# Package 'zenith'

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**Type** Package

**Title** Gene set analysis following differential expression using linear (mixed) modeling with dream

**Version** 1.6.0

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**Description** Zenith performs gene set analysis on the result of differential expression using linear (mixed) modeling with dream by considering the correlation between gene expression traits. This package implements the camera method from the limma package proposed by Wu and Smyth (2012). Zenith is a simple extension of camera to be compatible with linear mixed models implemented in variancePartition::dream().

**VignetteBuilder** knitr

**License** Artistic-2.0

**Encoding** UTF-8

**URL** https://DiseaseNeuroGenomics.github.io/zenith

**BugReports** https://github.com/DiseaseNeuroGenomics/zenith/issues

**Suggests** BiocStyle, BiocGenerics, knitr, pander, rmarkdown, tweeDEseqCountData, edgeR, kableExtra, RUnit

**biocViews** RNASeq, GeneExpression, GeneSetEnrichment, DifferentialExpression, BatchEffect, QualityControl, Regression, Epigenetics, FunctionalGenomics, Transcriptomics, Normalization, Preprocessing, Microarray, ImmunoOncology, Software

**Depends** R (>= 4.2.0), limma, methods

**Imports** variancePartition (>= 1.26.0), EnrichmentBrowser (>= 2.22.0), GSEABase (>= 1.54.0), msigdbr (>= 7.5.1), Rfast, ggplot2, tidyr, reshape2, progress, utils, Rdpack, stats

**RdMacros** Rdpack

**RoxygenNote** 7.2.3

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# Contents



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`.rankSumTestWithCorrelation` *Two Sample Wilcoxon-Mann-Whitney Rank Sum Test Allowing For Correlation*

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## Description

Same as `limma::.rankSumTestWithCorrelation`, but returns effect size.

## 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

See `limma::.rankSumTestWithCorrelation`

## Value

data.frame storing results of hypothesis test

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`corInGeneSet` *Evaluate mean correlation between residuals in gene set*

---

## Description

Evaluate mean correlation between residuals in gene set based on results from dream

## Usage

```
corInGeneSet(fit, idx, squareCorr = FALSE)
```

## Arguments

| | |
|---|---|
| `fit` | result of differential expression with dream |
| `idx` | indeces or rownames to extract |
| `squareCorr` | compute the mean squared correlation instead |

## Value

list storing correlation and variance inflation factor

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`get_GeneOntology` *Load Gene Ontology genesets*

---

## Description

Load Gene Ontology genesets

## Usage

```
get_GeneOntology(
  onto = c("BP", "MF", "CC"),
  to = "ENSEMBL",
  includeOffspring = TRUE,
  org = "hsa"
)
```

### Arguments

| | |
|---|---|
| onto | array of categories to load |
| to | convert gene names to this type using EnrichmentBrowser::idMap(). See EnrichmentBrowser::idTypes(org="hsa") for valid types |
| includeOffspring | if TRUE, follow the GO hierarchy down and include all genes in offspring sets for a given gene set |
| org | organism. human ('hsa'), mouse ('mmu'), etc |

### Details

This function loads the GO gene sets using the packages EnrichmentBrowser and GO.db It can take a mintute to load because converting gene name type is slow.

### Value

Gene sets stored as GeneSetCollection

### Examples

```
# load GO Biological Process
# gs = get_GeneOntology('BP')

# load all gene sets
# gs = get_GeneOntology()
```

---

## get_MSigDB *Load MSigDB genesets*

---

### Description

Load MSigDB genesets

### Usage

```
get_MSigDB(
  cat = unique(msigdbr_collections()$gs_cat),
  to = "ENSEMBL",
  org = "hsa"
)
```

### Arguments

| | |
|---|---|
| cat | array of categories to load. Defaults to array of all MSigDB categories |
| to | convert gene names to this type using EnrichmentBrowser::idMap(). See EnrichmentBrowser::idTypes(org="hsa") for valid types |
| org | organism. human ('hsa'), mouse ('mmu'), etc |

## Details

This function loads the MSigDB gene sets using the packages `EnrichmentBrowser` and `msigdbr`. It can take a mintute to load because converting gene name type is slow.

