CpGassoc

May 25, 2012

cpg.assoc	*Association Analysis Between Methylation Beta Values and Phenotype of Interest*

Usage

cpg.assoc(beta.val, indep, covariates = NULL, data = NULL, logit.transform = FALSE, chip.id = NULL, subset = NULL, random = FALSE, fdr.cutoff = 0.05, large.data = TRUE, fdr.method = "BH", logitperm = FALSE)

Arguments

beta.val	A vector, matrix, or data frame containing the beta values of interest (1 row per CpG site, 1 column per individual).
indep	A vector containing the variable to be tested for association. `cpg.assoc` will evaluate the association between the beta values (dependent variable) and indep (independent variable).
covariates	A data frame consisting of additional covariates to be included in the model. covariates can also be specified as a matrix if it takes the form of a model matrix with no intercept column, or can be specified as a vector if there is only one covariate of interest. Can also be a formula(e.g. `cov1+cov2`).
data	an optional data frame, list or environment (or object coercible by as.data.frame to a data frame) containing the variables in the model. If not found in data, the variables are taken from the environment from which cpg.assoc is called.
logit.transform	Logical. If `TRUE`, the logit transform of the beta values log(beta.val/(1-beta.val)) will be used. Any values equal to zero or one will be set to the next smallest or next largest value respectively; values <0 or >1 will be set to NA.

chip.id
An optional vector containing chip or batch identifiers. If specified, `chip.id` will be included as a factor in the model.

subset
An optional logical vector specifying a subset of observations to be used in the fitting process.

random
Logical. If `TRUE`, `chip.id` will be included in the model as a random effect, and a random intercept model will be fitted. If `FALSE`, `chip.id` will be included in the model as an ordinary categorical covariate, for a much faster analysis.

fdr.cutoff
The desired FDR threshold. The default setting is .05. The set of CpG sites with FDR < `fdr.cutoff` will be labeled as significant.

large.data
Logical. Enables analyses of large datasets. When `large.data=TRUE`, `cpg.assoc` avoids memory problems by performing the analysis in chunks.

fdr.method
Character. Method used to calculate False Discovery Rate. Choices include any of the methods available in `p.adjust()` or ”qvalue” for John Storey’s qvalue method (requires that *qvalue* package is installed). The default method is ”BH” for the Benjamini and Hochberg method.

logitperm
Logical. For internal use only.

Details

`cpg.assoc` is designed to test for association between an independent variable and methylation at a number of CpG sites, with the option to include additional covariates and factors. `cpg.assoc` assesses significance with the Holm (step-down Bonferroni) and FDR methods.

If `class(indep)=’factor’`, `cpg.assoc` will perform an ANOVA test of the variable conditional on the covariates specified. Covariates, if entered, should be in the form of a data frame, matrix, or vector. For example, `covariates=data.frame(weight,age,factor(city))`. The data frame can also be specified prior to calling `cpg.assoc`. The covariates should either be vectors or columns of a matrix or data.frame.

`cpg.assoc` is also designed to deal with large data sets. Setting `large.data=TRUE` will make `cpg.assoc` split up the data to enable efficient analysis of large datasets.

Value

`cpg.assoc` will return an object of class *cpg*. The functions `summary` and `plot` can be called to get a summary of results and to create QQ plots.

results

A data frame consisting of the t or F statistics and P-values for each CpG site, as well as indicators of Holm and FDR significance. CpG sites will be in the same order as the original input, but the sort() function can be used directly on the cpg.assoc object to sort CpG sites by p-value.

results

A data frame consisting of the t or F statistics and P-values for each CpG site, as well as indicators of Holm and FDR significance. CpG sites will be in the same order as the original input, but the `sort()` function can be used directly on the `cpg.assoc` object to sort CpG sites by p-value.

Holm.sig

A list of sites that met criteria for Holm significance.

FDR.sig

A data.frame of the CpG sites that were significant by the FDR method specified.

info

A data frame consisting of the minimum P-value observed, the FDR method that was used, the phenotype of interest, the number of covariates in the model, the name of the matrix or data frame the methylation beta values were taken from, the FDR cutoff value and whether a mixed effects analysis was performed.

indep

The independent variable that was tested for association.

covariates

Data.frame or matrix of covariates, if specified (otherwise `NULL`).

chip — chip.id vector, if specified (otherwise `NULL`).

coefficients — A data frame consisting of the residual degrees of freedom, the intercept effect adjusted for possible covariates in the model, the estimated effect size, and the standard error. This item will only be returned if indep is continuous. The degrees of freedom is used in plot.cpg to compute the expected t-statistics. If indep is a categorical variable this object will be `NULL`.

