for TMT groups in proteomics data.

Value

An `EList` object with the following components:

| | |
|------------------------|---|
| <code>E</code> | numeric matrix of log-expression values. Equal to <code>y</code> for vooma or log2-counts-per-million for voomaByGroup. |
| <code>weights</code> | numeric matrix of observation precision weights. |
| <code>design</code> | numeric matrix of experimental design. |
| <code>genes</code> | data-frame of gene annotation, only if <code>counts</code> was a <code>DGEList</code> object. |
| <code>voom.xy</code> | if <code>save.plot</code> , list containing x and y coordinates for points in mean-variance plot |
| <code>voom.line</code> | if <code>save.plot</code> , list containing coordinates of lowess line in the mean-variance plot |

Author(s)

Charity Law, Gordon Smyth and Mengbo Li. Mengbo Li contributed the functionality associated with the `predictor` argument.

References

Law CW (2013). *Precision weights for gene expression analysis*. PhD Thesis. University of Melbourne, Australia. <http://hdl.handle.net/11343/38150>

Ravindra KC, Vaidya VS, Wang Z, Federspiel JD, Virgen-Slane R, Everley RA, Grove JI, Stephens C, Ocana MF, Robles-Diaz M, Isabel Lucena M (2023). Tandem mass tag-based quantitative proteomic profiling identifies candidate serum biomarkers of drug-induced liver injury in humans. *Nature Communications* 14(1), 1215.

See Also

[voomaLmFit](#), [voom](#), [arrayWeights](#)

Examples

```
group <- gl(2,4)
design <- model.matrix(~group)
y <- matrix(rnorm(500*8),500,8)
u <- matrix(runif(length(y)),500,8)
yu <- y*u
v <- vooma(yu,design,plot=TRUE,predictor=u)
```

voomaLmFit

Apply vooma-lmFit Pipeline With Automatic Estimation of Sample Weights and Block Correlation

Description

Estimate the variance trend, use it to compute observational weights and use the weights to a fit a linear model. Includes automatic estimation of sample weights and block correlation. Equivalent to calling `vooma()`, `arrayWeights()`, `duplicateCorrelation()` and `lmFit()` iteratively.

Usage

```
voomaLmFit(y, design = NULL, prior.weights = NULL, block = NULL,
           sample.weights = FALSE, var.design = NULL, var.group = NULL, prior.n = 10,
           predictor = NULL, span = NULL, legacy.span = FALSE,
           plot = FALSE, save.plot = FALSE, keep.EList = TRUE)
```

Arguments

| | |
|---------------------|---|
| <code>y</code> | a numeric matrix, EList object, or any object containing log-expression data that can be coerced to a matrix. Rows correspond to genes and columns to samples. |
| <code>design</code> | design matrix with rows corresponding to samples and columns to coefficients to be estimated. Defaults to the unit vector meaning that samples are treated as replicates. |

| | |
|-----------------------------|---|
| <code>prior.weights</code> | prior weights. Can be a numeric matrix of individual weights of same dimensions as the counts, or a numeric vector of sample weights with length equal to <code>ncol(counts)</code> , or a numeric vector of gene weights with length equal to <code>nrow(counts)</code> . |
| <code>block</code> | vector or factor specifying a blocking variable on the arrays. Has length equal to <code>ncol(y)</code> . |
| <code>sample.weights</code> | logical value. If TRUE then empirical sample quality weights will be estimated. |
| <code>var.design</code> | design matrix for predicting the sample variances. Defaults to the sample-specific model whereby each sample has a different variance. |
| <code>var.group</code> | vector or factor indicating groups to have different sample weights. This is another way to specify <code>var.design</code> for groupwise sample weights. |
| <code>prior.n</code> | prior number of genes for squeezing the weights towards equality. Larger values squeeze the sample weights more strongly towards equality. |
| <code>predictor</code> | precision predictor. Either a column vector of length <code>nrow(y)</code> or a numeric matrix of the same dimensions as <code>y</code> that predicts the precision of each log-expression value. Is used as a second covariate together with the log-intensities to predict the variances and produce the final precision weights. |
| <code>span</code> | width of the smoothing window, as a proportion of the data set. Defaults to a value between 0.3 and 1 that depends the number of genes (<code>nrow(y)</code>). Equal to 1 if the number of genes is less than or equal to 50, then decreases slowly to 0.3 if the number of genes is very large. |
| <code>legacy.span</code> | logical. If TRUE, then the original default setting will be used for <code>span</code> , which is slightly smaller than the new default. |
| <code>plot</code> | logical. If TRUE, a plot of the mean-variance trend is displayed. |
| <code>save.plot</code> | logical, should the coordinates and line of the plot be saved in the output? |
| <code>keep.EList</code> | logical. If TRUE, then the <code>EList</code> object containing log-expression values and observation weights will be saved in the component <code>EList</code> of the output object. |

Details

This function is analogous to `voomLmFit` in the `edgeR` package but for microarray-like data with continuous log-expression values. The function is equivalent to calling `vooma()` followed by `lmFit()`, optionally with `arrayWeights()` and `duplicateCorrelation()` as well to estimate sample weights and block correlation. The function finishes with `lmFit()` and returns a fitted model object.

Like `vooma`, `voomaLmFit` estimates the mean-variance relationship in the data and uses it to compute appropriate precision weights for each observation. The mean-variance trend is estimated from gene-level data but is extrapolated back to individual observations to obtain a precision weight (inverse variance) for each observation. The weights are then used by `lmFit()` to adjust for heteroscedasticity.

If `span=NULL`, then an optimal span value is estimated depending on `nrow(y)`. The span is chosen by `chooseLowessSpan` with `n=nrow(y)`, `small.n=50`, `min.span=0.3` and `power=1/3`. If `legacy.span=TRUE`, then the `chooseLowessSpan` arguments are reset to `small.n=10`, `min.span=0.3` and `power=0.5` to match the settings used by `vooma` in `limma` version 3.59.1 and earlier.

If `predictor` is not `NULL`, then the variance trend is modeled as a function of both the mean log-expression and the predictor using a multiple linear regression with the two predictors. In this

case, the predictor is assumed to be some prior predictor of the precision or standard deviation of each log-expression value. Any predictor that is correlated with the precision of each observation should give good results. This ability to model the variance trend using two covariates (mean log-expression and the predictor covariate) was described for the first time by Li (2024).

Sample weights will be estimated using `arrayWeights` if `sample.weights = TRUE` or if either `var.design` or `var.group` are non-NULL. An intra-block correlation will be estimated using `duplicateCorrelation` if `block` is non-NULL. In either case, the whole estimation pipeline will be repeated twice to update the sample weights and/or block correlation.

Value

An `MArrayLM` object containing linear model fits for each row of data. If sample weights are estimated, then the output object will include a `targets.data.frame` component with the sample weights as a column with heading `"sample.weights"`.

