Package ‘DGEobj.utils’

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Type Package

Title Differential Gene Expression (DGE) Analysis Utility Toolkit

Version 1.0.4

Description Provides a function toolkit to facilitate reproducible RNA-Seq Differential Gene Expression (DGE) analysis (Law (2015) <doi:10.12688/f1000research.9005.3>). The tools include both analysis work-flow and utility functions: mapping/unit conversion, count normalization, accounting for unknown covariates, and more. This is a complement/cohort to the 'DGEobj' package that provides a flexible container to manage and annotate Differential Gene Expression analysis results.

Depends R (>= 3.5.0)

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Description

This package implements a set of utility functions to enable a limma/voom workflow capturing the results in DGEobj data structure. Aside from implementing a well developed and popular workflow in DGEobj format, the run* functions in the package illustrate how to wrap the individual processing steps in a workflow in functions that capture important metadata, processing parameters, and intermediate data items in the DGEobj data structure. This function-based approach to utilizing the DGEobj data structure insures consistency among a collection of projects processed by these methods and thus facilitates downstream automated meta-analysis.

More Information

browseVignettes(package = 'DGEobj.utils')
Convert count matrix to CPM, FPKM, FPK, or TPM

Description

Takes a count matrix as input and converts to other desired units. Supported units include CPM, FPKM, FPK, and TPM. Output units can be logged and/or normalized. Calculations are performed using edgeR functions except for the conversion to TPM which is converted from FPKM using the formula provided by Harold Pimental.

Usage

```r
convertCounts(
  countsMatrix, 
  unit, 
  geneLength, 
  log = FALSE, 
  normalize = "none", 
  prior.count = NULL 
)
```

Arguments

- **countsMatrix**: A numeric matrix or dataframe of N genes x M Samples. All columns must be numeric.
- **unit**: Required. One of CPM, FPKM, FPK or TPM.
- **geneLength**: A vector or matrix of gene lengths. Required for length-normalized units (TPM, FPKM or FPK). If geneLength is a matrix, the rowMeans are calculated and used.
- **log**: Default = FALSE. Set TRUE to return Log2 values. Employs edgeR functions which use an prior.count of 0.25 scaled by the library size.
- **normalize**: Default = "none". Invokes edgeR::calcNormFactors() for normalization. Other options are: "TMM", "RLE", "upperquartile" (uses 75th percentile), "TMMwzp" and are case-insensitive.
- **prior.count**: Average count to be added to each observation to avoid taking log of zero. Used only if log = TRUE. (Default dependent on method; 0 for TPM, 0.25 for CPM and FPKM) The prior.count is passed to edgeR cpm and rpkm functions and applies to logTPM, logCPM, and logFPKM calculations.

Details

geneLength is a vector where length(geneLength) == nrow(countsMatrix). If a RSEM effective-Length matrix is passed as input, rowMeans(effectiveLength) is used (because edgeR functions only accept a vector for effectiveLength).

Note that log2 values for CPM, TPM, and FPKM employ edgeR’s prior.count handling to avoid divide by zero.
Value

A matrix in the new unit space

Examples

# Simulate some data
counts <- trunc(matrix(runif(6000, min=0, max=2000), ncol=6))
geneLength <- rowMeans(counts)

# TMM normalized Log2FPKM
Log2FPKM <- convertCounts(counts,
    unit = "fpkm",
    geneLength = geneLength,
    log = TRUE,
    normalize = "tmm")

# Non-normalized CPM (not logged)
RawCPM <- convertCounts(counts,
    unit = "CPM",
    log = FALSE,
    normalize = "none")

extractCol

Extract a named column from a series of df or matrices

Description

Take a named list of dataframes where each dataframe has the same column names (e.g. a list of topTable dataframes), then extract the named column from each dataframe and return a matrix. The name of each dataframe is used as the column name in the resulting table.