## Value

Gene sets stored as GeneSetCollection

## Examples

```
# load Hallmark gene sets
gs = get_MSigDB('H')

# load all gene sets
# gs = get_MSigDB()
```

---

`plotZenithResults` *Heatmap of zenith results using ggplot2*

---

## Description

Heatmap of zenith results showing genesets that have the top and bottom t-statistics from each assay.

## Usage

```
plotZenithResults(
  df,
  ntop = 5,
  nbottom = 5,
  label.angle = 45,
  zmax = NULL,
  transpose = FALSE,
  sortByGeneset = TRUE
)
```

## Arguments

| | |
|---|---|
| `df` | result `data.frame` from zenith_gsa |
| `ntop` | number of gene sets with highest t-statistic to show |
| `nbottom` | number of gene sets with lowest t-statistic to show |
| `label.angle` | angle of x-axis label |
| `zmax` | maxium of the color scales. If not specified, used range of the observed t-statistics |
| `transpose` | transpose the axes of the plot |
| `sortByGeneset` | use hierarchical clustering to sort gene sets. Default is TRUE |

## Value

Heatmap showing enrichment for gene sets and cell types

## Examples

```
# Load packages
library(edgeR)
library(variancePartition)
library(tweeDEseqCountData)

# Load RNA-seq data from LCL's
data(pickrell)
geneCounts = exprs(pickrell.eset)
df_metadata = pData(pickrell.eset)

# Filter genes
# Note this is low coverage data, so just use as code example
dsgn = model.matrix(~ gender, df_metadata)
keep = filterByExpr(geneCounts, dsgn, min.count=5)

# Compute library size normalization
dge = DGEList(counts = geneCounts[keep,])
dge = calcNormFactors(dge)

# Estimate precision weights using voom
vobj = voomWithDreamWeights(dge, ~ gender, df_metadata)

# Apply dream analysis
fit = dream(vobj, ~ gender,df_metadata)
fit = eBayes(fit)

# Load Hallmark genes from MSigDB
# use gene 'SYMBOL', or 'ENSEMBL' id
# use get_GeneOntology() to load Gene Ontology
gs = get_MSigDB("H", to="ENSEMBL")

# Run zenith analysis
res.gsa = zenith_gsa(fit, gs, 'gendermale', progressbar=FALSE )

# Show top gene sets
head(res.gsa, 2)

# for each cell type select 3 genesets with largest t-statistic
# and 1 geneset with the lowest
# Grey boxes indicate the gene set could not be evaluted because
#    to few genes were represented
plotZenithResults(res.gsa)
```

`zenith` *Gene set analysis following differential expression with dream*

## Description

Perform gene set analysis on the result of differential expression using linear (mixed) modeling with `variancePartition::dream` by considering the correlation between gene expression traits. This package is a slight modification of `limma::camera` to 1) be compatible with dream, and 2) allow identification of gene sets with log fold changes with mixed sign.

## Usage

```
zenith(
  fit,
  coef,
  index,
  use.ranks = FALSE,
  allow.neg.cor = FALSE,
  progressbar = TRUE,
  inter.gene.cor = 0.01
)
```

## Arguments

| | |
|---|---|
| `fit` | result of differential expression with dream |
| `coef` | coefficient to test using `topTable(fit, coef)` |
| `index` | an index vector or a list of index vectors. Can be any vector such that `fit[index,]` selects the rows corresponding to the test set. The list can be made using `ids2indices`. |
| `use.ranks` | do a rank-based test (`TRUE`) or a parametric test ('FALSE')? |
| `allow.neg.cor` | should reduced variance inflation factors be allowed for negative correlations? |
| `progressbar` | if TRUE, show progress bar |
| `inter.gene.cor` | if NA, estimate correlation from data. Otherwise, use specified value |

## Details

`zenith` gives the same results as `camera(..., inter.gene.cor=NA)` which estimates the correlation with each gene set.