If `save.plot=TRUE` then the output object will include components `voom.xy` and `voom.line`. `voom.xy` contains the x and y coordinates of the points in the vooma variance-trend plot and `voom.line` contains the estimated trend line.

If `keep.EList=TRUE`, then the output includes component `EList` with sub-components `EList$E` and `EList$weights`. If `y` was an `EList` object, then the output `EList` preserves all the components of `y` and adds the weights.

Author(s)

Mengbo Li and Gordon Smyth

References

Li M (2024). Linear Models and Empirical Bayes Methods for Mass Spectrometry-based Proteomics Data. PhD Thesis, University of Melbourne. <http://hdl.handle.net/11343/351600>

See Also

[vooma](#), [lmFit](#), [voomLmFit](#) (in the `edgeR` package).

Examples

```
# Example with a precision predictor
group <- gl(2,4)
design <- model.matrix(~group)
y <- matrix(rnorm(500*8),500,8)
u <- matrix(runif(length(y)),500,8)
yu <- y*u
fit <- voomaLmFit(yu,design,plot=TRUE,predictor=u)

# Reproducing vooma plot from output object
fit <- voomaLmFit(yu,design,predictor=u,save.plot=TRUE)
do.call(plot,fit$voom.xy)
do.call(lines,fit$voom.line)
```

 voomWithQualityWeights

Transform RNA-Seq Counts for Linear Modeling With Precision and Sample Weights

Description

Combine voom observational-level precision weights with sample-specific quality weights in a designed experiment.

Usage

```
voomWithQualityWeights(counts, design = NULL, lib.size = NULL, normalize.method = "none",
  plot = FALSE, span = 0.5, adaptive.span = FALSE,
  var.design = NULL, var.group = NULL,
  method = "genebygene", maxiter = 50, tol = 1e-5, trace = FALSE,
  col = NULL, ...)
```

Arguments

| | |
|------------------|---|
| counts | a numeric matrix containing raw counts, or an ExpressionSet containing raw counts, or a DGEList object. Counts must be non-negative and NAs are not permitted. |
| design | design matrix with rows corresponding to samples and columns to coefficients to be estimated. Defaults to <code>model.matrix(~group, data=counts\$samples)</code> if counts is a DGEList, otherwise defaults to the unit vector meaning that all samples are treated as replicates. |
| lib.size | numeric vector containing total library sizes for each sample. If NULL and counts is a DGEList then, the normalized library sizes are taken from counts. Otherwise library sizes are calculated from the columnwise counts totals. |
| normalize.method | the microarray-style normalization method to be applied to the logCPM values. Choices are as for the method argument of <code>normalizeBetweenArrays</code> when the data is single-channel. |
| plot | logical, should a plot of the mean-variance trend and sample-specific weights be displayed? |
| span | width of the smoothing window used for the lowess mean-variance trend. Expressed as a proportion between 0 and 1. |
| adaptive.span | logical. If TRUE, then an optimal value for span will be chosen depending on the number of genes. |
| var.design | design matrix for the variance model. Defaults to the sample-specific model whereby each sample has a distinct quality weight. |
| var.group | vector or factor indicating groups to have different quality weights. This is another way to specify <code>var.design</code> for groupwise variance models. |

| | |
|---------|---|
| method | character string specifying the method used to estimate the quality weights. Choices are "genebygene" or "reml". |
| maxiter | maximum number of iterations allowed for quality weight estimation when method = "reml". |
| tol | convergence tolerance for quality weight estimation when method = "reml". |
| trace | logical. If TRUE then diagnostic information is output at each iteration of the "reml" algorithm, or at every 1000th iteration of the "genebygene" algorithm. |
| col | colors to use in the barplot of sample-specific weights if plot=TRUE). If NULL, then bars are plotted in grey. |
| ... | other arguments are passed to voom and hence to lmFit. |

Details

This function is an alternative to voom and, like voom, is intended to process RNA-seq data prior to linear modeling in limma. It combines observational-level weights from voom with sample-specific weights estimated using the arrayWeights function. The method is described by Liu et al (2015).

Value

An `EList` object similar to that from voom, with an extra column `sample.weights` containing the vector of sample quality factors added to the `targets` data.frame. The weights component combines the sample weights and the usual voom precision weights.

Note

Users are now recommended to use `edgeR::voomLmFit`, which is a further developed version of `voomWithQualityWeights` with extra capabilities. `voomLmFit` estimates both sample weights and intrablock correlation and also improves variance estimation for sparse data.

Author(s)

Matthew Ritchie, Cynthia Liu, Gordon Smyth

References

Liu R, Holik AZ, Su S, Jansz N, Chen K, Leong HS, Blewitt ME, Asselin-Labat ML, Smyth GK, Ritchie ME (2015). Why weight? Combining voom with estimates of sample quality improves power in RNA-seq analyses. *Nucleic Acids Research* 43, e97. doi:10.1093/nar/gkv412

See Also

[voom](#), [arrayWeights](#), [lmFit](#), [voomLmFit](#).

A summary of limma functions for RNA-seq analysis is given in [11.RNAseq](#).

| | |
|-----------------|------------------------|
| weighted.median | <i>Weighted Median</i> |
|-----------------|------------------------|

Description

Compute a weighted median of a numeric vector.

Usage

```
weighted.median(x, w, na.rm = FALSE)
```

Arguments

| | |
|-------|---|
| x | a numeric vector containing the values whose mean is to be computed. |
| w | a vector of weights the same length as x giving the weights to use for each element of x. |
| na.rm | a logical value indicating whether NA values in x should be stripped before the computation proceeds. |

Details

If w is missing then all elements of x are given the same weight.

Missing values in w are not handled.

The weighted median is the median of the discrete distribution with values given by x and probabilities given by $w/\text{sum}(w)$.

Value

numeric value giving the weighted median

See Also

[median](#), [weighted.mean](#)

Examples

```
## GPA from Siegel 1994
wt <- c(5, 5, 4, 1)/15
x <- c(3.7, 3.3, 3.5, 2.8)
xm <- weighted.median(x, wt)
```

| | |
|----------------|---|
| weightedLowess | <i>LOWESS Smoother with Prior Weights</i> |
|----------------|---|

Description

This function generalizes the original LOWESS smoother (locally-weighted regression) to incorporate prior weights while preserving the original algorithm design and efficiency as closely as possible.

Usage

```
weightedLowess(x, y, weights = NULL,
               delta = NULL, npts = 200, span = 0.3, iterations = 4,
               output.style = "loess")
```

Arguments

| | |
|--------------|--|
| x | a numeric vector of values for the covariate or x-axis coordinates. |
| y | a numeric vector of response values or y-axis coordinates, of same length as x. |
| weights | a numeric vector containing non-negative prior weights, of same length as x. Defaults to a constant vector. |
| delta | a numeric scalar specifying the maximum distance between successive anchor x-values where a local regression will be computed. Roughly corresponds to $\text{diff}(\text{range}(x))/\text{npts}$ if the x-values are equally spaced. Setting $\text{delta}=0$ forces every distinct x-value to be an anchor point. If NULL then a suitable delta value will be computed from npts. |
| npts | an integer scalar specifying the approximate number of anchor x-values at which local regressions will be computed. Ignored if delta is not NULL. |
| span | a numeric scalar between 0 and 1 specifying the width of the smoothing window as a proportion of the total weight. |
| iterations | an integer scalar specifying the number of iterations. $\text{iterations}=1$ corresponds to local least squares regression without robustifying weights. Each additional iteration incorporates robustifying weights. |
| output.style | character string indicating whether the output should be in the style of "loess" or of "lowess". |

Details

This function extends the LOWESS algorithm of Cleveland (1979, 1981) to handle non-negative prior weights.

