

Package ‘variancePartition’

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Type Package

Title Quantify and interpret divers of variation in multilevel gene expression experiments

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Description Quantify and interpret multiple sources of biological and technical variation in gene expression experiments. Uses a linear mixed model to quantify variation in gene expression attributable to individual, tissue, time point, or technical variables. Includes dream differential expression analysis for repeated measures.

VignetteBuilder knitr

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as.data.frame,varPartResults-method
Convert to data.frame

Description

Convert varPartResults to data.frame

Usage

```
## S4 method for signature 'varPartResults'
as.data.frame(x, row.names = NULL,
  optional = FALSE, ...)
```

Arguments

x	varPartResults
row.names	pass thru to generic
optional	pass thru to generic
...	other arguments.

Value

data.frame

Examples

```
# load library
# library(variancePartition)

# load simulated data:
# geneExpr: matrix of gene expression values
# info: information/metadata about each sample
data(varPartData)

# Specify variables to consider
# Age is continuous so we model it as a fixed effect
# Individual and Tissue are both categorical, so we model them as random effects
form <- ~ Age + (1|Individual) + (1|Tissue)

# Fit model
varPart <- fitExtractVarPartModel( geneExpr[1:5,], form, info )

# convert to matrix
as.data.frame(varPart)
```

as.matrix,varPartResults-method

Convert to matrix

Description

Convert varPartResults to matrix

Usage

```
## S4 method for signature 'varPartResults'
as.matrix(x, ...)
```

Arguments

x	varPartResults
...	other arguments.

Value

matrix

Examples

```
# load library
# library(variancePartition)

# load simulated data:
# geneExpr: matrix of gene expression values
# info: information/metadata about each sample
data(varPartData)

# Specify variables to consider
# Age is continuous so we model it as a fixed effect
# Individual and Tissue are both categorical, so we model them as random effects
form <- ~ Age + (1|Individual) + (1|Tissue)

# Fit model
varPart <- fitExtractVarPartModel( geneExpr[1:5,], form, info )

# convert to matrix
as.matrix(varPart)
```

calcVarPart

*Compute variance statistics***Description**

Compute fraction of variation attributable to each variable in regression model. Also interpretable as the intra-class correlation after correcting for all other variables in the model.

Usage

```
calcVarPart(fit, adjust = NULL, adjustAll = FALSE,
  showWarnings = TRUE, ...)

## S4 method for signature 'lm'
calcVarPart(fit, adjust = NULL, adjustAll = FALSE,
  showWarnings = TRUE, ...)

## S4 method for signature 'lmerMod'
calcVarPart(fit, adjust = NULL, adjustAll = FALSE,
  showWarnings = TRUE, ...)
```

Arguments

fit	model fit from lm() or lmer()
adjust	remove variation from specified variables from the denominator. This computes the adjusted ICC with respect to the specified variables

adjustAll	adjust for all variables. This computes the adjusted ICC with respect to all variables
showWarnings	show warnings about model fit (default TRUE)
...	additional arguments (not currently used)

Value

fraction of variance explained / ICC for each variable in the model

Examples

```
library(lme4)
data(varPartData)

# Linear mixed model
fit <- lmer( geneExpr[1,] ~ (1|Tissue) + Age, info)
calcVarPart( fit )

# Linear model
# Note that the two models produce slightly different results
# This is expected: they are different statistical estimates
# of the same underlying value
fit <- lm( geneExpr[1,] ~ Tissue + Age, info)
calcVarPart( fit )
```

canCorPairs

canCorPairs

Description

Assess correlation between all pairs of variables in a formula

Usage

```
canCorPairs(formula, data, showWarnings = TRUE)
```

Arguments

formula	standard linear model formula (doesn't support random effects currently, so just change the syntax)
data	data.frame with the data for the variables in the formula
showWarnings	default to true

Details

Canonical Correlation Analysis (CCA) is similar to correlation between two vectors, except that CCA can accommodate matrices as well. For a pair of variables, canCorPairs assesses the degree to which they co-vary and contain the same information. Variables in the formula can be a continuous variable or a discrete variable expanded to a matrix (which is done in the backend of a regression model). For a pair of variables, canCorPairs uses CCA to compute the correlation between these variables and returns the pairwise correlation matrix.

Statistically, let rho be the array of correlation values returned by the standard R function `cancor` to compute CCA. `canCorPairs` returns $\text{rho} / \text{sum}(\text{rho})$ which is the fraction of the maximum possible correlation.

Note that CCA returns correlations values between 0 and 1

Value

Matrix of correlation values between all pairs of variables.

Examples

```
# load library
# library(variancePartition)

# load simulated data:
data(varPartData)

# specify formula
form <- ~ Individual + Tissue + Batch + Age + Height

# Compute Canonical Correlation Analysis (CCA)
# between all pairs of variables
# returns absolute correlation value
C = canCorPairs( form, info)

# Plot correlation matrix
plotCorrMatrix( C )
```

colinearityScore

Collinearity score

Description

Collinearity score for a regression model indicating if variables are too highly correlated to give meaningful results

Usage

```
colinearityScore(fit)
```

Arguments

`fit` regression model fit from `lm()` or `lmer()`

Value

Returns the collinearity score between 0 and 1, where a score > 0.999 means the degree of collinearity is too high. This function reports the correlation matrix between coefficient estimates for fixed effects. The collinearity score is the maximum absolute correlation value of this matrix. Note that the values are the correlation between the parameter estimates, and not between the variables themselves.