Authors

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See Also

cpg.perm, cpg.work, plot.cpg scatterplot, cpg.combine, manhattan, plot.cpg.perm, sort.cpg.perm, sort.cpg, cpg.qc

Examples

```
> #Sample output from CpGassoc
> ###NOTE: If you are dealing with large data, do not specify large.data=FALSE.
> ##This will involve partitioning up the data and performing more gc() to clea
> library(CpGassoc)
> data(samplecpg,samplepheno,package="CpGassoc")
> results<-cpg.assoc(samplecpg,samplepheno$weight,large.data=FALSE)
> results
```

```
The top ten CpG sites were:
     CPG.Labels T.statistic      P.value Holm.sig       FDR
694      CpG694    3.454271 0.0006456268    FALSE 0.4318310
293      CpG293    3.412320 0.0007485123    FALSE 0.4318310
560      CpG560    3.313353 0.0010549618    FALSE 0.4318310
148      CpG148    3.133454 0.0019286973    FALSE 0.5645412
998      CpG998   -3.079596 0.0022986204    FALSE 0.5645412
1059    CpG1059   -2.883525 0.0042668430    FALSE 0.7693539
1182    CpG1182   -2.819710 0.0051827097    FALSE 0.7693539
100      CpG100    2.787987 0.0057015107    FALSE 0.7693539
751      CpG751   -2.759379 0.0062093208    FALSE 0.7693539
```

```
238      CpG238    2.756367 0.0062650966    FALSE 0.7693539

To access results for all  1228  CpG sites use object$results
or sort(object)$results to obtain results sorted by p-value.

General info:
  Min.P.Observed Num.Cov fdr.cutoff FDR.method Phenotype chipinfo num.Holm
1   0.0006456268       0       0.05         BH    weight     NULL        0
  num.fdr
1       0

0 sites were found significant by the Holm method
0 sites were found significant by BH method

The beta values were taken from: samplecpg
Effect sizes and standard error can be accessed using $coefficients
Other attributes are: results, Holm.sig, FDR.sig, info, indep, covariates, chip
 They can be accessed using the $

> #Analysis with covariates. There are multiple ways to do this. One can define
> #dataframe prior or do it in the function call or as a function such as ~Cov1
> #We will do it in the function call
> test<-cpg.assoc(samplecpg,samplepheno$weight,data.frame(samplepheno$Distance,
> #Doing a mixed effects model. This does take more time, so we will do a subse
> #the samplecpg
> randtest<-cpg.assoc(samplecpg[1:10,],samplepheno$weight,chip.id=samplepheno$c
>
> #summary function will work on items of class cpg.
>
>
```

cpg.combine *Combine various objects of class cpg*

Description

Takes a list containing objects of class *cpg* and combines them into one cpg item. Assumes that there are no repeated CpG sites bewtween the various objects (i.e. analysis wasn't performed on the same sites twice).

usage

cpg.combine(allvalues, fdr.method="BH",fdr.cutoff=.05)

Arguments

allvalues A list containing the *cpg* objects that are desired to be consolidated.

fdr.method FDR method that user wants to use. For options see the `cpg.assoc` help page.

fdr.cutoff The desired FDR threshold. The default setting is .05. The set of CpG sites with FDR < fdr.cutoff will be labeled as significant.

Value

indo.data An object of class *cpg* that is the consolidated version of the objects of class cpg that were passed in.

Authors

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Note

This is designed to be used by `cpg.assoc` when it does analysis on large data sets or by the user if they split up the analysis by chromosome or some other such partition.