The LOWESS method consists of computing a series of local linear regressions, with each local regression restricted to a window of x-values. Smoothness is achieved by using overlapping windows and by gradually down-weighting points in each regression according to their distance from the anchor point of the window (tri-cube weighting).

To conserve running time and memory, locally-weighted regressions are computed at only a limited number of anchor x -values, either `npts` or the number of distinct x -values, whichever is smaller. Anchor points are defined exactly as in the original LOWESS algorithm. Any x -value within distance `delta` of an anchor point is considered adjacent to it. The first anchor point is $\min(x)$. With the x -values sorted in ascending order, successive anchor points are defined as follows. The next anchor point is the smallest x -value not adjacent to any previous anchor points. The last anchor point is $\max(x)$.

For each anchor point, a weighted linear regression is performed for a window of neighboring points. The neighboring points consist of the smallest set of closest neighbors such as the sum of weights is greater than or equal to `span` times the total weight of all points. Each local regression produces a fitted value for that anchor point. Fitted values for other x -values are then obtained by linear interpolation between anchor points.

For the first iteration, the local linear regressions use weights equal to prior weights times the tri-cube distance weights. Subsequent iterations multiple these weights by robustifying weights. Points with residuals greater than 6 times the median absolute residual are assigned weights of zero and otherwise Tukey's biweight function is applied to the residuals to obtain the robust weights. More iterations produce greater robustness.

In summary, the prior weights are used in two ways. First, the prior weights are used during the span calculations such that the points included in the window for each local regression must account for the specified proportion of the total sum of weights. Second, the weights used for the local regressions are the product of the prior weights, tri-cube local weights and biweight robustifying weights. Hence a point with prior weight equal to an integer n has the same influence as n points with unit weight and the same x and y -values.

See also `loessFit`, which is essentially a wrapper function for `lowess` and `weightedLowess` with added error checking.

Relationship to lowess and loess

The stats package provides two functions `lowess` and `loess`. `lowess` implements the original LOWESS algorithm of Cleveland (1979, 1981) designed for scatterplot smoothing with single x -variable while `loess` implements the more complex algorithm by Cleveland et al (1988, 1992) designed to fit multivariate surfaces. The `loess` algorithm is more general than `lowess` in a number of ways, notably because it allows prior weights and up to four numeric predictors. On the other hand, `loess` is necessarily slower and uses more memory than `lowess`. Furthermore, it has less accurate interpolation than `lowess` because it uses a cruder algorithm to choose the anchor points whereby anchor points are equi-spaced in terms of numbers of points rather than in terms of x -value spacing. `lowess` and `loess` also have different defaults and input parameters. See Smyth (2003) for a detailed discussion.

Another difference between `lowess` and `loess` is that `lowess` returns the x and y coordinates of the fitted curve, with x in ascending order, whereas `loess` returns fitted values and residuals in the original data order.

The purpose of the current function is to incorporate prior weights but keep the algorithmic advantages of the original `lowess` code for scatterplot smoothing. The current function therefore generalizes the span and interpolation concepts of `lowess` differently to `loess`.

When `output.style="loess"`, `weightedLowess` outputs results in original order similar to `loessFit` and `loess`. When `output.style="lowess"`, `weightedLowess` outputs results in sorted order the same as `lowess`.

The span argument corresponds to the f argument of lowess and the span argument of loess. The delta argument is the same as the delta argument of lowess. The npts argument is new and amounts to a more convenient way to specify delta. The iterations argument is the same as the corresponding argument of loess and is equivalent to iter+1 where iter is the lowess argument.

Value

If `output.style="loess"`, then a list with the following components:

| | |
|-----------|---|
| fitted | numeric vector of smoothed y-values (in the same order as the input vectors). |
| residuals | numeric vector or residuals. |
| weights | numeric vector of robustifying weights used in the most recent iteration. |
| delta | the delta used, either the input value or the value derived from npts. |

If `output.style="lowess"`, then a list with the following components:

| | |
|-------|--|
| x | numeric vector of x-values in ascending order. |
| y | numeric vector or smoothed y-values. |
| delta | the delta used, either the input value or the value derived from npts. |

Author(s)

C code and R function by Aaron Lun.

References

- Cleveland, W.S. (1979). Robust Locally Weighted Regression and Smoothing Scatterplots. *Journal of the American Statistical Association* 74(368), 829-836.
- Cleveland, W.S. (1981). LOWESS: A program for smoothing scatterplots by robust locally weighted regression. *The American Statistician* 35(1), 54.
- Cleveland, W.S., and Devlin, S.J. (1988). Locally-weighted regression: an approach to regression analysis by local fitting. *Journal of the American Statistical Association* 83(403), 596-610.
- Cleveland, W.S., Grosse, E., and Shyu, W.M. (1992). Local regression models. Chapter 8 In: *Statistical Models in S* edited by J.M. Chambers and T.J. Hastie, Chapman & Hall/CRC, Boca Raton.
- Smyth, G.K. 2003. *lowess vs. loess*. Answer on the Bioconductor Support forum <https://support.bioconductor.org/p/2323/>.

See Also

[lowess](#), [loess](#), [loessFit](#), [tricubeMovingAverage](#).

Examples

```

y <- rt(100,df=4)
x <- runif(100)
w <- runif(100)
l <- weightedLowess(x, y, w, span=0.7, output.style="lowess")
plot(x, y, cex=w)
lines(l, col = "red")

```

write.fit

Write MArrayLM Object to a File

Description

Write a microarray linear model fit to a file.

Usage

```

write.fit(fit, results = NULL, file, digits = NULL,
         adjust = "none", method = "separate", F.adjust = "none",
         quote = FALSE, sep = "\t", row.names = TRUE, ...)