For differential expression with dream using linear (mixed) models see Hoffman and Roussos (2020). For the original camera gene set test see Wu and Smyth (2012).

## Value

- NGenes: number of genes in this set
- Correlation: mean correlation between expression of genes in this set
- delta: difference in mean t-statistic for genes in this set compared to genes not in this set
- se: standard error of delta
- p.less: p-value for hypothesis test of H0: delta < 0
- p.greater: p-value for hypothesis test of H0: delta > 0
- PValue: p-value for hypothesis test H0: delta != 0
- Direction: direction of effect based on sign(delta)
- FDR: false discovery rate based on Benjamini-Hochberg method in p.adjust

## References

Hoffman GE, Roussos P (2020). "dream: Powerful differential expression analysis for repeated measures designs." *Bioinformatics*. doi:10.1093/bioinformatics/btaa687.

Wu D, 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.

## Examples

```
library(variancePartition)

# simulate meta-data
info <- data.frame(Age=c(20, 31, 52, 35, 43, 45),Group=c(0,0,0,1,1,1))

# simulate expression data
y <- matrix(rnorm(1000*6),1000,6)
rownames(y) = paste0("gene", 1:1000)
colnames(y) = rownames(info)

# 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

# perform differential expression analysis with dream
fit = dream(y, ~ Age + Group, info)
fit = eBayes(fit)

# perform gene set analysis testing Age
res = zenith(fit, "Age", list(set1=index1,set2=index2) )

head(res)
```

zenithPR_gsa *Gene set analysis using pre-computed test statistic*

## Description

Perform gene set analysis on the result of a pre-computed test statistic. Test whether statistics in a gene set are larger/smaller than statistics not in the set.

## Usage

```
zenithPR_gsa(
  statistics,
  ids,
  geneSets,
  use.ranks = FALSE,
  n_genes_min = 10,
  progressbar = TRUE,
  inter.gene.cor = 0.01,
  coef.name = "zenithPR"
)
```

## Arguments

| | |
|---|---|
| statistics | pre-computed test statistics |
| ids | name of gene for each entry in statistics |
| geneSets | GeneSetCollection |
| use.ranks | do a rank-based test TRUE or a parametric test FALSE? default: FALSE |
| n_genes_min | minumum number of genes in a geneset |
| progressbar | if TRUE, show progress bar |
| inter.gene.cor | correlation of test statistics with in gene set |
| coef.name | name of column to store test statistic |

## Details

This is the same as zenith_gsa(), but uses pre-computed test statistics. Note that zenithPR_gsa() may give slightly different results for small samples sizes, if zenithPR_gsa() is fed t-statistics instead of z-statistics.

## Value

- NGenes: number of genes in this set
- Correlation: mean correlation between expression of genes in this set
- delta: difference in mean t-statistic for genes in this set compared to genes not in this set
- se: standard error of delta

- p.less: p-value for hypothesis test of H0: delta < 0
- p.greater: p-value for hypothesis test of H0: delta > 0
- PValue: p-value for hypothesis test H0: delta != 0
- Direction: direction of effect based on sign(delta)
- FDR: false discovery rate based on Benjamini-Hochberg method in p.adjust
- coef.name: name for pre-computed test statistics. Default: zenithPR

## See Also

zenith_gsa(), limma::cameraPR()

