Package 'kimma'

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Type Package

Title Kinship In Mixed Model Analysis of RNA-seq

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Description Data analysis and linear mixed effects models with pairwise kinship for RNA-seq data.

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biocViews

Imports broom, car, coxme, data.table, doParallel, dplyr, edgeR, emmeans, forcats, foreach, limma, lme4, magrittr, stats, tibble, tidyr, tidyselect

RoxygenNote 7.1.1

Depends R (>= 2.10)

R topics documented:

`dat.example` *kimma example DGEList.*

Description

RNA-seq of human dendritic cells cultured with and without virus. Samples from 3 donors and a random subset of 1000 genes were selected. Counts are unnormalized.

Usage

```
dat.example
```

Format

Formal class 'DGEList' [package "edgeR"] with 1 slot:

1. **counts** A matrix with 1000 rows and 6 columns

   rownames character. ENSEMBL gene ID.

   lib1 integer. Counts in library 1.

   lib2 integer. Counts in library 2.

   lib3 integer. Counts in library 3.

   lib4 integer. Counts in library 4.

   lib5 integer. Counts in library 5.

   lib6 integer. Counts in library 6.

2. **samples** A data frame with 6 rows and 7 columns

   group factor. No grouping was provided. All = 1.

   lib.size numeric. Total library size for this 1000 gene subset.

   norm.factors numeric. Normalizatin factors. No normalization was completed. All = 1.

   libID character. Unique library ID. Matches column names in counts.

   donorID character. Donor ID.

   median_cv_coverage numeric. Median coefficient of variation of coverage. Quality metric for sequencing libraries calculated from original full data set.

   virus character. A for media samples with no virus. B for virus-infected samples.

3. **genes** A data frame with 1000 rows and 5 columns

   hgnc_symbol character. Current approved HGNC symbol.

   Previous symbols character. Previous HGNC symbols.

   Alias symbols character. Alias HGNC symbols.

   gene_biotype character. Gene product type. All = protein-coding.

   geneName character. ENSEMBL gene ID. Matches row names in counts.

Details

An edgeR DGEList data set containing unnormalized RNA-seq counts.

Source

https://github.com/altman-lab/P259_pDC_public

References

Dill-McFarland et al. 2021. Eosinophil-mediated suppression and Anti-IL-5 enhancement of plasmacytoid dendritic cell interferon responses in asthma. J Allergy Clin Immunol. In revision

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`dat.voom.example` *kimma example EList.*

Description

RNA-seq of human dendritic cells cultured with and without virus. Samples from 3 donors and a random subset of 1000 genes were selected. Counts are TMM normalized log2 counts per million (CPM).

Usage

```
dat.voom.example
```

Format

Formal class 'EList' [package "limma"] with 1 slot:

1. **genes** A data frame with 1000 rows and 5 columns

   hgnc_symbol character. Current approved HGNC symbol.

   Previous symbols character. Previous HGNC symbols.

   Alias symbols character. Alias HGNC symbols.

   gene_biotype character. Gene product type. All = protein-coding.

   geneName character. ENSEMBL gene ID. Matches row names in E.

2. **targets** A data frame with 6 rows and 7 columns

   group factor. No grouping was provided. All = 1.

   lib.size numeric. Total library size for this 1000 gene subset.

   norm.factors numeric. TMM normalizatin factors.

   libID character. Unique library ID. Matches column names in E.

   donorID character. Donor ID.

   median_cv_coverage numeric. Median coefficient of variation of coverage. Quality metric for sequencing libraries calculated from original full data set.

   virus character. A for media samples with no virus. B for virus-infected samples.

3. **E** A matrix with 1000 rows and 6 columns

   rownames character. ENSEMBL gene ID.

   lib1 integer. log2 CPM in library 1.

   lib2 integer. log2 CPM in library 2.

   lib3 integer. log2 CPM in library 3.

   lib4 integer. log2 CPM in library 4.

   lib5 integer. log2 CPM in library 5.

   lib6 integer. log2 CPM in library 6.

   #'

4. **weights** A matrix with 1000 rows and 6 columns

- **1** numeric. limma gene weights for library 1.
- **2** numeric. limma gene weights for library 2.
- **3** numeric. limma gene weights for library 3.
- **4** numeric. limma gene weights for library 4.
- **5** numeric. limma gene weights for library 5.
- **6** numeric. limma gene weights for library 6.