```

Arguments

| | |
|-----------|--|
| fit | object of class MArrayLM containing the results of a linear model fit. |
| results | object of class TestResults. |
| file | character string giving path name for the output file. |
| digits | integer indicating rounding precision for output values. If NULL, then no rounding is done. |
| adjust | character string specifying multiple-testing adjustment method for the t-statistic P-values, e.g., "BH". See p.adjust for the available options. If NULL or "none" then the P-values are not adjusted. |
| method | character string, should the P-value adjustment be "global" or "separate" for each contrast. Ignored if adjust = "none". |
| F.adjust | character string specifying adjustment method for the F-statistic P-values. |
| quote | logical value. If TRUE, any character or factor columns will be surrounded by double quotes. |
| sep | the field separator string. Values in the output file will be separated by this string. |
| row.names | logical value, whether to include row names in the output file. |
| ... | other arguments are passed to write.table. |

Details

This function writes a delimited text file containing for each gene (1) the average log₂-intensity (AveExpr), (2) the coefficients or contrasts (log₂-fold-changes, Coef), (3) moderated t-statistics, (4) t-statistic P-values, (5) F-statistic if available, (6) F-statistic P-values if available, (7) decideTests results if available and (8) gene names and annotation.

The results argument is optional. If supplied, it should be the output from decideTests for the same fit object, which indicates whether each contrast for each gene is considered statistically significant or not (coded 1 or -1 for positive or negative significant differences and 0 for non-significant values).

If fit contains row names and row.names=TRUE, then the row names will be the first column of the output file with a blank column heading. This behaviour is analogous to that of write.csv or to write.table with col.names=NA.

Value

No value is produced but a file is written to the current working directory.

Author(s)

Gordon Smyth

See Also

[write.table](#) or [write.csv](#) in the base library.

An overview of linear model functions in limma is given by [06.LinearModels](#).

Examples

```
## Not run:
# The following three alternatives are equivalent:
write.fit(fit, file = "temp.csv", sep = ",")
write.csv(fit, file = "temp.csv")
a <- as.data.frame(fit)
write.csv(fit, file = "temp.csv")

## End(Not run)
```

Description

Calculate surrogate variables from the singular vectors of the linear model residual space.

Usage

```
wsva(y, design, n.sv = 1L, weight.by.sd = FALSE, plot = FALSE, ...)
```

Arguments

| | |
|---------------------------|--|
| <code>y</code> | numeric matrix giving log-expression or log-ratio values for a series of microarrays, or any object that can be coerced to a matrix including <code>ExpressionSet</code> , <code>MAList</code> , <code>EList</code> or <code>PLMSet</code> objects. Rows correspond to genes and columns to samples. |
| <code>design</code> | design matrix |
| <code>n.sv</code> | number of surrogate variables required. |
| <code>weight.by.sd</code> | logical, should the surrogate variables be especially tuned to the more variable genes? |
| <code>plot</code> | logical. If <code>TRUE</code> , plots the proportion of variance explained by each surrogate variable. |
| <code>...</code> | other arguments can be included that would be suitable for <code>lmFit</code> . |

Details

The function constructs surrogate variables that explain a high proportion of the residual variability for many of the genes. The surrogate variables can be included in the design matrix to remove unwanted variation. The surrogate variables are constructed from the singular vectors of a representation of the linear model residual space.

If `weight.by.sd=FALSE`, then the method is a simplification of the approach by Leek and Storey (2007).

Value

Numeric matrix with `ncol(y)` rows and `n.sv` columns containing the surrogate variables.

Author(s)

Gordon Smyth and Yifang Hu

References

Leek, JT, Storey, JD (2007). Capturing heterogeneity in gene expression studies by surrogate variable analysis. *PLoS Genetics* 3, 1724-1735.

zscore

Transform non-normal random deviates to standard normal

Description

Compute z-score equivalents of non-normal random deviates.

Usage

```
zscore(q, distribution, ...)
zscoreGamma(q, shape, rate = 1, scale = 1/rate)
zscoreHyper(q, m, n, k)
```

Arguments

| | |
|--------------|--|
| q | numeric vector or matrix giving deviates of a random variable |
| distribution | character name of probability distribution for which a cumulative distribution function exists |
| ... | other arguments specify distributional parameters and are passed to the cumulative distribution function |
| shape | gamma shape parameter (>0) |
| rate | gamma rate parameter (>0) |
| scale | gamma scale parameter (>0) |
| m | as for qhyper |
| n | as for qhyper |
| k | as for qhyper |

Details

These functions compute the standard normal deviates which have the same quantiles as the given values in the specified distribution. For example, if `z <- zscoreGamma(x, shape, rate)` then `pnorm(z)` equals `pgamma(x, shape, rate)`.

`zscore` works for any distribution for which a cumulative distribution function (like `pnorm`) exists in R. The argument `distribution` is the name of the cumulative distribution function with the "p" removed.

`zscoreGamma` and `zscoreHyper` are specific functions for the gamma and hypergeometric distributions respectively.

The transformation to z-scores is done by converting to log tail probabilities, and then using `qnorm`. For numerical accuracy, the left or right tail is used, depending on which is likely to be smaller.

Value

Numeric vector or matrix of equivalent deviates from the standard normal distribution.

Author(s)

Gordon Smyth

See Also

[zscoreT](#).

`zscoreNBinom` in the `edgeR` package.

`qnorm` in the `stats` package.

Examples

```
# These are all equivalent
zscore(c(1,2.5), dist="gamma", shape=0.5, scale=2)
zscore(c(1,2.5), dist="chisq", df=1)
zscoreGamma(c(1,2.5), shape=0.5, scale=2)
```

zscoreT

*Transform t-statistics to standard normal***Description**

Compute z-score equivalents of t-distributed random deviates.

Usage

```
zscoreT(x, df, approx=FALSE, method = "bailey")
tZscore(z, df)
```

Arguments

| | |
|--------|--|
| x | numeric vector or matrix of values from a t-distribution. |
| df | degrees of freedom (>0) of the t-distribution. |
| approx | logical. If TRUE then a fast approximation is used otherwise exact z-scores are computed. |
| method | character string specifying transformation to be used when approx=TRUE, options being "bailey", "hill" or "wallace". |
| z | numeric vector or matrix of values from the standard normal distribution. |

Details

zscoreT transforms t-distributed values to standard normal. Each value is converted to the equivalent quantile of the normal distribution so that if $z <- \text{zscoreT}(x, \text{df}=\text{df})$ then $\text{pnorm}(z)$ equals $\text{pt}(x, \text{df}=\text{df})$.

tZscore is the inverse of zscoreT and computes t-distribution equivalents of standard normal deviates.

If approx=FALSE, the transformation is done by converting to log tail probabilities using pt or pnorm and then converting back to quantiles using qnorm or qt. For numerical accuracy, the smaller of the two tail probabilities is used for each deviate.

If approx=TRUE, then an approximate closed-form transformation is used to convert t-statistics to z-scores directly without computing tail probabilities. The method argument provides a choice of three transformations. method="bailey" is equation (5) of Bailey (1980) or equation (7) of Brophy (1987). method="hill" is from Hill (1970) as given by equation (5) of Brophy (1987). method="wallace" is from Wallace (1959) as given by equation (2) of Brophy (1987). Bailey's transformation is a modification of Wallace's approximation. The Hill approximation is generally the most accurate for $\text{df} > 2$ but is poor for $\text{df} < 1$. Bailey's approximation is faster than Hill's and gives acceptable two-figure accuracy throughout. Bailey's approximation also works for some extreme values, with very large x or df, for which Hill's approximation fails due to overflow.