Usage

extractCol(contrastList, colName, robust = TRUE)

Arguments

contrastList  A list of data.frames which all have the same colnames and same row counts. The dataframes in the list should have geneIDs as rownames.

colName  The name of the data column to extract into a matrix.

robust  Default = TRUE; TRUE forces use of a joins to merge columns which is more reliable and allows for combination of contrasts from different projects, but may not return items in the same row order as the source table. Setting to FALSE invokes a cbind() approach that requires all data.frames to have the same row count and row order but preserves the original row order.
Details

The common use case for this is to provide a list of `topTable` data frames and extract one column from each file to create a matrix of LogRatios or P-values (genes x contrasts).

This should work as long as the requested column name is present in every dataframe. The default robust = TRUE should be used unless it has been verified that each dataframe in the input list has the same row count and row order.

Value

A dataframe containing the extracted columns

Examples

```r
dgeObj <- readRDS(system.file("exampleObj.RDS", package = "DGEobj"))
TopTableList <- DGEobj::getType(dgeObj, type = "topTable")
MyPvalues <- extractCol(TopTableList, colName = "P.Value")
head(MyPvalues)
```

lowIntFilter

Apply low intensity filters to a DGEobj

Description

Takes a DGEobj as input and applies a combination of low intensity filters as specified by the user. Raw count, zFPKM, TPM, and/or FPK filters are supported. A gene must pass all active filters. Not setting a threshold argument inactivates that threshold.

Usage

```r
lowIntFilter(
  dgeObj,
  countThreshold,
  zfpkmThreshold,
  fpkThreshold,
  tpmThreshold,
  sampleFraction = 0.5,
  geneLength,
  verbose = FALSE
)
```

Arguments

dgeObj A DGEobj with RNA-Seq (counts) data (Required)
countThreshold Genes below this threshold are removed (10 is recommended).
zfpkmThreshold Genes below this threshold are removed. (-3.0 is recommended)
rsqCalc

Calculate R-squared for each gene fit

Description
Takes a Log2CPM numeric matrix and MArrayLM fit object from limma::lmFit and calculates R-squared for each gene fit.

Usage
rsqCalc(normMatrix, fit)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>normMatrix</td>
<td>A normalized log2cpm matrix.</td>
</tr>
<tr>
<td>fit</td>
<td>A MArrayLM object from limma::lmFit.</td>
</tr>
</tbody>
</table>

Examples

```r
myDGEobj <- readRDS(system.file("exampleObj.RDS", package = "DGEobj"))
dim(myDGEobj)

# Simple count threshold in at least 3/4ths the samples
myDGEobj <- lowIntFilter(myDGEobj,
  countThreshold = 10,
  sampleFraction = 0.5)
dim(myDGEobj)

# Count and FPK thresholds
myDGEobj <- lowIntFilter(myDGEobj,
  countThreshold = 10,
  fpkThreshold = 5,
  sampleFraction = 0.5)
dim(myDGEobj)
```
runContrasts

Value

A vector of R-squared values for each gene fit.

Examples

```r
dgeObj <- readRDS(system.file("exampleObject.RDS", package = "DGEobj"))
log2cpm <- convertCounts(dgeObj$counts, unit = "cpm", log = TRUE, normalize = "tmm")
fitObject <- dgeObj$ReplicateGroupDesign_fit
rsq <- rsqCalc (log2cpm, fitObject)
```

runContrasts

Build contrast matrix and calculate contrast fits

Description

Takes a DGEobj and a named list of contrasts to build. The DGEobj must contain a limma Fit object and associated designMatrix. Returns the DGEobj with contrast fit(s), contrast matrix, and topTable/topTreat dataframes added.

Usage

```r
runContrasts(
  dgeObj,
  designMatrixName,
  contrastList,
  contrastSetName = NULL,
  runTopTable = TRUE,
  runTopTreat = FALSE,
  foldChangeThreshold = 1.5,
  runEBayes = TRUE,
  robust = TRUE,
  proportion = 0.01,
  qValue = FALSE,
  IHW = FALSE,
  verbose = FALSE
)
```

Arguments

dgeObj A DGEobj object containing a Fit object and design matrix. (Required)
designMatrixName The name of the design matrix within dgeObj to use for contrast analysis. (Required)
contrastList A named list of contrasts. (Required)
The contrastSet is a named list. The values are composed of column names from the designMatrix of the DGEobj. Each contrast is named to give it a short, recognizable name to be used for display purposes.