Examples

```
# load library
# library(variancePartition)

# load simulated data:
data(varPartData)
form <- ~ Age + (1|Individual) + (1|Tissue)

res <- fitVarPartModel( geneExpr[1:10,], form, info )

# evaluate the collinearity score on the first model fit
# this reports the correlation matrix between coefficients estimates
# for fixed effects
# the collinearity score is the maximum absolute correlation value
# If the collinearity score > .999 then the variance partition
# estimates may be problematic
# In that case, a least one variable should be omitted
colinearityScore(res[[1]])
```

dream

Differential expression with linear mixed model

Description

Fit linear mixed model for differential expression and perform hypothesis test on fixed effects as specified in the contrast matrix L

Usage

```
dream(exprObj, formula, data, L, ddf = c("Satterthwaite",
    "Kenward-Roger"), REML = TRUE, useWeights = TRUE,
    weightsMatrix = NULL, control = lme4::lmerControl(calc.derivs =
    FALSE, check.rankX = "stop.deficient"), suppressWarnings = FALSE, ...)
```

Arguments

exprObj	matrix of expression data (g genes x n samples), or ExpressionSet, or EList returned by voom() from the limma package
formula	specifies variables for the linear (mixed) model. Must only specify covariates, since the rows of exprObj are automatically used as a response. e.g.: ~ a + b + (1 c) Formulas with only fixed effects also work, and lmFit() followed by contrasts.fit() are run.
data	data.frame with columns corresponding to formula
L	contrast matrix specifying a linear combination of fixed effects to test
ddf	Specify "Satterthwaite" or "Kenward-Roger" method to estimate effective degrees of freedom for hypothesis testing in the linear mixed model. Note that Kenward-Roger is more accurate, but is *much* slower. Satterthwaite is a good enough approximation for most datasets.

REML	use restricted maximum likelihood to fit linear mixed model. default is TRUE. Strongly discourage against changing this option
useWeights	if TRUE, analysis uses heteroskedastic error estimates from voom(). Value is ignored unless exprObj is an EList() from voom() or weightsMatrix is specified
weightsMatrix	matrix the same dimension as exprObj with observation-level weights from voom(). Used only if useWeights is TRUE
control	control settings for lmer()
suppressWarnings	if TRUE, do not stop because of warnings or errors in model fit
...	Additional arguments for lmer() or lm()

Details

A linear (mixed) model is fit for each gene in `exprObj`, using formula to specify variables in the regression. If categorical variables are modeled as random effects (as is recommended), then a linear mixed model is used. For example if formula is `~ a + b + (1|c)`, then the model is

```
fit <- lmer( exprObj[j,] ~ a + b + (1|c), data=data)
```

`useWeights=TRUE` causes `weightsMatrix[j,]` to be included as weights in the regression model.

Note: Fitting the model for 20,000 genes can be computationally intensive. To accelerate computation, models can be fit in parallel using `foreach/dopar` to run loops in parallel. Parallel processing must be enabled before calling this function. See below.

The regression model is fit for each gene separately. Samples with missing values in either gene expression or metadata are omitted by the underlying call to `lmer`.

Hypothesis tests and degrees of freedom are produced by `lmerTest` and `pBkrTest` packages

Value

MArrayLM2 object (just like MArrayLM from `limma`), and the directly estimated p-value (without `eBayes`)

Examples

```
# load library
# library(variancePartition)

# optional step to run analysis in parallel on multicore machines
# Here, we used 4 threads
library(doParallel)
cl <- makeCluster(4)
registerDoParallel(cl)
# or by using the doSNOW package

# load simulated data:
# geneExpr: matrix of gene expression values
# info: information/metadata about each sample
data(varPartData)

# get contrast matrix testing if the coefficient for Batch2 is zero
# The variable of interest must be a fixed effect
form <- ~ Batch + (1|Individual) + (1|Tissue)
L = getContrast( geneExpr, form, info, "Batch3")
```



```
# plot contrasts
plotContrasts( L )

# Fit linear mixed model for each gene
# run on just 10 genes for time
fit = dream( geneExpr[1:10,], form, info, L)

# Run empirical Bayes post processing from limma
fitEB = eBayes( fit )

# view top genes
topTable( fitEB )

# stop cluster
stopCluster(cl)
```

eBayes,MArrayLM2-method

eBayes for MArrayLM2

Description

eBayes for MArrayLM2

Usage

```
## S4 method for signature 'MArrayLM2'
eBayes(fit, proportion = 0.01,
       stdev.coef.lim = c(0.1, 4), trend = FALSE, robust = FALSE,
       winsor.tail.p = c(0.05, 0.1))
```

Arguments

fit	fit
proportion	proportion
stdev.coef.lim	stdev.coef.lim
trend	trend
robust	robust
winsor.tail.p	winsor.tail.p

Value

resold of eBayes

ESS

*Effective sample size***Description**

Compute effective sample size based on correlation structure in linear mixed model

Usage

```
ESS(fit, method = "full")
```

```
## S4 method for signature 'lmerMod'
ESS(fit, method = "full")
```

Arguments

<code>fit</code>	model fit from <code>lmer()</code>
<code>method</code>	"full" uses the full correlation structure of the model. The "approximate" method makes the simplifying assumption that the study has a mean of m samples in each of k groups, and computes m based on the study design. When the study design is evenly balanced (i.e. the assumption is met), this gives the same results as the "full" method.

Details

Effective sample size calculations are based on: Liu, G., and Liang, K. Y. (1997). Sample size calculations for studies with correlated observations. *Biometrics*, 53(3), 937-47.

"full" method: if $V_x = \text{var}(Y;x)$ is the variance-covariance matrix of Y , the response, based on the covariate x , then the effective sample size corresponding to this covariate is $\sum_{i,j} (V_x^{-1})_{i,j}$. In R notation, this is: `sum(solve(V_x))`. In practice, this can be evaluated as `sum(w)`, where R

"approximate" method: Letting m be the mean number of samples per group, k be the number of groups, and ρ be the intraclass correlation, the effective sample size is $m*k / (1+\rho*(m-1))$

Note that these values are equal when there are exactly m samples in each group. If m is only an average then this is an approximation.

Value

effective sample size for each random effect in the model

Examples

```
library(lme4)
data(varPartData)

# Linear mixed model
fit <- lmer( geneExpr[1,] ~ (1|Individual) + (1|Tissue) + Age, info)

# Effective sample size
ESS( fit )
```

extractVarPart	<i>Extract variance statistics</i>
----------------	------------------------------------

Description

Extract variance statistics from list of models fit with `lm()` or `lmer()`

Usage

```
extractVarPart(modellist, adjust = NULL, adjustAll = FALSE,
  showWarnings = TRUE, ...)
```

Arguments

<code>modellist</code>	list of <code>lmer()</code> model fits
<code>adjust</code>	remove variation from specified variables from the denominator. This computes the adjusted ICC with respect to the specified variables
<code>adjustAll</code>	adjust for all variables. This computes the adjusted ICC with respect to all variables. This overrides the previous argument, so all variables are include in <code>adjust</code> .
<code>showWarnings</code>	show warnings about model fit (default TRUE)
<code>...</code>	other arguments

Value

data.frame of fraction of variance explained by each variable, after correcting for all others.