See Also

cpg.perm, cpg.work, plot.cpg scatterplot, cpg.assoc, manhattan, plot.cpg.perm, sort.cpg.perm, sort.cpg

Examples

```
> library(CpGassoc)
> data(samplecpg,samplepheno,package="CpGassoc")
> ###NOTE: If you are dealing with large data, do not specify large.data=FALSE.
> ##This will involve partitioning up the data and performing more gc() to clea
> test1<-cpg.assoc(samplecpg[1:100,],samplepheno$weight,large.data=FALSE)
> test2<-cpg.assoc(samplecpg[101:200,],samplepheno$weight,large.data=FALSE)
> overall<-cpg.combine(list(test1,test2))
> overall

The top ten CpG sites were:
    CPG.Labels T.statistic     P.value Holm.sig       FDR
148     CpG148    3.133454 0.001928697    FALSE 0.3857395
100     CpG100    2.787987 0.005701511    FALSE 0.5701511
52       CpG52   -2.400358 0.017093566    FALSE 0.6753972
3         CpG3   -2.307436 0.021828750    FALSE 0.6753972
85       CpG85    2.289916 0.022840129    FALSE 0.6753972
72       CpG72   -2.093410 0.037296699    FALSE 0.6753972
153     CpG153   -2.080196 0.038502367    FALSE 0.6753972
178     CpG178   -2.055509 0.040844281    FALSE 0.6753972
70       CpG70   -2.023648 0.044045272    FALSE 0.6753972
35       CpG35   -2.000859 0.046463353    FALSE 0.6753972

To access results for all  200  CpG sites use object$results
or sort(object)$results to obtain results sorted by p-value.

General info:
  Min.P.Observed Num.Cov fdr.cutoff FDR.method Phenotype chipinfo num.Holm
1    0.001928697       0       0.05         BH    weight     NULL        0
  num.fdr
1       0

0 sites were found significant by the Holm method
0 sites were found significant by BH method

The beta values were taken from: samplecpg
Effect sizes and standard error can be accessed using $coefficients
Other attributes are: results, Holm.sig, FDR.sig, info, indep
 They can be accessed using the $
```

cpg.perm — *Perform a Permutation Test of the Association Between Methylation and a Phenotype of Interest*

Description

Calls `cpg.assoc` to get the observed P-values from the study and then performs a user-specified number of permutations to calculate an emperical p-value. In addition to the same test statistics computed by `cpg.assoc`, `cpg.perm` will compute the permutation p-values for the observed p-value, the number of Holm significant sites, and the number of FDR significant sites.

Usage

cpg.perm(beta.values, indep, covariates = NULL, nperm, data = NULL, seed = NULL, logit.transform = FALSE, chip.id = NULL, subset = NULL, random = FALSE, fdr.cutoff = 0.05, fdr.method = ”BH”,large.data=TRUE)

Arguments

beta.values
A vector, matrix, or data frame containing the beta values of interest (1 row per CpG site, 1 column per individual).

indep
A vector containing the main variable of interest. `cpg.assoc` will evaluate the association between indep and the beta values.

covariates
A data frame consisting of the covariates of interest. covariates can also be a matrix if it is a model matrix minus the intercept column. It can also be a vector if there is only one covariate of interest. Can also be a formula(e.g. `cov1+cov2`).

nperm
The number of permutations to be performed.

data
an optional data frame, list or environment (or object coercible by `as.data.frame` to a data frame) containing the variables in the model. If not found in data, the variables are taken from the environment from which `cpg.perm` is called.

seed
The required seed for random number generation. If not input, will use R’s internal seed.

logit.transform
Logical. If `TRUE`, the logit transform of the beta values log(beta.val/(1-beta.val)) will be used. Any values equal to zero or one will be set to the next smallest or next largest value respectively; values <0 or >1 will be set to NA.

chip.id
An optional vector containing the chip information. If specified, chip id will be included as a factor in the model.

subset
An optional logical vector specifying a subset of observations to be used in the fitting process.

random
Logical. If `TRUE`, the `chip.id` will be processed as a random effect, and a random intercept model will be fitted.

fdr.cutoff
The threshold at which to compare the FDR values. The default setting is .05. Any FDR values less than .05 will be considered significant.

fdr.method
Character. Method used to calculate False Discovery Rate. Can be any of the methods listed in `p.adjust` or ”qvalue” for John Storey’s qvalue method (required to have *qvalue* package installed). The default method is ”BH” for the Benjamini and Hochberg method.

large.data — Logical. Enables analyses of large datasets. When `large.data=TRUE`, `cpg.assoc` avoids memory problems by performing the analysis in chunks.