Example contrastList

```r
contrastList = list(
  T1 = "treatment1 - control",
  T2 = "treatment2 - control"
)
```

where treatment1, treatment2, and control are column names in the designMatrix.

The returned DGEobj list contains the new items:

- "contrastMatrix" a matrix
- "Fit.Contrasts" a Fit object
- "topTableList" a List of dataframes
- "topTreatList" a List of dataframes: if enabled

Value

The DGEobj with contrast matrix, fit and topTable/topTreat dataframes added.
runEdgeRNorm

Run edgeR normalization on DGEobj

Description

Takes a DGEobj and adds a normalized DGEList object representing the result of edgeR normalization (calcNormFactors).

Usage

runEdgeRNorm(
  dgeObj,
  normMethod = "TMM",
  itemName = "DGEList",
  includePlot = FALSE,
  plotLabels = NULL
)

Arguments

dgeObj A DGEobj containing counts, design data, and gene annotation.
normMethod One of "TMM", "RLE", "upperquartile", or "none". (Default = "TMM")
runIHW

**itemName**

optional string represents the name of the new DGEList. It must be unique and not exist in the passed DGEobj (Default = "DGEList")

**includePlot**

Enable returning a "canvasXpress" or "ggplot" bar plot of the norm.factors produced (Default = FALSE). Possible values to pass:

- **FALSE or NULL**: Disable plot
- **TRUE or "canvasXpress"**: returns "canvasXpress" bar plot of the norm.factors produced.
- **"ggplot"**: returns "ggplot" bar plot of the norm.factors produced.

**plotLabels**

Sample text labels for the plot. Length must equal the number of samples. (Default = NULL; sample number will be displayed)

**Value**

A DGEobj with a normalized DGEList added or a list containing the normalized DGEobj and a plot

**Examples**

```r
myDGEobj <- readRDS(system.file("exampleObj.RDS", package = "DGEobj"))
myDGEobj <- DGEobj::resetDGEobj(myDGEobj)

# Default TMM normalization
myDGEobj <- runEdgeRNorm(myDGEobj)

# With some options and plot output
require(canvasXpress)
myDGEobj <- DGEobj::resetDGEobj(myDGEobj)
obj_plus_plot <- runEdgeRNorm(myDGEobj,
    normMethod = "upperquartile",
    includePlot = TRUE)
myDGEobj <- obj_plus_plot[[1]]
obj_plus_plot[[2]]
```

---

runIHW  

Apply *Independent Hypothesis Weighting (IHW)* to a list of *topTable* dataframes

**Description**

This is a wrapper around the independent hypothesis weighting package that takes a list of *topTable* data frames and applies *Independent Hypothesis Weighting (IHW)* to each *topTable* data frame in the list.

**Usage**

```r
runIHW(contrastList, alpha = 0.1, FDRthreshold = 0.1, ...)
```
runIHW

Arguments

contrastList    A named list of topTable dataframes.
alpha           Alpha should be the desired FDR level to interrogate (range 0-1; Default = 0.1)
FDRthreshold    Threshold value for the p-values of a dataframe (Default = 0.1)
...             other arguments are passed directly to the ihw function (see ?ihw)

Details

IHW is a method developed by N. Ignatiadis (http://dx.doi.org/10.1101/034330) to weight FDR values based on a covariate (AveExpr in this case).

The IHW FDR values are added as additional columns to the topTable data frames.

Function runIHW is normally called by runContrasts with argument IHW=T. It can also be used independently on a list of topTable dataframes. A list of topTable dataframes is conveniently retrieved with the DGEobj::getType function with the type argument set to "topTable".

This function expects the following columns are present in each data frame: P.value, adj.P.Val, AveExpr.