Examples

```
# library(variancePartition)

# optional step to run analysis in parallel on multicore machines
# Here, we used 4 threads
library(doParallel)
cl <- makeCluster(4)
registerDoParallel(cl)
# or by using the doSNOW package

# load simulated data:
# geneExpr: matrix of gene expression values
# info: information/metadata about each sample
data(varPartData)

# Specify variables to consider
# Age is continuous so we model it as a fixed effect
# Individual and Tissue are both categorical, so we model them as random effects
form <- ~ Age + (1|Individual) + (1|Tissue)

# Step 1: fit linear mixed model on gene expression
# If categorical variables are specified, a linear mixed model is used
# If all variables are modeled as continuous, a linear model is used
# each entry in results is a regression model fit on a single gene
```

```

# Step 2: extract variance fractions from each model fit
# for each gene, returns fraction of variation attributable to each variable
# Interpretation: the variance explained by each variable
# after correction for all other variables
varPart <- fitExtractVarPartModel( geneExpr, form, info )

# violin plot of contribution of each variable to total variance
plotVarPart( sortCols( varPart ) )

# Advanced:
# Fit model and extract variance in two separate steps
# Step 1: fit model for each gene, store model fit for each gene in a list
results <- fitVarPartModel( geneExpr, form, info )

# Step 2: extract variance fractions
varPart <- extractVarPart( results )

# stop cluster
stopCluster(cl)

```

```
fitExtractVarPartModel
```

Fit linear (mixed) model, report variance fractions

Description

Fit linear (mixed) model to estimate contribution of multiple sources of variation while simultaneously correcting for all other variables. Report fraction of variance attributable to each variable

Usage

```

fitExtractVarPartModel(exprObj, formula, data, REML = FALSE,
  useWeights = TRUE, weightsMatrix = NULL, adjust = NULL,
  adjustAll = FALSE, showWarnings = TRUE,
  control = lme4::lmerControl(calc.derivs = FALSE, check.rankX =
    "stop.deficient"), ...)

## S4 method for signature 'matrix'
fitExtractVarPartModel(exprObj, formula, data,
  REML = FALSE, useWeights = TRUE, weightsMatrix = NULL,
  adjust = NULL, adjustAll = FALSE, showWarnings = TRUE,
  control = lme4::lmerControl(calc.derivs = FALSE, check.rankX =
    "stop.deficient"), ...)

## S4 method for signature 'data.frame'
fitExtractVarPartModel(exprObj, formula, data,
  REML = FALSE, useWeights = TRUE, weightsMatrix = NULL,
  adjust = NULL, adjustAll = FALSE, showWarnings = TRUE,
  control = lme4::lmerControl(calc.derivs = FALSE, check.rankX =
    "stop.deficient"), ...)

```

```
## S4 method for signature 'EList'
fitExtractVarPartModel(exprObj, formula, data,
  REML = FALSE, useWeights = TRUE, weightsMatrix = NULL,
  adjust = NULL, adjustAll = FALSE, showWarnings = TRUE,
  control = lme4::lmerControl(calc.derivs = FALSE, check.rankX =
    "stop.deficient"), ...)

## S4 method for signature 'ExpressionSet'
fitExtractVarPartModel(exprObj, formula, data,
  REML = FALSE, useWeights = TRUE, weightsMatrix = NULL,
  adjust = NULL, adjustAll = FALSE, showWarnings = TRUE,
  control = lme4::lmerControl(calc.derivs = FALSE, check.rankX =
    "stop.deficient"), ...)
```

Arguments

exprObj	matrix of expression data (g genes x n samples), or ExpressionSet, or EList returned by voom() from the limma package
formula	specifies variables for the linear (mixed) model. Must only specify covariates, since the rows of exprObj are automatically used as a response. e.g.: ~ a + b + (1 c)
data	data.frame with columns corresponding to formula
REML	use restricted maximum likelihood to fit linear mixed model. default is FALSE. Strongly discourage against changing this option
useWeights	if TRUE, analysis uses heteroskedastic error estimates from voom(). Value is ignored unless exprObj is an EList() from voom() or weightsMatrix is specified
weightsMatrix	matrix the same dimension as exprObj with observation-level weights from voom(). Used only if useWeights is TRUE
adjust	remove variation from specified variables from the denominator. This computes the adjusted ICC with respect to the specified variables
adjustAll	adjust for all variables. This computes the adjusted ICC with respect to all variables. This overrides the previous argument, so all variables are included in adjust.
showWarnings	show warnings about model fit (default TRUE)
control	control settings for lmer()
...	Additional arguments for lmer() or lm()

Details

A linear (mixed) model is fit for each gene in exprObj, using formula to specify variables in the regression. If categorical variables are modeled as random effects (as is recommended), then a linear mixed model is used. For example if formula is ~ a + b + (1|c), then the model is

```
fit <- lmer( exprObj[j,] ~ a + b + (1|c), data=data)
```

If there are no random effects, so formula is ~ a + b + c, a 'standard' linear model is used:

```
fit <- lm( exprObj[j,] ~ a + b + c, data=data)
```

In both cases, useWeights=TRUE causes weightsMatrix[j,] to be included as weights in the regression model.

Note: Fitting the model for 20,000 genes can be computationally intensive. To accelerate computation, models can be fit in parallel using `foreach/dopar` to run loops in parallel. Parallel processing must be enabled before calling this function. See below.

The regression model is fit for each gene separately. Samples with missing values in either gene expression or metadata are omitted by the underlying call to `lm/lmer`.