Value

Numeric vector or matrix of z-scores or t-distribution deviates.

Note

The default approximation used when `approx=TRUE` was changed from Hill to Bailey in limma version 3.41.13.

Author(s)

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See Also

[zscore](#).

`zscoreNBinom` in the `edgeR` package.

Examples

```
zscoreT(4, df=3)
zscoreT(4, df=3, approx=TRUE)
zscoreT(4, df=Inf)
tZscore(2.2, df=3)
```

Index

- * **IO**
 - controlStatus, 54
 - getLayout, 87
 - getSpacing, 89
 - gridr, 98
 - printorder, 184
 - readGAL, 206
 - readHeader, 207
 - readSpotTypes, 209
 - readTargets, 210
 - write.fit, 264
- * **algebra**
 - is.fullrank, 107
- * **array**
 - as.data.frame, 24
 - as.matrix, 26
 - avedups, 30
 - avereps, 31
 - dim, 65
 - dimnames, 66
 - exprs.MA, 75
 - head, 98
 - uniquegenelist, 244
 - unwrapdups, 245
- * **background correction**
 - backgroundCorrect, 32
 - detectionPValues, 61
 - kooperberg, 109
 - ma3x3, 122
 - nec, 135
 - propexpr, 187
- * **character**
 - makeUnique, 124
 - protectMetachar, 191
 - removeExt, 213
 - strsplit2, 228
 - trimWhiteSpace, 244
- * **classes**
 - as.MAList, 25
 - EList-class, 73
 - LargeDataObject-class, 110
 - MAList-class, 125
 - MArrayLM-class, 126
 - PrintLayout, 183
 - RGList-class, 215
 - TestResults-class, 233
- * **data**
 - as.MAList, 25
 - LargeDataObject-class, 110
 - MAList-class, 125
 - PrintLayout, 183
 - RGList-class, 215
- * **distributions**
 - qqt, 192
 - zscore, 266
 - zscoreT, 268
- * **distribution**
 - fitGammaIntercept, 78
 - tmixture, 234
- * **documentation**
 - 01.Introduction, 6
 - 02.Classes, 7
 - 03.ReadingData, 8
 - 04.Background, 9
 - 05.Normalization, 10
 - 06.LinearModels, 11
 - 07.SingleChannel, 13
 - 08.Tests, 14
 - 09.Diagnostics, 15
 - 10.GeneSetTests, 16
 - 11.RNAseq, 17
 - changeLog, 47
 - limmaUsersGuide, 111
- * **empirical Bayes**
 - eBayes, 69
 - fitFDist, 75
 - predFCm, 181
 - squeezeVar, 226

- * **file**
 - readGAL, 206
 - readHeader, 207
 - readIdeaGeneHeader, 208
 - removeExt, 213
- * **gene annotation**
 - alias2Symbol, 18
- * **gene set enrichment analysis**
 - cumOverlap, 57
- * **gene set tests**
 - 10.GeneSetTests, 16
 - camera, 43
 - geneSetTest, 84
 - goana, 91
 - goanaTrend, 96
 - ids2indices, 102
 - roast, 216
 - romer, 221
 - topGO, 235
 - topRomer, 236
- * **hplot**
 - imageplot, 103
 - imageplot3by2, 104
 - modifyWeights, 133
 - plotExonJunc, 158
 - plotExons, 160
 - plotMA, 163
 - plotMA3by2, 165
 - plotPrintTipLoess, 172
 - plotRLDF, 173
 - plotSplice, 176
 - printHead, 182
 - venn, 246
- * **htest**
 - auROC, 28
 - classifyTestsF, 49
 - contrasts.fit, 52
 - decideTests, 58
 - propTrueNull, 189
 - rankSumTestWithCorrelation, 194
 - TestResults-class, 233
- * **illumina beadchips**
 - detectionPValues, 61
 - nec, 135
 - propexpr, 187
 - read.idat, 197
 - read.ilmn, 199
 - read.ilmn.targets, 201
- * **locally-weighted regression smoothing**
 - chooseLowessSpan, 48
 - loessFit, 117
 - normalizeCyclicLoess, 140
 - tricubeMovingAverage, 241
 - weightedLowess, 261
- * **manip**
 - cbind, 46
 - merge, 128
- * **mathematical functions**
 - logcosh, 120
 - trigammaInverse, 243
- * **methods**
 - helpMethods, 101
 - summary, 230
- * **microarray data file**
 - read.idat, 197
 - read.ilmn, 199
 - read.maimages, 202
 - readHeader, 207
- * **models**
 - anova.MAList-method, 20
 - arrayWeights, 21
 - bwss, 41
 - bwss.matrix, 42
 - dupcor, 67
 - fitted.MArrayLM, 80
 - gls.series, 90
 - lm.series, 112
 - lmFit, 113
 - lmvcFit, 116
 - mergeScans, 129
 - mrlm, 134
 - normalizeVSN, 147
 - normexp.fit, 150
 - normexp.fit.control, 152
 - normexp.fit.detection.p, 154
 - normexp.signal, 156
 - printtipWeights, 185
 - residuals.MArrayLM, 214
 - selectModel, 224
- * **multivariate**
 - intraspotCorrelation, 105
 - normalizeVSN, 147
- * **normalization**
 - normalizeBetweenArrays, 137
 - normalizeCyclicLoess, 140
 - normalizeForPrintorder, 141