Note that it is impractical to run IHW on a list of genes less than ~5000. Operationally, IHW breaks the data into bins of 1500 genes for the analysis. If bins = 1, IHW converges on the BH FDR value. Instead, run IHW on the complete set of detected genes from topTable (not topTreat) results.

Value

A list of lists. The first element is the original contrastList with additional IHW columns added to each dataframe. The topTable dataframes will contain additional columns added by the IHW analysis and prefixed with "ihw." The second list element is the IHW result dataframe.

Examples

```r
if (requireNamespace("IHW", quietly = TRUE)) {
  dgeObj <- readRDS(system.file("exampleObj.RDS", package = "DGEobj"))
  contrastList <- DGEobj::getType(dgeObj, type = "topTable")
  contrastList <- lapply(contrastList, dplyr::select,
                          -ihw.adj_pvalue,
                          -ihw.weight,
                          -ihw.weighted_pvalue)
  colnames(contrastList[[1]])
  contrastList <- runIHW(contrastList)
  # note new columns added
  colnames(contrastList[[1]]["contrasts"])
}
```
runPower  

Run a power analysis on counts and design matrix

Description

Take a counts matrix and design matrix and return a power analysis using the RNASeqPower package. The counts matrix should be pre-filtered to remove non-expressed genes using an appropriate filtering criteria. The design matrix should describe the major sources of variation so the procedure can dial out those known effects for the power calculations.

Usage

runPower(
  countsMatrix,  
  designMatrix,  
  depth = c(10, 100, 1000),  
  N = c(3, 6, 10, 20),  
  FDR = c(0.05, 0.1),  
  effectSize = c(1.2, 1.5, 2),  
  includePlots = FALSE
)

Arguments

countsMatrix  A counts matrix or dataframe of numeric data. (Required)
designMatrix  A design matrix or dataframe of numeric data. (Required)
depth  A set of depth to use in the calculations. The default depths of c(10, 100, 1000) respectively represent a detection limit, below average expression, and median expression levels, expressed in read count units.

N  A set of N value to report power for. (Default = c(3, 6, 10, 20))

FDR  FDR thresholds to filter for for FDR vs. Power graph. (Default = c(0.05, 0.1))
effectSize  A set of fold change values to test. (Default = c(1.2, 1.5, 2))

includePlots  controls adding tow plots to the returned dataframe (Default = FALSE). The two plots are; a ROC curve (FDR vs. Power) and a plot of N vs. Power. Possible values to pass:

  • FALSE or NULL: Disable plots  
  • TRUE or "canvasXpress": returns "canvasXpress" plots.  
  • "ggplot": returns "ggplot" plots.

Details

Note, both ‘RNASeqPower’ and ‘statmod’ packages are required to run this function as follow:

  • ‘RNASeqPower’ package is required to run power analysis on the given counts matrix and design matrix.
• 'statmod' package is required to run estimate dispersion calculations

If includePlots = FALSE (the default) or NULL, the function will return a tall skinny dataframe of power calculations for various requested combinations of N and significance thresholds.
If includePlots = TRUE, "canvasXpress" or "ggplot", a list is returned with an additional two "canvasXpress" or ggplots (plots) to the dataframe.

Value

a dataframe of power calculations or a list of the dataframe and defined plots as defined by the "includePlots" argument.

Examples

if (requireNamespace("RNASeqPower", quietly = TRUE) & &
requireNamespace("statmod", quietly = TRUE)) {

dgeObj <- readRDS(system.file("exampleObj.RDS", package = "DGEobj"))
counts <- dgeObj$counts
dm <- DGEobj::getType(dgeObj, type = "designMatrix")[[1]]

resultList <- runPower(countsMatrix = counts,
designMatrix = dm,
includePlots = TRUE)

head(resultList[[1]]) # dataframe
resultList[[2]] # ROC Curves Plot
resultList[[3]] # N vs Power Plot
}

runQvalue

Calculate and add q-value and lFDR to dataframe

Description

Takes an list of contrasts (e.g. topTable output or other dataframes that contain a p-value column). Adds a q-value and local FDR (lFDR) column to each dataframe.