Value

list() of where each entry is a model fit produced by `lmer()` or `lm()`

Examples

```
# load library
# library(variancePartition)

# optional step to run analysis in parallel on multicore machines
# Here, we used 4 threads
library(doParallel)
cl <- makeCluster(4)
registerDoParallel(cl)
# or by using the doSNOW package

# load simulated data:
# geneExpr: matrix of gene expression values
# info: information/metadata about each sample
data(varPartData)

# Specify variables to consider
# Age is continuous so we model it as a fixed effect
# Individual and Tissue are both categorical, so we model them as random effects
form <- ~ Age + (1|Individual) + (1|Tissue)

# Step 1: fit linear mixed model on gene expression
# If categorical variables are specified, a linear mixed model is used
# If all variables are modeled as continuous, a linear model is used
# each entry in results is a regression model fit on a single gene
# Step 2: extract variance fractions from each model fit
# for each gene, returns fraction of variation attributable to each variable
# Interpretation: the variance explained by each variable
# after correction for all other variables
varPart <- fitExtractVarPartModel( geneExpr, form, info )

# violin plot of contribution of each variable to total variance
plotVarPart( sortCols( varPart ) )

# Note: fitExtractVarPartModel also accepts ExpressionSet
data(sample.ExpressionSet, package="Biobase")

# ExpressionSet example
form <- ~ (1|sex) + (1|type) + score
info2 <- pData(sample.ExpressionSet)
varPart2 <- fitExtractVarPartModel( sample.ExpressionSet, form, info2 )

# stop cluster
stopCluster(cl)
```

fitVarPartModel	<i>Fit linear (mixed) model</i>
-----------------	---------------------------------

Description

Fit linear (mixed) model to estimate contribution of multiple sources of variation while simultaneously correcting for all other variables.

Usage

```
fitVarPartModel(exprObj, formula, data, REML = FALSE,
  useWeights = TRUE, weightsMatrix = NULL, showWarnings = TRUE,
  fxn = identity, control = lme4::lmerControl(calc.derivs = FALSE,
  check.rankX = "stop.deficient"), ...)
```

```
## S4 method for signature 'matrix'
fitVarPartModel(exprObj, formula, data, REML = FALSE,
  useWeights = TRUE, weightsMatrix = NULL, showWarnings = TRUE,
  fxn = identity, control = lme4::lmerControl(calc.derivs = FALSE,
  check.rankX = "stop.deficient"), ...)
```

```
## S4 method for signature 'data.frame'
fitVarPartModel(exprObj, formula, data,
  REML = FALSE, useWeights = TRUE, weightsMatrix = NULL,
  showWarnings = TRUE, fxn = identity,
  control = lme4::lmerControl(calc.derivs = FALSE, check.rankX =
  "stop.deficient"), ...)
```

```
## S4 method for signature 'EList'
fitVarPartModel(exprObj, formula, data, REML = FALSE,
  useWeights = TRUE, weightsMatrix = NULL, showWarnings = TRUE,
  fxn = identity, control = lme4::lmerControl(calc.derivs = FALSE,
  check.rankX = "stop.deficient"), ...)
```

```
## S4 method for signature 'ExpressionSet'
fitVarPartModel(exprObj, formula, data,
  REML = FALSE, useWeights = TRUE, weightsMatrix = NULL,
  showWarnings = TRUE, fxn = identity,
  control = lme4::lmerControl(calc.derivs = FALSE, check.rankX =
  "stop.deficient"), ...)
```

Arguments

exprObj	matrix of expression data (g genes x n samples), or ExpressionSet, or EList returned by voom() from the limma package
formula	specifies variables for the linear (mixed) model. Must only specify covariates, since the rows of exprObj are automatically used as a response. e.g.: ~ a + b + (1 c)
data	data.frame with columns corresponding to formula

REML	use restricted maximum likelihood to fit linear mixed model. default is FALSE. Strongly discourage against changing this option
useWeights	if TRUE, analysis uses heteroskedastic error estimates from voom(). Value is ignored unless exprObj is an EList() from voom() or weightsMatrix is specified
weightsMatrix	matrix the same dimension as exprObj with observation-level weights from voom(). Used only if useWeights is TRUE
showWarnings	show warnings about model fit (default TRUE)
fxn	apply function to model fit for each gene. Defaults to identify function so it returns the model fit itself
control	control settings for lmer()
...	Additional arguments for lmer() or lm()

Details

A linear (mixed) model is fit for each gene in exprObj, using formula to specify variables in the regression. If categorical variables are modeled as random effects (as is recommended), then a linear mixed model is used. For example if formula is $\sim a + b + (1|c)$, then the model is

```
fit <- lmer( exprObj[j,] ~ a + b + (1|c), data=data)
```

If there are no random effects, so formula is $\sim a + b + c$, a 'standard' linear model is used:

```
fit <- lm( exprObj[j,] ~ a + b + c, data=data)
```

In both cases, useWeights=TRUE causes weightsMatrix[j,] to be included as weights in the regression model.

Note: Fitting the model for 20,000 genes can be computationally intensive. To accelerate computation, models can be fit in parallel using foreach/dopar to run loops in parallel. Parallel processing must be enabled before calling this function. See below.

The regression model is fit for each gene separately. Samples with missing values in either gene expression or metadata are omitted by the underlying call to lm/lmer.

Since this function returns a list of each model fit, using this function is slower and uses more memory than fitExtractVarPartModel().

Value

list() of where each entry is a model fit produced by lmer() or lm()

Examples

```
# load library
# library(variancePartition)

# optional step to run analysis in parallel on multicore machines
# Here, we used 4 threads
library(doParallel)
cl <- makeCluster(4)
registerDoParallel(cl)
# or by using the doSNOW package

# load simulated data:
# geneExpr: matrix of gene expression values
# info: information/metadata about each sample
```



```

data(varPartData)

# Specify variables to consider
# Age is continuous so we model it as a fixed effect
# Individual and Tissue are both categorical, so we model them as random effects
form <- ~ Age + (1|Individual) + (1|Tissue)

# Step 1: fit linear mixed model on gene expression
# If categorical variables are specified, a linear mixed model is used
# If all variables are modeled as continuous, a linear model is used
# each entry in results is a regression model fit on a single gene
# Step 2: extract variance fractions from each model fit
# for each gene, returns fraction of variation attributable to each variable
# Interpretation: the variance explained by each variable
# after correction for all other variables
varPart <- fitExtractVarPartModel( geneExpr, form, info )

# violin plot of contribution of each variable to total variance
# also sort columns
plotVarPart( sortCols( varPart ) )

# Advanced:
# Fit model and extract variance in two separate steps
# Step 1: fit model for each gene, store model fit for each gene in a list
results <- fitVarPartModel( geneExpr, form, info )

# Step 2: extract variance fractions
varPart <- extractVarPart( results )

# Note: fitVarPartModel also accepts ExpressionSet
data(sample.ExpressionSet, package="Biobase")

# ExpressionSet example
form <- ~ (1|sex) + (1|type) + score
info2 <- pData(sample.ExpressionSet)
results2 <- fitVarPartModel( sample.ExpressionSet, form, info2 )

# stop cluster
stopCluster(cl)