- normalizeMedianAbsValues, 143
- normalizeQuantiles, 144
- normalizeRobustSpline, 145
- normalizeWithinArrays, 148
- * **plots**
 - heatdiagram, 99
 - mdplot, 127
 - plotDensities, 157
 - plotFB, 161
 - plotlines, 162
 - plotMD, 167
 - plotMDS, 169
 - plotSA, 175
 - volcanoplot, 248
- * **programming**
 - isNumeric, 108
- * **reading data**
 - QualityWeights, 193
 - read.columns, 196
 - read.idat, 197
 - read.ilmn, 199
 - read.ilmn.targets, 201
 - read.maimages, 202
 - sampleInfoFromGEO, 223
- * **regression**
 - arrayWeights, 21
 - contrastAsCoef, 51
 - dupcor, 67
 - fitted.MArrayLM, 80
 - gls.series, 90
 - lm.series, 112
 - lmFit, 113
 - lmscFit, 116
 - makeContrasts, 123
 - MArrayLM-class, 126
 - modelMatrix, 131
 - mrlm, 134
 - printtipWeights, 185
 - residuals.MArrayLM, 214
 - selectModel, 224
 - voomWithQualityWeights, 258
- * **rna-seq**
 - diffSplice, 63
 - plotExonJunc, 158
 - plotExons, 160
 - plotSplice, 176
 - topSplice, 237
 - voom, 249
 - voomWithQualityWeights, 258
- * **separate channel analysis**
 - designI2M, 60
 - lmscFit, 116
 - targetsA2C, 231
- * **univar**
 - weighted.median, 260
- [.EList (subsetting), 229
- [.EListRaw (subsetting), 229
- [.MAList (subsetting), 229
- [.MArrayLM (subsetting), 229
- [.RGList (subsetting), 229
- [.TestResults (subsetting), 229
- 01. Introduction, 6, 8–11, 13, 15–17, 47
- 02. Classes, 7, 7, 8–11, 13, 15–17, 24–26, 30–32, 65, 67, 74, 75, 87, 99, 111, 125, 127, 183, 215, 230, 231, 233
- 03. ReadingData, 7, 8, 8, 9–11, 13, 15–17, 23, 46, 55, 62, 88, 89, 129, 185, 191, 194, 197, 205, 207–211, 214, 224, 229, 244
- 04. Background, 7–9, 9, 10, 11, 13, 15–17, 34, 110, 122, 137, 152, 153, 155, 156
- 05. Normalization, 7–10, 10, 11, 13, 15–17, 119, 133, 137, 139, 141, 143–146, 148, 150, 212
- 06. LinearModels, 7–11, 11, 13–17, 22, 24, 27, 40, 52, 53, 68, 73, 91, 113, 115, 123, 132, 135, 163, 187, 225, 228, 241, 247, 249, 265
- 07. SingleChannel, 7–11, 13, 13, 14–17, 61, 106, 117, 232
- 08. Tests, 7–13, 14, 15–17, 51, 60, 190, 195, 233
- 09. Diagnostics, 7–11, 13, 15, 15, 16, 17, 20, 57, 104, 105, 128, 158, 162, 165, 166, 169, 171, 172, 176, 179
- 10. GeneSetTests, 7–11, 13, 15, 16, 16, 17, 19, 37, 45, 86, 95, 97, 102, 220, 223, 236, 237
- 11. RNAseq, 7–11, 13, 15–17, 17, 64, 160, 177, 238, 252, 259
- alias2Symbol, 14, 16, 18, 92, 93
- alias2SymbolTable, 14, 16
- alias2SymbolTable (alias2Symbol), 18
- alias2SymbolUsingNCBI, 14, 16
- alias2SymbolUsingNCBI (alias2Symbol), 18
- anova, 15, 20, 42

- anova.MAList, [42](#)
- anova.MAList (anova.MAList-method), [20](#)
- anova.MAList-method, [20](#)
- array2channel (targetsA2C), [231](#)
- arrayWeights, [15](#), [21](#), [23](#), [255](#), [259](#)
- arrayWeightsQuick, [22](#), [23](#)
- as.data.frame, [7](#), [24](#), [24](#)
- as.MAList, [8](#), [25](#)
- as.matrix, [26](#), [26](#)
- asMatrixWeights, [27](#)
- auROC, [14](#), [28](#)
- avearrays, [29](#), [32](#)
- avedups, [30](#), [32](#)
- avereps, [30](#), [31](#), [31](#)

- backgroundCorrect, [9](#), [32](#), [149](#), [157](#)
- backgroundCorrect.matrix, [9](#)
- barcodeplot, [14](#), [16](#), [34](#), [86](#), [242](#)
- beadCountWeights, [38](#)
- bg.parameters, [152](#)
- blockDiag, [40](#)
- bwss, [15](#), [41](#), [42](#)
- bwss.matrix, [15](#), [20](#), [41](#), [42](#)

- camera, [16](#), [37](#), [43](#), [85](#), [86](#), [102](#), [217](#), [223](#)
- cameraPR, [16](#), [85](#), [86](#)
- cameraPR (camera), [43](#)
- cbind, [9](#), [46](#), [46](#), [129](#)
- changeLog, [6](#), [47](#)
- chooseLowessSpan, [48](#)
- classifyTestsF, [14](#), [49](#), [59](#), [127](#)
- coerce, RGList, exprSet2-method (RGList-class), [215](#)
- combined, [7](#), [74](#), [125](#), [215](#)
- contrastAsCoef, [51](#)
- contrasts.fit, [12](#), [44](#), [52](#), [83](#), [123](#), [182](#), [217](#)
- controlStatus, [9](#), [54](#), [178](#)
- convest (propTrueNull), [189](#)
- coolmap, [55](#)
- cumOverlap, [57](#)

- decideTests, [14](#), [50](#), [58](#), [100](#), [127](#), [233](#), [246](#)
- density, [157](#), [158](#)
- designI2A (designI2M), [60](#)
- designI2M, [60](#)
- detectionPValues, [8](#), [61](#), [198](#)
- diffSplice, [17](#), [63](#), [159](#), [177](#), [238](#)
- dim, [65](#), [65](#), [74](#), [125](#), [127](#), [215](#)
- dimnames, [66](#), [66](#), [67](#)
- dimnames<- .EList (dimnames), [66](#)
- dimnames<- .EListRaw (dimnames), [66](#)
- dimnames<- .MAList (dimnames), [66](#)
- dimnames<- .RGList (dimnames), [66](#)
- dist, [57](#)
- dupcor, [67](#)
- duplicateCorrelation, [11](#), [91](#)
- duplicateCorrelation (dupcor), [67](#)

- eBayes, [12](#), [43](#), [49](#), [69](#), [78](#), [83](#), [126](#), [127](#), [160](#), [176](#), [182](#), [217](#), [218](#), [228](#), [235](#), [239](#), [251](#), [252](#)
- EList, [7](#), [46](#), [128](#), [251](#), [259](#)
- EList-class, [73](#)
- EListRaw, [7](#), [32](#), [46](#), [128](#), [138](#), [204](#)
- EListRaw-class (EList-class), [73](#)
- estimate.m0 (propTrueNull), [189](#)
- ExpressionSet, [74](#)
- exprs, [26](#)
- exprs.MA, [75](#)
- Extract, [230](#)

- filter, [242](#)
- fitFDist, [12](#), [73](#), [75](#), [228](#), [243](#)
- fitFDistRobustly, [12](#), [49](#), [228](#)
- fitFDistRobustly (fitFDist), [75](#)
- fitFDistUnequalDF1, [12](#), [71](#), [228](#)
- fitFDistUnequalDF1 (fitFDist), [75](#)
- fitGammaIntercept, [78](#)
- fitmixture, [79](#)
- fitted, [81](#)
- fitted.MArrayLM, [80](#)
- fry, [16](#), [45](#)
- fry (roast), [216](#)

- genas, [12](#), [14](#), [79](#), [81](#)
- geneSetTest, [14](#), [16](#), [37](#), [45](#), [84](#)
- getDupSpacing (getLayout), [87](#)
- getEAWP, [43](#), [45](#), [67](#), [86](#), [113](#), [115](#), [211](#)
- getGeneKEGGLinks (goana), [91](#)
- getKEGGPathwayNames (goana), [91](#)
- getLayout, [8](#), [87](#), [183](#)
- getLayout2 (getLayout), [87](#)
- getSpacing, [9](#), [89](#)
- gls.series, [11](#), [90](#)
- goana, [16](#), [91](#), [95](#), [97](#), [235](#), [236](#)
- goanaTrend, [94](#), [96](#)
- gridc, [8](#), [16](#)
- gridc (gridr), [98](#)