Usage

runQvalue(contrastList, pvalField = "P.Value", ...)

Arguments

contrastList A list of dataframes with a p-value column (all tables must use the same colname for the p-value column.)
pvalField Define the colname of the p-value field in each dataframe. Not needed if using topTable output. (Optional. Default = "P.Value")
... Optional arguments passed to the qvalue function (See ?qvalue)
Details

The qvalue package from John Storey at Princeton takes a list of p-values and calculates a q-value and a Local FDR (lFDR). The q-value is essentially a less conservative FDR estimate compared to the default Benjamini-Hochberg FDR produced by topTable analysis (i.e. will give more differential genes at the same nominal cutoff). The q-value function also produces a Local FDR (lFDR) column which answers a slightly different and possibly more relevant question. The BH FDR (adj.P.Val in topTable data.frames) and q-value gives the false discovery rate is for a list of genes at a given threshold. The local FDR attempts to answer the question: what is the probability that this particular gene is a false discovery? See doi: 10.1007/9783642048982_248 for a brief introduction to FDRs and q-values.

Value

The input contrastList now containing q-value and lFDR columns in each dataframe.

Examples

dgeObj <- readRDS(system.file("exampleObj.RDS", package = "DGEobj"))
contrastList <- DGEobj::getType(dgeObj, type = "topTable")
contrastList <- lapply(contrastList, dplyr::select,
                        -Qvalue,
- -qvalue.lfdr)

colnames(contrastList[[1]])

contrastList <- runQvalue(contrastList)
# note new columns added

colnames(contrastList[[1]])

---

runSVA

Test for surrogate variables

Description

Takes a DGEobj from runVoom and tests for surrogate variables. Adds a new design matrix to the DGEobj with the surrogate variable columns appended using cbind. runVoom should then be run again with the new design matrix to complete the analysis.

Usage

runSVA(dgeObj, designMatrixName, n.sv, method = "leek")

Arguments

dgeObj A DGEobj with normalized counts and a designMatrix.
designMatrixName The itemName of the design matrix in DGEobj.
runVoom

n.sv Optional; Use to override the default n.sv returned by num.sv for the number of SV to analyze.

method Method passed to num.sv. Supports "leek" or "be". (Default = "leek")

Value
dgeObj containing an updated design table, the svobj and a new design matrix.

Examples
dgeObj <- readRDS(system.file("exampleObj.RDS", package = "DGEobj"))

### Create a model based on surgery status, intentionally omitting the compound treatments
dgeObj$design$SurgeryStatus <- "BDL"
dgeObj$design$SurgeryStatus[geObj$design$ReplicateGroup == "Sham"] <- "Sham"

formula <- ~ 0 + SurgeryStatus
designMatrix <- model.matrix(as.formula(formula), dgeObj$design)

# Make sure the column names in the design matrix are legal
colnames(designMatrix) <- make.names(colnames(designMatrix))

# capture the formula as an attribute of the design matrix
attr(designMatrix, "formula") <- formula

# add the designMatrix to the DGEobj
dgeObj <- DGEobj::addItem(dgeObj, designMatrix)

dgeObj <- runSVA(dgeObj, designMatrixName = "SurgeryStatusDesign")

runVoom

Run functions in a typical voom/lmFit workflow

Description

In the default workflow, this function runs voomWithQualityWeights followed by lmFit and optionally eBayes. If the contrasts of interest are already represented in the model, enable eBayes. To use contrasts.fit downstream, run eBayes after that step instead. eBayes should always be run last.