```

getContrast

Extract contrast matrix for linear mixed model

Description

Extract contrast matrix, L, testing a single variable. Contrasts involving more than one variable can be constructed by modifying L directly

Usage

```
getContrast(exprObj, formula, data, coefficient)
```

Arguments

exprObj	matrix of expression data (g genes x n samples), or ExpressionSet, or EList returned by voom() from the limma package
formula	specifies variables for the linear (mixed) model. Must only specify covariates, since the rows of exprObj are automatically used as a response. e.g.: ~ a + b + (1 c) Formulas with only fixed effects also work
data	data.frame with columns corresponding to formula
coefficient	the coefficient to use in the hypothesis test

Value

Contrast matrix testing one variable

Examples

```
# load simulated data:
# geneExpr: matrix of gene expression values
# info: information/metadata about each sample
data(varPartData)

# get contrast matrix testing if the coefficient for Batch2 is zero
# The variable of interest must be a fixed effect
form <- ~ Batch + (1|Individual) + (1|Tissue)
L = getContrast( geneExpr, form, info, "Batch3")

# get contrast matrix testing if Batch3 - Batch2 = 0
form <- ~ Batch + (1|Individual) + (1|Tissue)
L = getContrast( geneExpr, form, info, c("Batch3", "Batch2"))

# To test against Batch1 use the formula:
# ~ 0 + Batch + (1|Individual) + (1|Tissue)
# to estimate Batch1 directly instead of using it as the baseline
```

getVarianceComponents *Extract variance terms*

Description

Extract variance terms from a model fit with lm() or lmer()

Usage

```
getVarianceComponents(fit)
```

Arguments

fit	list of lmer() model fits
-----	---------------------------

Value

variance explained by each variable

Examples

```
# library(variancePartition)

# optional step to run analysis in parallel on multicore machines
# Here, we used 4 threads
library(doParallel)
cl <- makeCluster(4)
registerDoParallel(cl)
# or by using the doSNOW package

# load simulated data:
# geneExpr: matrix of gene expression values
# info: information/metadata about each sample
data(varPartData)

# Specify variables to consider
# Age is continuous so we model it as a fixed effect
# Individual and Tissue are both categorical, so we model them as random effects
form <- ~ Age + (1|Individual) + (1|Tissue)

# Fit model and extract variance in two separate steps
# Step 1: fit model for each gene, store model fit for each gene in a list
modellist <- fitVarPartModel( geneExpr, form, info )

fit <- modellist[[1]]
getVarianceComponents( fit )

# stop cluster
stopCluster(cl)
```

ggColorHue

Default colors for ggplot

Description

Return an array of n colors the same as the default used by ggplot2

Usage

```
ggColorHue(n)
```

Arguments

n number of colors

Value

array of colors of length n

Examples

```
ggColorHue(4)
```

MArrayLM2-class	<i>Class MArrayLM2</i>
-----------------	------------------------

Description

Class MArrayLM2

plotCompareP	<i>Compare p-values from two analyses</i>
--------------	---

Description

Plot $-\log_{10}$ p-values from two analyses and color based on donor component from variancePartition analysis

Usage

```
plotCompareP(p1, p2, vpDonor, dupcorvalue, fraction = 0.2,
  xlabel = bquote(duplicateCorrelation ~ (-log[10] ~ p)),
  ylabel = bquote(dream ~ (-log[10] ~ p)))
```

Arguments

p1	p-value from first analysis
p2	p-value from second analysis
vpDonor	donor component for each gene from variancePartition analysis
dupcorvalue	scalar donor component from duplicateCorrelation
fraction	fraction of highest/lowest values to use for best fit lines
xlabel	for x-axis
ylabel	label for y-axis

Value

ggplot2 plot

Examples

```
# load library
# library(variancePartition)

# optional step to run analysis in parallel on multicore machines
# Here, we used 4 threads
library(doParallel)
cl <- makeCluster(4)
registerDoParallel(cl)
# or by using the doSNOW package

# load simulated data:
```

```
# geneExpr: matrix of gene expression values
# info: information/metadata about each sample
data(varPartData)

# Perform very simple analysis for demonstration

# Analysis 1
form <- ~ Batch
L = getContrast( geneExpr, form, info, "Batch3")
fit = dream( geneExpr, form, info, L)
fitEB = eBayes( fit )
res = topTable( fitEB, number=Inf )

# Analysis 2
form <- ~ Batch + (1|Tissue)
L = getContrast( geneExpr, form, info, "Batch3")
fit = dream( geneExpr, form, info, L)
fitEB = eBayes( fit )
res2 = topTable( fitEB, number=Inf )

# Compare p-values
plotCompareP( res$P.Value, res2$P.Value, runif(nrow(res)), .3 )

# stop cluster
stopCluster(cl)
```

plotContrasts

Plot representation of contrast matrix

Description

Plot contrast matrix to clarify interpretation of hypothesis tests with linear contrasts

Usage

```
plotContrasts(L)
```

Arguments

L contrast matrix

Value

ggplot2 object

Examples

```
# load library
# library(variancePartition)

# load simulated data:
# geneExpr: matrix of gene expression values
```

```

# info: information/metadata about each sample
data(varPartData)

# get contrast matrix testing if the coefficient for Batch2 is zero
form <- ~ Batch + (1|Individual) + (1|Tissue)
L1 = getContrast( geneExpr, form, info, "Batch3")

# get contrast matrix testing if the coefficient for Batch2 is different from Batch3
form <- ~ Batch + (1|Individual) + (1|Tissue)
L2 = getContrast( geneExpr, form, info, c("Batch2", "Batch3"))

# combine contrasts into single matrix
L_combined = cbind(L1, L2)

# plot contrasts
plotContrasts( L_combined )