- gridr, [8](#), [16](#), [98](#)
- hclust, [57](#)
- head, [98](#), [99](#)
- heatDiagram, [14](#), [233](#)
- heatDiagram(heatdiagram), [99](#)
- heatdiagram, [14](#), [99](#)
- heatmap.2, [56](#), [57](#)
- helpMethods, [101](#)
- ids2indices, [16](#), [43](#), [45](#), [102](#), [217](#), [221](#), [223](#)
- image, [101](#), [104](#)
- imageplot, [15](#), [103](#)
- imageplot3by2, [15](#), [104](#)
- interGeneCorrelation(camera), [43](#)
- intraspotCorrelation, [13](#), [105](#), [116](#)
- is.fullrank, [107](#)
- is.numeric, [108](#)
- isNumeric, [108](#)
- kegga, [16](#), [97](#), [235](#), [236](#)
- kegga(goana), [91](#)
- kooperberg, [9](#), [34](#), [109](#)
- labels.TestResults(TestResults-class), [233](#)
- LargeDataObject, [7](#), [74](#), [125](#), [215](#)
- LargeDataObject-class, [110](#)
- legend, [157](#), [178](#)
- levels.TestResults(TestResults-class), [233](#)
- limma(01.Introduction), [6](#)
- LIMMA User's Guide, [10](#), [158](#)
- limma-package(01.Introduction), [6](#)
- limmaUsersGuide, [6](#), [111](#)
- lm.fit, [113](#), [117](#)
- lm.series, [11](#), [112](#)
- lmFit, [11](#), [44](#), [53](#), [83](#), [90](#), [107](#), [112](#), [113](#), [126](#), [134](#), [160](#), [182](#), [212](#), [217](#), [250](#), [252](#), [257](#), [259](#)
- lmscFit, [13](#), [114](#), [116](#)
- locfit.raw, [119](#)
- loess, [49](#), [119](#), [263](#)
- loessByCol, [242](#)
- loessFit, [10](#), [49](#), [117](#), [149](#), [262](#), [263](#)
- logcosh, [120](#), [121](#)
- logsumexp, [120](#), [121](#)
- lowess, [49](#), [119](#), [263](#)
- MA.RG, [10](#), [125](#)
- MA.RG(normalizeWithinArrays), [148](#)
- ma3x3, [122](#)
- ma3x3.matrix, [9](#)
- ma3x3.spottedarray, [9](#)
- make.names, [123](#)
- make.unique, [124](#)
- makeContrasts, [12](#), [123](#)
- makeUnique, [9](#), [124](#)
- MAList, [7](#), [25](#), [46](#), [106](#), [116](#), [128](#), [138](#), [139](#), [147](#), [149](#)
- MAList-class, [125](#)
- MArrayLM, [7](#), [11](#), [52](#), [53](#), [114](#), [116](#)
- MArrayLM-class, [126](#)
- Math, [108](#)
- matplot, [158](#)
- mdplot, [15](#), [127](#), [164](#), [168](#), [169](#), [179](#)
- MDS-class(plotMDS), [169](#)
- mean, [106](#)
- median, [260](#)
- merge, [9](#), [128](#), [129](#)
- merge.RGList, [124](#)
- merged, [215](#)
- mergeScans, [129](#)
- mergeScansRG(mergeScans), [129](#)
- mixedModel2Fit, [68](#)
- model.matrix, [12](#), [52](#), [61](#), [132](#)
- modelMatrix, [12](#), [40](#), [131](#)
- modifyWeights, [10](#), [27](#), [133](#)
- mr1m, [11](#), [134](#)
- mroast, [16](#), [102](#)
- mroast(roast), [216](#)
- ncol, [74](#), [125](#), [127](#), [215](#)
- nec, [135](#), [153](#), [155](#)
- neqc, [9](#), [10](#), [34](#), [40](#), [62](#), [137](#), [139](#), [198](#), [200](#)
- neqc(nec), [135](#)
- news, [47](#)
- nlminb, [151](#)
- nonEstimable(is.fullrank), [107](#)
- normalize.loess, [141](#)
- normalizeBetweenArrays, [10](#), [73](#), [125](#), [137](#), [144](#), [150](#), [215](#)
- normalizeCyclicLoess, [10](#), [138](#), [140](#)
- normalizeForPrintorder, [10](#), [125](#), [141](#), [185](#), [215](#)
- normalizeMedianAbsValues, [10](#), [143](#)
- normalizeMedianValues, [10](#)
- normalizeMedianValues(normalizeMedianAbsValues), [143](#)