Usage

runVoom(
  dgeObj,
  designMatrixName,
runVoom

dupCorBlock, runDupCorTwice = TRUE, qualityWeights = TRUE, var.design, mvPlot = TRUE, runEBayes = TRUE, robust = TRUE, proportion = 0.01
)

Arguments

dgeObj A DGEobj containing a DGEList (e.g. from runEdgeRNorm.) (Required)
designMatrixName Name of a design matrix within dgeObj. (Required)
dupCorBlock Supply a block argument to trigger duplicateCorrelation. (Optional) Should be a vector the same length as ncol with values to indicate common group membership for duplicateCorrelation. Also, 'statmod' package must be installed to run duplicate correlation calculations.
runDupCorTwice Default = TRUE. Gordon Smyth recommends running duplicateCorrelation twice. Set this to false to run duplicateCorrelation just once.
qualityWeights Runs limma::voomWithQualityWeights if set to TRUE (Default = TRUE). This should normally be set to TRUE.
var.design Provide a design matrix (from model.matrix) to identify replicate groups (e.g. "~ ReplicateGroup") for quality weight determination. Causes quality weights to be determined on a group basis. If omitted limma::voomWithQualityWeights treats each sample individually.
mvPlot Enables the voom mean-variance plot. (Default = TRUE)
runEBayes Runs eBayes after lmFit. (Default = TRUE) Note, 'statmod' package must be installed to run eBayes calculations.
robust Used by eBayes. (Default = TRUE) Note, 'statmod' package must be installed to run eBayes calculations.
proportion Proportion of genes expected to be differentially expressed (used by eBayes) (Default = 0.01) Modify the prior accordingly if the 1st pass analysis shows a significantly higher or lower proportion of genes regulated than the default.

Details

Input is minimally a DGEobj containing a DGEList (typically TMM-normalized), and a formula (character representation). Other arguments can invoke the duplicateCorrelation method and modify use of quality weights.

Returns a DGEobj class object containing the VoomElist (voom output), and Fit object (lmFit output).

Quality weights should be enabled unless there is a good reason to turn them off. If all samples are equal quality, the weights will all approach 1.0 with no consequence on the results. More typically, some samples are better than others and using quality weights improves the overall result.
summarizeSigCounts

Use `var.design` if the quality weights are correlated with some factor in the experiment. This will cause the quality weights to be calculated as a group instead of individually.

Use `duplicate correlation (dupCorBlock)` when there are subjects that have been sampled more than once (e.g. before and after some treatment). This calculates a within-subject correlation and includes this in the model.

Value

A DGEobj now containing designMatrix, Elist, and fit object.

Examples

```r
dgeObj <- readRDS(system.file("exampleObj.RDS", package = "DGEobj"))
for (name in names(dgeObj)[11:length(dgeObj)]) {
  dgeObj <- DGEobj::rmItem(dgeObj, name)
}

dgeObj <- runVoom(dgeObj,
  designMatrixName = "ReplicateGroupDesign",
  mvPlot = TRUE)

# Note the Elist and fit objects have been added
DGEobj::inventory(dgeObj)
```

summarizeSigCounts  Summarize a contrast list

Description

Takes a contrast list produced by `runContrasts`. Defaults are provided to specify columns to summarize and thresholds for each column, though they can be adjusted. A fold change threshold can optionally be specified. The function queries the `topTable` results and returns a dataframe with the summary results, but only includes gene counts that meet the specified conditions.

Usage

```r
summarizeSigCounts(
  contrastList,
  columns = c("P.Value", "adj.P.Val", "Qvalue", "qvalue.lfdr", "ihw.adj_pvalue"),
  sigThresholds = c(0.01, 0.05, 0.05, 0.05, 0.05),
  fcThreshold = 0
)
```
Arguments

- **contrastList**: A list of topTable dataframes.
- **columns**: Vector of column names to summarize from topTable dataframes. Default = c("P.Value", "adj.P.Val", "Qvalue", "qvalue.lfdr", "ihw.adj_pvalue")
- **sigThresholds**: Thresholds to use for each column specified in columns. Must be same length at columns argument. Default = c(0.01, 0.05, 0.05, 0.05, 0.05)
- **fcThreshold**: Fold-change threshold (absolute value, not logged.)

Details

Any specified column names that don’t exist will be ignored. Normally the defaults cover all the p-value and FDR related columns. However, a fcThreshold can be added and the p-value/FDR thresholds can be modified using the fcThreshold and sigThresholds arguments, respectively.