5. **design** A matrix with 6 rows and 1 column

   GrandMean numeric. limma default design matrix.

Details

A limma EList data set containing normalized log2 RNA-seq counts.

Source

https://github.com/altman-lab/P259_pDC_public

References

Dill-McFarland et al. 2021. Eosinophil-mediated suppression and Anti-IL-5 enhancement of plasmacytoid dendritic cell interferon responses in asthma. J Allergy Clin Immunol. In revision

`extract_lmFit` *Extract lmFit model results*

Description

Extract model fit and significance for all individual variables and/or contrasts

Usage

```
extract_lmFit(
  design,
  fit,
  contrast.mat = NULL,
  dat.genes = NULL,
  name.genes = "geneName"
)
```

Arguments

`design` model matrix output by model.matrix( )

`fit` MArrayLM model fit output by limma::eBayes( )

`contrast.mat` contrast matrix output by limma::makeContrasts( )

`dat.genes` data frame with additional gene annotations. Optional. Default is NULL

`name.genes` character for variable name in dat.genes that matches gene names in fit. Default is "geneName"

Value

Data frame with model fit and significant for all variable and genes

Examples

```
# Run limma model
design <- model.matrix(~ virus, data = dat.voom.example$targets)
fit <- limma::eBayes(limma::lmFit(dat.voom.example$E, design))

## Get results
result <- extract_lmFit(design = design, fit = fit)
## Get results and add gene annotations
fdr <- extract_lmFit(design = design, fit = fit,
                         dat.genes = dat.voom.example$genes, name.genes = "geneName")

# Run limma contrasts model
design <- model.matrix(~ 0 + virus, data = dat.voom.example$targets)
fit <- limma::lmFit(dat.voom.example$E, design)
contrast.mat <- limma::makeContrasts(virusB-virusA, levels = design)
fit <- eBayes(contrasts.fit(fit, contrast.mat))

## Get contrast results
fdr <- extract_lmFit(design = design, fit = fit, contrast.mat = contrast.mat)
```

`filter_rare` *Filter rare and low abundance genes*

Description

Filter genes at a specified minimum counts per million (CPM) in a minmum number or percent of total samples.

Usage

```
filter_rare(
  dat,
  min.CPM,
  gene.var = "geneName",
  min.sample = NULL,
  min.pct = NULL
)
```

Arguments

`dat`	DGEList output by edgeR::DEGList( )
`min.CPM`	numeric minimum counts per million (CPM)
`gene.var`	character name for column with gene names in dat$genes that matches names in expression data dat$E. Default "geneName"
`min.sample`	numeric minimum number of samples
`min.pct`	numeric minimum percent of samples (0-100)

Value

DGEList object filtered to not rare genes

Examples

```
filter_rare(dat = dat.example, min.CPM = 0.1, min.sample = 3)
filter_rare(dat = dat.example, min.CPM = 0.1, min.pct = 10)
```

`kin.example` *kimma example kinship.*

Description

Pairwise kinship values between donor 1,2,3. Values are dummy data with 1 for self comparison, 0.5 for siblings, and 0.1 for unrelated.

Usage

```
kin.example
```

Format

A matrix with 3 rows and 3 variables:

rowname Donor ID. Same as column names

donor1 numeric kinship (0-1) with donor 1

donor2 numeric kinship (0-1) with donor 2

donor3 numeric kinship (0-1) with donor 3

Details

Matrix of pairwise kinship values between participants

`kmFit` *Linear mixed effects models with kinship for RNA-seq*

Description

Run lmekin and corresponding lm or lme without kinships models for all genes

Usage

```
kmFit(
  dat = NULL,
  kin = NULL,
  patientID = "ptID",
  libraryID = "libID",
  counts = NULL,
  meta = NULL,
  genes = NULL,
  subset.var = NULL,
  subset.lvl = NULL,
  subset.genes = NULL,
  model,
  compare.lm = FALSE,
  compare.lme = FALSE,
  contrast = FALSE,
  contrast.mat = NULL,
  processors = 1,
  p.method = "BH"
)
```

Arguments

`dat` EList object output by voom( ). Contains counts (dat$E), meta (dat$targets), and genes (dat$genes).

`kin` Matrix with pairwise kinship values between individuals. Must be numeric with rownames.

`patientID` Character of variable name to match dat$targets to kinship row and column names.