```

plotCorrMatrix

plotCorrMatrix

Description

Plot correlation matrix

Usage

```
plotCorrMatrix(C, dendrogram = "both", sort = TRUE, margins = c(13,
  13), key.xlab = "correlation", ...)
```

Arguments

C	correlation matrix: R or R ² matrix
dendrogram	character string indicating whether to draw 'both' or none'
sort	sort rows and columns based on clustering
margins	spacing of plot
key.xlab	label of color gradient
...	additional arguments to heatmap.2

Details

Plots image of correlation matrix using customized call to heatmap.2

Value

Image of correlation matrix

Examples

```
# simulate simple matrix of 10 variables
mat = matrix(rnorm(1000), ncol=10)

# compute correlation matrix
C = cor(mat)

# plot correlations
plotCorrMatrix( C )

# plot squared correlations
plotCorrMatrix( C^2, dendrogram="none" )
```

plotCorrStructure *plotCorrStructure*

Description

Plot correlation structure of a gene based on random effects

Usage

```
plotCorrStructure(fit, varNames = names(coef(fit)), reorder = TRUE,
  pal = colorRampPalette(c("white", "red", "darkred")),
  hclust.method = "complete")
```

Arguments

fit	linear mixed model fit of a gene produced by lmer() or fitVarPartModel()
varNames	variables in the metadata for which the correlation structure should be shown. Variables must be random effects
reorder	how to reorder the rows/columns of the correlation matrix. reorder=FALSE gives no reorder. reorder=TRUE reorders based on hclust. reorder can also be an array of indices to reorder the samples manually
pal	color palette
hclust.method	clustering methods for hclust

Value

Image of correlation structure between each pair of experiments for a single gene

Examples

```
# load library
# library(variancePartition)

# optional step to run analysis in parallel on multicore machines
# Here, we used 4 threads
```

```

library(doParallel)
cl <- makeCluster(4)
registerDoParallel(cl)
# or by using the doSNOW package

# load simulated data:
data(varPartData)

# specify formula
form <- ~ Age + (1|Individual) + (1|Tissue)

# fit and return linear mixed models for each gene
fitList <- fitVarPartModel( geneExpr[1:10,], form, info )

# Focus on the first gene
fit = fitList[[1]]

# plot correlation structure based on Individual, reordering samples with hclust
plotCorrStructure( fit, "Individual" )

# don't reorder
plotCorrStructure( fit, "Individual", reorder=FALSE )

# plot correlation structure based on Tissue, reordering samples with hclust
plotCorrStructure( fit, "Tissue" )

# don't reorder
plotCorrStructure( fit, "Tissue", FALSE )

# plot correlation structure based on all random effects
# reorder manually by Tissue and Individual
idx = order(info$Tissue, info$Individual)
plotCorrStructure( fit, reorder=idx )

# plot correlation structure based on all random effects
# reorder manually by Individual, then Tissue
idx = order(info$Individual, info$Tissue)
plotCorrStructure( fit, reorder=idx )

# stop cluster
stopCluster(cl)

```

plotPercentBars

Bar plot of variance fractions

Description

Bar plot of variance fractions for a subset of genes

Usage

```

plotPercentBars(varPart, col = c(ggColorHue(ncol(varPart) - 1),
  "grey85"))

```


Arguments

varPart object returned by extractVarPart() or fitExtractVarPartModel()
 col color of bars for each variable

Value

Returns ggplot2 barplot

Examples

```
# library(variancePartition)

# optional step to run analysis in parallel on multicore machines
# Here, we used 4 threads
library(doParallel)
cl <- makeCluster(4)
registerDoParallel(cl)
# or by using the doSNOW package

# load simulated data:
# geneExpr: matrix of gene expression values
# info: information/metadata about each sample
data(varPartData)

# Specify variables to consider
form <- ~ Age + (1|Individual) + (1|Tissue)

# Fit model
varPart <- fitExtractVarPartModel( geneExpr, form, info )

# Bar plot for a subset of genes showing variance fractions
plotPercentBars( varPart[1:5,] )

# Move the legend to the top
plotPercentBars( varPart[1:5,] ) + theme(legend.position="top")

# stop cluster
stopCluster(cl)
```

plotStratify

plotStratify

Description

Plot gene expression stratified by another variable

Usage

```
plotStratify(formula, data, xlab, ylab, main, sortBy, colorBy,
  sort = TRUE, text = NULL, text.y = 1, text.size = 5,
  pts.cex = 1, ylim = NULL, legend = TRUE, x.labels = FALSE)
```

Arguments

formula	specify variables shown in the x- and y-axes. Y-axis should be continuous variable, x-axis should be discrete.
data	data.frame storing continuous and discrete variables specified in formula
xlab	label x-axis. Defaults to value of xval
ylab	label y-axis. Defaults to value of yval
main	main label
sortBy	name of column in geneExpr to sort samples by. Defaults to xval
colorBy	name of column in geneExpr to color box plots. Defaults to xval
sort	if TRUE, sort boxplots by median value, else use default ordering
text	plot text on the top left of the plot
text.y	indicate position of the text on the y-axis as a fraction of the y-axis range
text.size	size of text
pts.cex	size of points
ylim	specify range of y-axis
legend	show legend
x.labels	show x axis labels

Value

ggplot2 object

Examples

```
# Note: This is a newer, more convient interface to plotStratifyBy()

# load library
# library(variancePartition)

# load simulated data:
data(varPartData)

# Create data.frame with expression and Tissue information for each sample
GE = data.frame( Expression = geneExpr[1,], Tissue = info$Tissue)

# Plot expression stratified by Tissue
plotStratify( Expression ~ Tissue, GE )

# Omit legend and color boxes grey
plotStratify( Expression ~ Tissue, GE, colorBy = NULL)

# Specify colors
col = c( B="green", A="red", C="yellow")
plotStratify( Expression ~ Tissue, GE, colorBy=col, sort=FALSE)
```

plotStratifyBy	<i>plotStratifyBy</i>
----------------	-----------------------

Description

Plot gene expression stratified by another variable

Usage

```
plotStratifyBy(geneExpr, xval, yval, xlab = xval, ylab = yval,  
  main = NULL, sortBy = xval, colorBy = xval, sort = TRUE,  
  text = NULL, text.y = 1, text.size = 5, pts.cex = 1,  
  ylim = NULL, legend = TRUE, x.labels = FALSE)
```