## Examples

```
# Load packages
library(edgeR)
library(variancePartition)
library(tweeDEseqCountData)

# Load RNA-seq data from LCL's
data(pickrell)
geneCounts = exprs(pickrell.eset)
df_metadata = pData(pickrell.eset)

# Filter genes
# Note this is low coverage data, so just use as code example
dsgn = model.matrix(~ gender, df_metadata)
keep = filterByExpr(geneCounts, dsgn, min.count=5)

# Compute library size normalization
dge = DGEList(counts = geneCounts[keep,])
dge = calcNormFactors(dge)

# Estimate precision weights using voom
vobj = voomWithDreamWeights(dge, ~ gender, df_metadata)

# Apply dream analysis
fit = dream(vobj, ~ gender, df_metadata)
fit = eBayes(fit)

# Load Hallmark genes from MSigDB
# use gene 'SYMBOL', or 'ENSEMBL' id
# use get_GeneOntology() to load Gene Ontology
gs = get_MSigDB("H", to="ENSEMBL")

# Run zenithPR analysis with a test statistic for each gene
tab = topTable(fit, coef='gendermale', number=Inf)

res.gsa = zenithPR_gsa(tab$t, rownames(tab), gs)
```

zenith_gsa *Perform gene set analysis using zenith*

## Description

Perform a competitive gene set analysis accounting for correlation between genes.

## Usage

```
zenith_gsa(
  fit,
  geneSets,
  coefs,
  use.ranks = FALSE,
  n_genes_min = 10,
  inter.gene.cor = 0.01,
  progressbar = TRUE,
  ...
)

## S4 method for signature 'MArrayLM,GeneSetCollection'
zenith_gsa(
  fit,
  geneSets,
  coefs,
  use.ranks = FALSE,
  n_genes_min = 10,
  inter.gene.cor = 0.01,
  progressbar = TRUE,
  ...
)
```

## Arguments

| | |
|---|---|
| fit | results from dream() |
| geneSets | GeneSetCollection |
| coefs | list of coefficients to test using topTable(fit, coef=coefs[[i]]) |
| use.ranks | do a rank-based test TRUE or a parametric test FALSE? default: FALSE |
| n_genes_min | minumum number of genes in a geneset |
| inter.gene.cor | if NA, estimate correlation from data. Otherwise, use specified value |
| progressbar | if TRUE, show progress bar |
| ... | other arguments |

## Details

This code adapts the widely used `camera()` analysis (Wu and Smyth 2012) in the `limma` package (Ritchie et al. 2015) to the case of linear (mixed) models used by `variancePartition::dream()`.

## Value

`data.frame` of results for each gene set and cell type

## References

Ritchie ME, Phipson B, Wu DI, Hu Y, Law CW, Shi W, Smyth GK (2015). "limma powers differential expression analyses for RNA-sequencing and microarray studies." *Nucleic acids research*, **43**(7), e47–e47.

Wu D, 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.

## See Also

`limma::camera`

## Examples

```
# Load packages
library(edgeR)
library(variancePartition)
library(tweeDEseqCountData)

# Load RNA-seq data from LCL's
data(pickrell)
geneCounts = exprs(pickrell.eset)
df_metadata = pData(pickrell.eset)

# Filter genes
# Note this is low coverage data, so just use as code example
dsgn = model.matrix(~ gender, df_metadata)
keep = filterByExpr(geneCounts, dsgn, min.count=5)

# Compute library size normalization
dge = DGEList(counts = geneCounts[keep,])
dge = calcNormFactors(dge)

# Estimate precision weights using voom
vobj = voomWithDreamWeights(dge, ~ gender, df_metadata)

# Apply dream analysis
fit = dream(vobj, ~ gender, df_metadata)
fit = eBayes(fit)

# Load Hallmark genes from MSigDB
# use gene 'SYMBOL', or 'ENSEMBL' id
```

```
# use get_GeneOntology() to load Gene Ontology
gs = get_MSigDB("H", to="ENSEMBL")

# Run zenith analysis
res.gsa = zenith_gsa(fit, gs, 'gendermale', progressbar=FALSE )

# Show top gene sets
head(res.gsa, 2)

# for each cell type select 3 genesets with largest t-statistic
# and 1 geneset with the lowest
# Grey boxes indicate the gene set could not be evaluted because
#    to few genes were represented
plotZenithResults(res.gsa)
```

# Index