- normalizeQuantiles, [10](#), [144](#)
- normalizeRobustSpline, [10](#), [145](#), [149](#)
- normalizeVSN, [10](#), [139](#), [147](#)
- normalizeWithinArrays, [10](#), [33](#), [125](#), [146](#), [148](#), [215](#)
- normexp.fit, [9](#), [33](#), [137](#), [150](#), [153](#), [155](#), [156](#)
- normexp.fit.control, [9](#), [136](#), [137](#), [152](#), [152](#), [154](#), [155](#), [200](#)
- normexp.fit.detection.p, [136](#), [137](#), [153](#), [154](#)
- normexp.signal, [9](#), [136](#), [152](#), [153](#), [155](#), [156](#)
- nrow, [74](#), [125](#), [127](#), [215](#)
- openPDF, [111](#)
- openVignette, [111](#)
- p.adjust, [59](#), [217](#), [239–241](#), [264](#)
- par, [104](#)
- plotDensities, [15](#), [16](#), [157](#)
- plotExonJunc, [158](#)
- plotExons, [17](#), [160](#)
- plotFB, [15](#), [161](#)
- plotlines, [12](#), [162](#)
- plotMA, [15](#), [125](#), [163](#), [166](#), [179](#)
- plotMA3by2, [15](#), [165](#)
- plotMD, [15](#), [128](#), [164](#), [167](#), [179](#)
- plotMDS, [16](#), [169](#)
- plotPrintorder, [15](#)
- plotPrintorder
(normalizeForPrintorder), [141](#)
- plotPrintTipLoess, [15](#), [125](#), [172](#)
- plotRLDF, [173](#)
- plotSA, [16](#), [71](#), [73](#), [175](#)
- plotSplice, [17](#), [64](#), [160](#), [176](#), [238](#)
- plotWithHighlights, [15](#), [127](#), [128](#), [164](#), [165](#), [168](#), [169](#), [177](#)
- points, [161](#), [170](#), [173](#), [176](#), [178](#)
- poolVar, [179](#)
- predFCm, [181](#)
- printHead, [7](#), [182](#)
- PrintLayout, [125](#), [183](#), [205](#), [215](#)
- PrintLayout-class (PrintLayout), [183](#)
- printorder, [8](#), [142](#), [143](#), [184](#)
- prnttipWeights, [185](#)
- prnttipWeightsSimple
(prnttipWeights), [185](#)
- propexpr, [187](#), [198](#), [200](#)
- propTrueNull, [14](#), [181](#), [189](#)
- protectMetachar, [191](#)
- qhyper, [267](#)
- qnorm, [267](#)
- qqf (qqt), [192](#)
- qqnorm, [193](#)
- qqt, [192](#)
- QualityWeights, [8](#), [193](#), [204](#)
- rankSumTestWithCorrelation, [45](#), [194](#)
- rbind, [9](#)
- rbind.EList (cbind), [46](#)
- rbind.EListRaw (cbind), [46](#)
- rbind.MAList (cbind), [46](#)
- rbind.RGList (cbind), [46](#)
- read.columns, [8](#), [196](#), [199](#), [205](#)
- read.idat, [8](#), [40](#), [62](#), [197](#)
- read.ilmn, [8](#), [40](#), [136](#), [188](#), [197](#), [198](#), [199](#), [201](#), [203](#), [205](#)
- read.ilmn.targets, [200](#), [201](#)
- read.imagene, [8](#), [209](#)
- read.imagene (read.maimages), [202](#)
- read.maimages, [8](#), [73](#), [196](#), [197](#), [202](#), [208](#), [215](#)
- read.table, [197](#), [205](#), [210](#)
- readGAL, [8](#), [54](#), [206](#)
- readGenericHeader, [8](#)
- readGenericHeader (readHeader), [207](#)
- readGPRHeader, [8](#)
- readGPRHeader (readHeader), [207](#)
- readHeader, [207](#)
- readImaGeneHeader, [8](#), [208](#)
- readSMDHeader (readHeader), [207](#)
- readSpotTypes, [9](#), [209](#)
- readTargets, [8](#), [200](#), [201](#), [210](#), [232](#)
- remlscore, [106](#)
- removeBatchEffect, [10](#), [211](#)
- removeExt, [8](#), [213](#)
- residuals, [214](#)
- residuals.MArrayLM, [214](#)
- RG.MA, [16](#)
- RG.MA (normalizeWithinArrays), [148](#)
- RGList, [7](#), [32](#), [46](#), [128](#), [138](#), [147](#), [204](#)
- RGList-class, [215](#)
- rlm, [135](#)
- roast, [14](#), [16](#), [37](#), [44](#), [45](#), [84](#), [86](#), [216](#), [223](#)
- Roast-class (roast), [216](#)
- roast.default (roast), [216](#)
- romer, [14](#), [16](#), [37](#), [45](#), [102](#), [217](#), [221](#), [236](#), [237](#)
- rowsum, [32](#)
- sampleInfoFromGEO, [223](#)

- selectModel, [12](#), [14](#), [224](#)
- show, [74](#), [125](#), [215](#), [233](#)
- show, LargeDataObject-method
(LargeDataObject-class), [110](#)
- show, MDS-method (plotMDS), [169](#)
- show, Roast-method (roast), [216](#)
- show, TestResults-method
(TestResults-class), [233](#)
- showMethods, [101](#)
- spotc, [8](#)
- spotc (gridr), [98](#)
- spotr, [8](#)
- spotr (gridr), [98](#)
- squeezeVar, [12](#), [49](#), [71](#), [73](#), [78](#), [225](#), [226](#)
- strsplit, [228](#), [229](#)
- strsplit2, [9](#), [228](#)
- subsetListOfArrays (subsetting), [229](#)
- subsetting, [7](#), [74](#), [125](#), [215](#)
- subsetting, [107](#), [229](#)
- summary, [230](#), [231](#)
- summary.TestResults
(TestResults-class), [233](#)
- Sweave, [111](#)
- Sys.getenv, [111](#)
- Sys.putenv, [111](#)

- tail.EList (head), [98](#)
- tail.EListRaw (head), [98](#)
- tail.MAList (head), [98](#)
- tail.MArrayLM (head), [98](#)
- tail.RGList (head), [98](#)
- tail.TestResults (head), [98](#)
- targetsA2C, [13](#), [231](#)
- TestResults, [7](#), [14](#), [50](#), [60](#)
- TestResults-class, [233](#)
- text, [171](#)
- tmixture, [234](#)
- tmixture.matrix, [12](#), [73](#)
- tmixture.vector, [12](#)
- topGO, [16](#), [95](#), [235](#)
- topKEGG, [16](#), [95](#)
- topKEGG (topGO), [235](#)
- topRomer, [14](#), [16](#), [223](#), [236](#)
- topSplice, [17](#), [64](#), [159](#), [177](#), [237](#)
- topTable, [12](#), [238](#)
- topTableF, [12](#)
- topTableF (topTable), [238](#)
- topTreat, [70](#)
- topTreat (topTable), [238](#)

- treat, [12](#), [78](#), [176](#), [239](#), [251](#)
- treat (eBayes), [69](#)
- tricubeMovingAverage, [37](#), [94](#), [97](#), [241](#), [263](#)
- trigamma, [243](#)
- trigammaInverse, [78](#), [243](#)
- trimWhiteSpace, [244](#)
- tZscore (zscoreT), [268](#)

- unique.TestResults (TestResults-class),
[233](#)
- uniquegenelist, [9](#), [244](#)
- uniqueTargets (modelMatrix), [131](#)
- unwrapdups, [11](#), [245](#), [245](#)

- venn, [246](#)
- vennCounts, [14](#), [233](#)
- vennCounts (venn), [246](#)
- vennDiagram, [14](#), [233](#)
- vennDiagram (venn), [246](#)
- vignette, [111](#)
- volcanoplot, [12](#), [248](#)
- voom, [17](#), [71](#), [73](#), [249](#), [255](#), [259](#)
- vooma, [49](#), [252](#), [252](#), [257](#)
- voomaByGroup (vooma), [252](#)
- voomaLmFit, [255](#), [255](#)
- voomWithQualityWeights, [17](#), [22](#), [252](#), [258](#)
- vsnMatrix, [148](#)

- weighted.mean, [260](#)
- weighted.median, [260](#)
- weightedLowess, [49](#), [118](#), [119](#), [261](#), [262](#)
- wilcox.test, [86](#), [195](#)
- wilcoxGST, [14](#), [16](#), [223](#)
- wilcoxGST (geneSetTest), [84](#)
- write, [240](#)
- write.csv, [265](#)
- write.fit, [12](#), [14](#), [233](#), [240](#), [264](#)
- write.table, [265](#)
- wsva, [265](#)
- wtarea (QualityWeights), [193](#)
- wtfFlags (QualityWeights), [193](#)
- wtIgnore.Filter (QualityWeights), [193](#)

- zscore, [266](#), [269](#)
- zscoreGamma (zscore), [266](#)
- zscoreHyper (zscore), [266](#)
- zscoreT, [16](#), [219](#), [267](#), [268](#)