Arguments

geneExpr	data.frame of gene expression values and another variable for each sample. If there are multiple columns, the user can specify which one to use
xval	name of column in geneExpr to be used along x-axis to stratify gene expression
yval	name of column in geneExpr indicating gene expression
xlab	label x-axis. Defaults to value of xval
ylab	label y-axis. Defaults to value of yval
main	main label
sortBy	name of column in geneExpr to sort samples by. Defaults to xval
colorBy	name of column in geneExpr to color box plots. Defaults to xval
sort	if TRUE, sort boxplots by median value, else use default ordering
text	plot text on the top left of the plot
text.y	indicate position of the text on the y-axis as a fraction of the y-axis range
text.size	size of text
pts.cex	size of points
ylim	specify range of y-axis
legend	show legend
x.labels	show x axis labels

Value

ggplot2 object

Examples

```
# load library  
# library(variancePartition)  
  
# load simulated data:  
data(varPartData)
```

```
# Create data.frame with expression and Tissue information for each sample
GE = data.frame( Expression = geneExpr[1,], Tissue = info$Tissue)

# Plot expression stratified by Tissue
plotStratifyBy( GE, "Tissue", "Expression")

# Omit legend and color boxes grey
plotStratifyBy( GE, "Tissue", "Expression", colorBy = NULL)

# Specify colors
col = c( B="green", A="red", C="yellow")
plotStratifyBy( GE, "Tissue", "Expression", colorBy=col, sort=FALSE)
```

plotVarPart

Violin plot of variance fractions

Description

Violin plot of variance fraction for each gene and each variable

Usage

```
plotVarPart(obj, col = c(ggColorHue(ncol(obj) - 1), "grey85"),
  label.angle = 20, main = "", ylab = "", convertToPercent = TRUE,
  ...)
```

```
## S4 method for signature 'matrix'
plotVarPart(obj, col = c(ggColorHue(ncol(obj) - 1),
  "grey85"), label.angle = 20, main = "", ylab = "",
  convertToPercent = TRUE, ...)
```

```
## S4 method for signature 'data.frame'
plotVarPart(obj, col = c(ggColorHue(ncol(obj) -
  1), "grey85"), label.angle = 20, main = "", ylab = "",
  convertToPercent = TRUE, ...)
```

```
## S4 method for signature 'varPartResults'
plotVarPart(obj, col = c(ggColorHue(ncol(obj)
  - 1), "grey85"), label.angle = 20, main = "", ylab = "",
  convertToPercent = TRUE, ...)
```

Arguments

obj	varParFrac object returned by fitExtractVarPart or extractVarPart
col	vector of colors
label.angle	angle of labels on x-axis
main	title of plot
ylab	text on y-axis
convertToPercent	multiply fractions by 100 to convert to percent values
...	additional arguments

Value

Makes violin plots of variance components model. This function uses the graphics interface from ggplot2. Warnings produced by this function usually ggplot2 warning that the window is too small.

Examples

```
# load library
# library(variancePartition)

# optional step to run analysis in parallel on multicore machines
# Here, we used 4 threads
library(doParallel)
cl <- makeCluster(4)
registerDoParallel(cl)
# or by using the doSNOW package

# load simulated data:
# geneExpr: matrix of gene expression values
# info: information/metadata about each sample
data(varPartData)

# Specify variables to consider
# Age is continuous so we model it as a fixed effect
# Individual and Tissue are both categorical, so we model them as random effects
form <- ~ Age + (1|Individual) + (1|Tissue)

varPart <- fitExtractVarPartModel( geneExpr, form, info )

# violin plot of contribution of each variable to total variance
plotVarPart( sortCols( varPart ) )

# stop cluster
stopCluster(cl)
```

residuals, VarParFitList-method

Residuals from model fit

Description

Extract residuals for each gene from model fit with fitVarPartModel()

Usage

```
## S4 method for signature 'VarParFitList'
residuals(object, ...)
```

Arguments

object	object produced by fitVarPartModel()
...	other arguments.

Details

If model is fit with missing data, residuals returns NA for entries that were missing in the original data

Value

Residuals extracted from model fits stored in object

Examples

```
# load library
# library(variancePartition)

# optional step to run analysis in parallel on multicore machines
# Here, we used 4 threads
library(doParallel)
cl <- makeCluster(4)
registerDoParallel(cl)
# or by using the doSNOW package

# load simulated data:
# geneExpr: matrix of gene expression values
# info: information/metadata about each sample
data(varPartData)

# Specify variables to consider
# Age is continuous so we model it as a fixed effect
# Individual and Tissue are both categorical, so we model them as random effects
form <- ~ Age + (1|Individual) + (1|Tissue)

# Fit model
modelFit <- fitVarPartModel( geneExpr, form, info )

# Extract residuals of model fit
res <- residuals( modelFit )

# stop cluster
stopCluster(cl)
```

sortCols

Sort variance partition statistics

Description

Sort columns returned by `extractVarPart()` or `fitExtractVarPartModel()`

Usage

```
sortCols(x, FUN = median, decreasing = TRUE, last = c("Residuals",
  "Measurement.error"), ...)
```

```
## S4 method for signature 'matrix'
```

```

sortCols(x, FUN = median, decreasing = TRUE,
  last = c("Residuals", "Measurement.error"), ...)

## S4 method for signature 'data.frame'
sortCols(x, FUN = median, decreasing = TRUE,
  last = c("Residuals", "Measurement.error"), ...)

## S4 method for signature 'varPartResults'
sortCols(x, FUN = median, decreasing = TRUE,
  last = c("Residuals", "Measurement.error"), ...)

