

Package ‘kimma’

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Type Package

Title Kinship In Mixed Model Analysis of RNA-seq

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Description 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, ggplot2, limma, lme4, magrittr, readr, stats, tibble, tidyr, tidyselect

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example.dat

*kimma example DGEList.***Description**

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

example.dat

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. Normalization 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.

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

example.kin	<i>limma example kinship.</i>
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Description

Matrix of 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

```
example.kin
```

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

example.voom	<i>limma example EList.</i>
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Description

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

```
example.voom
```

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 normalization 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.

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 in a limma model

Usage

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

Arguments

design	model matrix output by <code>model.matrix()</code>
fit	MArrayLM model fit output by <code>limma::eBayes()</code>
contrast.mat	contrast matrix output by <code>limma::makeContrasts()</code>
dat.genes	data frame with additional gene annotations. Optional.
name.genes	character for variable name in <code>dat.genes</code> that matches gene names in <code>fit</code>

Value

Data frame with model fit and significance for all variable and genes. Format as in `limma::topTable()`

Examples

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

# Run limma contrasts model
design <- model.matrix(~ 0 + virus, data = example.voom$targets)
fit <- limma::lmFit(example.voom$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)
```

kmFit

*Linear mixed effects models with kinship for RNA-seq***Description**

Run `lmekin` and corresponding `lm` or `lme` without kinship of gene expression in RNA-seq data

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,
run.lm = FALSE,
run.lme = FALSE,
run.lmekin = FALSE,
run.contrast = FALSE,
contrast.mat = NULL,
processors = 1,
p.method = "BH"
)

```

Arguments

<code>dat</code>	EList object output by <code>voom()</code> . Contains counts (<code>dat\$E</code>), meta (<code>dat\$targets</code>), and genes (<code>dat\$genes</code>).
<code>kin</code>	Matrix with pairwise kinship values between individuals. Must be numeric with rownames.
<code>patientID</code>	Character of variable name to match <code>dat\$targets</code> to kinship row and column names.
<code>libraryID</code>	Character of variable name to match <code>dat\$targets</code> to <code>dat\$E</code> colnames
<code>counts</code>	Matrix of normalized expression. Rows are genes, columns are libraries.
<code>meta</code>	Matrix or data frame of sample and individual metadata.
<code>genes</code>	Matrix or data frame of gene metadata.
<code>subset.var</code>	Character list of variable name(s) to filter data by.
<code>subset.lvl</code>	Character list of variable value(s) or level(s) to filter data to. Must match order of <code>subset.var</code>
<code>subset.genes</code>	Character vector of genes to include in models.
<code>model</code>	Character vector of model starting with <code>~</code> Should include (1 <code>patientID</code>) if mixed effects will be run
<code>run.lm</code>	Logical if should run lm model without kinship
<code>run.lme</code>	Logical if should run lme model without kinship
<code>run.lmekin</code>	Logical if should run lmekin model with kinship
<code>run.contrast</code>	Logical if should run pairwise contrasts. If no matrix provided, all possible pairwise comparisons are completed.
<code>contrast.mat</code>	Numeric contrast matrix created <code>limma::makeContrasts()</code>
<code>processors</code>	Numeric processor to run in parallel
<code>p.method</code>	Character of FDR adjustment method. Values as in <code>p.adjust()</code>

Value

Dataframe with model fit and significance for each gene

Examples

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

# Subset samples and genes
kmFit(dat = example.voom,
      patientID = "donorID", libraryID = "libID",
      run.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 = example.voom,
      patientID = "donorID", libraryID = "libID",
      kin = example.kin,
      run.lme = TRUE, run.contrast = TRUE,
      subset.genes = c("ENSG00000250479", "ENSG00000250510", "ENSG00000255823"),
      model = "~ virus + (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),
  contrast = FALSE,
  FCgroup = FALSE,
  intercept = FALSE
)
```

Arguments

fdr	data.frame output by kimma::kmFit()
fdr.cutoff	numeric vector of FDR cutoffs to summarise at
contrast	logical if should separate summary by pairwise contrasts within variables
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 at various FDR cutoffs

Examples

```
# Run kimma model
fdr <- kmFit(dat = example.voom,
  patientID = "donorID", libraryID = "libID",
  kin = example.kin,
  run.lme = TRUE, run.lmekin=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)
```

summarise_lmFit	<i>Summarise lmFit FDR results</i>
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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 at various FDR cutoffs

Examples

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