`libraryID` Character of variable name to match dat$targets to dat$E colnames

Alternate data if not using EList object

`counts` Matrix of normalized expression. Rows are genes, columns are libraries.

`meta` Matrix or data frame of sample and individual metadata.

`genes` Matrix or data frame of gene metadata.

Subset data (optional)

`subset.var` Character list of variable name(s) to filter data by.

`subset.lvl` Character list of variable value(s) or level(s) to filter data to. Must match order of subset.var

`subset.genes` Character vector of genes to include in models. Model

`model` Character vector of model starting with ~ Should include (1|patientID) if mixed effects will be run

`compare.lm` Logical if should run corresponding lm model without kinship

`compare.lme` Logical if should run corresponding lme model without kinship

`contrast` Logical if should run pairwise contrasts. If no matrix provided, all possible pairwise comparisons are completed.

`contrast.mat` Numeric contrast matrix created limma::makeContrasts( ) Other

`processors` Numeric processor to run in parallel

`p.method` Character of FDR adjustment method. Values as in p.adjust( )

Details

Data

Value

data.frame

Examples

```
# All samples and all genes
# Not run
# kmFit(dat = dat.voom.example,
#       patientID = "donorID", libraryID = "libID",
#       kin = kin.example, compare.lme = TRUE,
#       model = "~ virus + (1|donorID)")

# Subset samples and genes
kmFit(dat = dat.voom.example,
      patientID = "donorID", libraryID = "libID",
      kin = kin.example,
      compare.lme = TRUE,
      subset.var = list("donorID"), subset.lvl = list(c("donor1","donor2")),
      subset.genes = c("ENSG00000250479","ENSG00000250510","ENSG00000255823"),
      model = "~ virus + (1|donorID)")
# Pairwise contrasts
kmFit(dat = dat.voom.example,
      patientID = "donorID", libraryID = "libID",
      kin = kin.example,
      compare.lm = TRUE, contrast = TRUE,
      subset.genes = c("ENSG00000250479","ENSG00000250510","ENSG00000255823"),
      model = "~ donorID + (1|donorID)")
```

summarise_kmFit   *Summarise kmFit FDR results*

Description

Summarise number of significant genes at various FDR cutoffs. Can split by up/down fold change as well.

Usage

```
summarise_kmFit(
  fdr,
  fdr.cutoff = c(0.05, 0.1, 0.2, 0.3, 0.4, 0.5),
  FCgroup = FALSE,
  intercept = FALSE
)
```

Arguments

`fdr`	data.frame output by kimma::extract_lmFit( )
`fdr.cutoff`	numeric vector of FDR cutoffs to summarise at
`FCgroup`	logical if should separate summary by up/down fold change groups
`intercept`	logical if should include intercept variable in summary

Value

Data frame with total significant genes for each variable are various FDR cutoffs

Examples

```
# Run kimma model
fdr <- kmFit(dat = dat.voom.example,
      patientID = "donorID", libraryID = "libID",
      kin = kin.example,
      compare.lme = TRUE,
      subset.genes = c("ENSG00000250479","ENSG00000250510","ENSG00000255823"),
      model = "~ virus + (1|donorID)")

# Summarise results
fdr.summary <- summarise_kmFit(fdr = fdr, fdr.cutoff = c(0.05, 0.5), FCgroup = TRUE)
```

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summarise_lmFit *Summarise lmFit FDR results*

Description

Summarise number of significant genes at various FDR cutoffs. Can split by up/down fold change as well.

Usage

```
summarise_lmFit(
  fdr,
  fdr.cutoff = c(0.05, 0.1, 0.2, 0.3, 0.4, 0.5),
  FCgroup = FALSE,
  intercept = FALSE
)
```

Arguments

`fdr`	data.frame output by kimma::extract_lmFit( )
`fdr.cutoff`	numeric vector of FDR cutoffs to summarise at
`FCgroup`	logical if should separate summary by up/down fold change groups
`intercept`	logical if should include intercept variable in summary

Value

Data frame with total significant genes for each variable are various FDR cutoffs

Examples

```
# Run limma model
design <- model.matrix(~ virus, data = dat.voom.example$targets)
fit <- limma::eBayes(limma::lmFit(dat.voom.example$E, design))

## Get results
fdr <- extract_lmFit(design = design, fit = fit)

# Summarise results
fdr.summary <- summarise_lmFit(fdr = fdr, fdr.cutoff = c(0.05, 0.5), FCgroup = TRUE)
```

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