```

Arguments

x	object returned by extractVarPart() or fitExtractVarPartModel()
FUN	function giving summary statistic to sort by. Defaults to median
decreasing	logical. Should the sorting be increasing or decreasing?
last	columns to be placed on the right, regardless of values in these columns
...	other arguments to sort

Value

data.frame with columns sorted by mean value, with Residuals in last column

Examples

```

# library(variancePartition)

# optional step to run analysis in parallel on multicore machines
# Here, we used 4 threads
library(doParallel)
cl <- makeCluster(4)
registerDoParallel(cl)
# or by using the doSNOW package

# load simulated data:
# geneExpr: matrix of gene expression values
# info: information/metadata about each sample
data(varPartData)

# Specify variables to consider
# Age is continuous so we model it as a fixed effect
# Individual and Tissue are both categorical, so we model them as random effects
form <- ~ Age + (1|Individual) + (1|Tissue)

# Step 1: fit linear mixed model on gene expression
# If categorical variables are specified, a linear mixed model is used
# If all variables are modeled as continuous, a linear model is used
# each entry in results is a regression model fit on a single gene
# Step 2: extract variance fractions from each model fit
# for each gene, returns fraction of variation attributable to each variable
# Interpretation: the variance explained by each variable
# after correction for all other variables
varPart <- fitExtractVarPartModel( geneExpr, form, info )

```

```
# violin plot of contribution of each variable to total variance
# sort columns by median value
plotVarPart( sortCols( varPart ) )

# stop cluster
stopCluster(cl)
```

VarParCIList-class *Class VarParCIList*

Description

Class VarParCIList

VarParFitList-class *Class VarParFitList*

Description

Class VarParFitList

varParFrac-class *Class varParFrac*

Description

Class varParFrac

varPartConfInf *Linear mixed model confidence intervals*

Description

Fit linear mixed model to estimate contribution of multiple sources of variation while simultaneously correcting for all other variables. Then perform parametric bootstrap sampling to get a 95% confidence intervals for each variable for each gene.

Usage

```
varPartConfInf(exprObj, formula, data, REML = FALSE, useWeights = TRUE,
  weightsMatrix = NULL, adjust = NULL, adjustAll = FALSE,
  showWarnings = TRUE, colinearityCutoff = 0.999,
  control = lme4::lmerControl(calc.derivs = FALSE, check.rankX =
    "stop.deficient"), nsim = 1000, ...)
```


Arguments

exprObj	matrix of expression data (g genes x n samples), or ExpressionSet, or EList returned by voom() from the limma package
formula	specifies variables for the linear (mixed) model. Must only specify covariates, since the rows of exprObj are automatically used as a response. e.g.: ~ a + b + (1 c)
data	data.frame with columns corresponding to formula
REML	use restricted maximum likelihood to fit linear mixed model. default is FALSE. Strongly discourage against changing this option
useWeights	if TRUE, analysis uses heteroskedastic error estimates from voom(). Value is ignored unless exprObj is an EList() from voom() or weightsMatrix is specified
weightsMatrix	matrix the same dimension as exprObj with observation-level weights from voom(). Used only if useWeights is TRUE
adjust	remove variation from specified variables from the denominator. This computes the adjusted ICC with respect to the specified variables
adjustAll	adjust for all variables. This computes the adjusted ICC with respect to all variables. This overrides the previous argument, so all variables are included in adjust.
showWarnings	show warnings about model fit (default TRUE)
colinearityCutoff	cutoff used to determine if model is computationally singular
control	control settings for lmer()
nsim	number of bootstrap datasets
...	Additional arguments for lmer() or lm()

Details

A linear mixed model is fit for each gene, and bootMer() is used to generate parametric bootstrap confidence intervals. use.u=TRUE is used so that the \hat{u} values from the random effects are used as estimated and are not re-sampled. This gives confidence intervals as if additional data were generated from these same current samples. Conversely, use.u=FALSE assumes that this dataset is a sample from a larger population. Thus it simulates \hat{u} based on the estimated variance parameter. This approach gives confidence intervals as if additional data were collected from the larger population from which this dataset is sampled. Overall, use.u=TRUE gives smaller confidence intervals that are appropriate in this case.

Value

list() of where each entry is the result for a gene. Each entry is a matrix of the 95% confidence interval of the variance fraction for each variable

Examples

```
# load library
# library(variancePartition)

# optional step to run analysis in parallel on multicore machines
# Here, we used 4 threads
```

```

library(doParallel)
cl <- makeCluster(4)
registerDoParallel(cl)
# or by using the doSNOW package

# load simulated data:
# geneExpr: matrix of gene expression values
# info: information/metadata about each sample
data(varPartData)

# Specify variables to consider
# Age is continuous so we model it as a fixed effect
# Individual and Tissue are both categorical, so we model them as random effects
form <- ~ Age + (1|Individual) + (1|Tissue)

# Compute bootstrap confidence intervals for each variable for each gene
resCI <- varPartConfInf( geneExpr[1:5,], form, info, nsim=100 )

# stop cluster
stopCluster(cl)

```

varPartData

Simulation dataset for examples

Description

A simulated dataset of gene expression and metadata
A simulated dataset of gene counts
info about study design
Normalized expression data

Usage

```

data(varPartData)

data(varPartData)

data(varPartData)

data(varPartData)

```

Format

A dataset of 100 samples and 200 genes

Details

- geneCounts gene expression in the form of RNA-seq counts
- geneExpr gene expression on a continuous scale
- info metadata about the study design

- geneCounts gene expression in the form of RNA-seq counts
- geneExpr gene expression on a continuous scale
- info metadata about the study design
- geneCounts gene expression in the form of RNA-seq counts
- geneExpr gene expression on a continuous scale
- info metadata about the study design
- geneCounts gene expression in the form of RNA-seq counts
- geneExpr gene expression on a continuous scale
- info metadata about the study design

varPartDEdata *Simulation dataset for dream example*

Description

Gene counts from RNA-seq
 metadata matrix of sample information

Usage

data(varPartDEdata)

data(varPartDEdata)

Format

A dataset of 24 samples and 19,364 genes

Details

- countMatrix gene expression in the form of RNA-seq counts
- metadata metadata about the study design
- countMatrix gene expression in the form of RNA-seq counts
- metadata metadata about the study design

varPartResults-class *Class varPartResults*

Description

Class varPartResults

`[.MArrayLM2`*Subsetting for MArrayLM2*

Description

Enable subsetting on MArrayLM2 object. Same as for MArrayLM, but apply column subsetting to df.residual and pValue

Arguments

object	MArrayLM2
i	row
j	col

Value

subset

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