Value

data.frame with one summary row per contrast.

Examples

```r
dgeObj <- readRDS(system.file("exampleObj.RDS", package = "DGEobj"))
contrastList <- DGEobj::getType(dgeObj, type = "topTable")

#all defaults
sigSummary <- summarizeSigCounts(contrastList)

#add the fold-chage threshold
sigSummary <- summarizeSigCounts(contrastList, fcThreshold = 2)

#change the significance thresholds
sigSummary <- summarizeSigCounts(contrastList,
       sigThresholds = c(0.01, 0.1, 0.1, 0.1, 0.1))
```

topTable.merge

Merge specified topTable df cols

Description

Take a named list of topTable dataframes and cbinds the requested columns from each file. To avoid column name conflicts the names are used as suffixes to the colnames. Although written for topTable data, this should work on any named list of dataframes where each member of the list has the same columns.
tpm.direct

Usage
topTable.merge(
  contrastList,
  colNames = c("logFC", "AveExpr", "P.Value", "adj.P.Val"),
  digits = c(2, 2, 4, 3)
)

Arguments
contrastList A named list of topTable data.frames which all have the same colnames and
same row counts. The dataframes in the list should have rownames (geneIDs).
colNames The list of column names of the data column to extract to a matrix (Default =
c("logFC", "AveExpr", "P.Value", "adj.P.Val")
digits Number of decimal places for specified columns. Should be same length as
colNames. (Default = c(2, 2, 4, 3)). If one value supplied, it is used for all
columns.

Value
A matrix containing the extracted columns.

Examples
dgeObj <- readRDS(system.file("exampleObj.RDS", package = "DGEobj"))
contrastList <- DGEobj::getType(dgeObj, type = "topTable")
mergedData <- topTable.merge(contrastList)
colnames(mergedData)

---

tpm.direct Convert countsMatrix and geneLength to TPM units

Description
Takes a countsMatrix and geneLength as input and converts to TPM units using the equation from
Harold Pimental.

Usage
tpm.direct(countsMatrix, geneLength, collapse = FALSE)

Arguments
countsMatrix A numeric matrix of N genes x M samples. All columns must be numeric.
geneLength Numeric matrix of gene lengths. Often the ExonLength item of a DGEobj.
collapse Default = FALSE. TRUE or FALSE determines whether to use rowMeans on
the geneLength matrix.
### Details

The result should be the same as using `convertCounts` with `normalize = 'tpm'` and `log = FALSE`. `geneLength` can be a vector (length == nrow(countsMatrix)) or a matrix (same dim as countsMatrix). The `geneLength` is used as is, or optionally collapsed to a vector by `rowMeans`.

### Value

A matrix of TPM values

### Examples

```r
dgeObj <- readRDS(system.file("exampleObj.RDS", package = "DGEobj"))

counts <- DGEobj::getItem(dgeObj, "counts")
exonLength <- dgeObj$geneData$ExonLength
tpm <- tpm.direct(counts, geneLength = exonLength)
```

### Description

Calculates TPM for a subsetted DGEobj. The function will calculate TPM using the original data but returns a DGEobj with the subset.

### Usage

```r
tpm.on.subset(dgeObj, applyFilter = TRUE)
```

### Arguments

- **dgeObj** A DGEobj data structure
- **applyFilter** Default = TRUE. If TRUE, reduces to the filtered gene list. FALSE returns all genes in the raw data.

### Details

TPM should be calculated on a full dataset with only low signal genes removed. `tpm.on.subset` therefore allows calculation of TPM after heavy filtering of a DGEobj.

Internally, `convertCounts` uses `edgeR::fpkm()` to calculate FPKM and converts to TPM using the formula provided by [Harold Pimental](https://haroldpimentel.wordpress.com/2014/05/08/what-the-fpkm-a-review-rna-seq-expression-units/).

### Value

A matrix of TPM values
Examples

dgeObj <- readRDS(system.file("exampleObj.RDS", package = "DGEobj"))

tpm <- tpm.on.subset(dgeObj)
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