Value

The item returned will be of class *cpg.perm*. It will contain all of the values of class
cpg cpg.assoc and a few more:

permutation.matrix — A matrix consisting of the minimum observed P-value, the number of Holm significant CpG sites, and the number of FDR significant sites for each permutation.

perm.p.values — A data frame consisting of the permutation P-values, and the number of permutations performed.

perm.tstat — If one hundred or more permutations were performed and indep is a continuous variable, consists of the quantile .025 and .975 of observed t-statistcs for each permutation, ordered from smallest to largest. perm.tstat is used by `plot.cpg.perm` to compute the confidence intervals for the QQ plot of t-statistics. Otherwise `NULL`.

perm.pval — If one hundred or more permutations were performed, consists of the observed p-values for each permutation, ordered from smallest to largest. perm.pval is usd by `plot.cpg.perm` to compute the confidence intervals for the QQ plot of the p-values. Otherwise `NULL`.

Authors

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See Also

cpg.assoc, cpg.work, plot.cpg scatterplot, cpg.combine, manhattan, plot.cpg.perm,
sort.cpg.perm, sort.cpg, cpg.qc

Examples

```
> ##Loading the data
> library(CpGassoc)
> data(samplecpg,samplepheno,package="CpGassoc")
> ###NOTE: If you are dealing with large data, do not specify large.data=FALSE. The default option i
> ##This will involve partitioning up the data and performing more gc() to clear up space
> #Performing a permutation 10 times
> Testperm<-cpg.perm(samplecpg,samplepheno$weight,data.frame(samplepheno$Dose,samplepheno$Distance),
+                 seed=2314,nperm=10,large.data=FALSE)
> Testperm
```

```
The permutation P-values, number of permutations and seed:
  p.value.p p.value.holm p.value.FDR nperm seed
```

```
1       0.3            1           1    10 2314

Other information:
  Min.P.Observed Num.Cov fdr.cutoff FDR.method num.real.Holm num.real.fdr
1   0.0006002142       2       0.05         BH             0            0

The top ten CpG sites were:
     CPG.Labels T.statistic      P.value Holm.sig       FDR
694      CpG694    3.475160 0.0006002142    FALSE 0.3833341
293      CpG293    3.464076 0.0006243226    FALSE 0.3833341
560      CpG560    3.333678 0.0009848497    FALSE 0.4031318
148      CpG148    3.187753 0.0016135434    FALSE 0.4953578
238      CpG238    3.012760 0.0028504303    FALSE 0.5921086
998      CpG998   -3.008091 0.0028930386    FALSE 0.5921086
1059    CpG1059   -2.932014 0.0036749081    FALSE 0.6295151
100      CpG100    2.889847 0.0041873059    FALSE 0.6295151
1006    CpG1006   -2.831992 0.0049965867    FALSE 0.6295151
1182    CpG1182   -2.823521 0.0051263442    FALSE 0.6295151

To access results for all  1228  CpG sites use object$results
 or sort(object)$results to obtain results sorted by p-value.

0 sites were found significant by the Holm method
0 sites were found significant by BH method

The beta values were taken from: samplecpg
Other attributes are: permutation.matrix, perm.p.values, results, Holm.sig, FDR.sig ,
 info, indep, covariates, chip, coefficients.
They can be accessed using the $
```

```
> #All the contents of CpGassoc are included in the output from Testperm
> #Using the output from CpGassoc in the example
> test<-cpg.assoc(samplecpg,samplepheno$weight,data.frame(samplepheno$Distance,samplepheno$Dose),lar
> all.equal(Testperm$results,test$results)
```

```
[1] TRUE
```

```
> #summary function works on objects of class cpg.perm
> summary(Testperm)
```

```
The permutation P-values, number of permutations and seed:
  p.value.p p.value.holm p.value.FDR nperm seed
1       0.3            1           1    10 2314

Other information:
  Min.P.Observed Num.Cov fdr.cutoff FDR.method num.real.Holm num.real.fdr
1   0.0006002142       2       0.05         BH             0            0

The top ten CpG sites were:
     CPG.Labels T.statistic      P.value Holm.sig       FDR
694      CpG694    3.475160 0.0006002142    FALSE 0.3833341
293      CpG293    3.464076 0.0006243226    FALSE 0.3833341
560      CpG560    3.333678 0.0009848497    FALSE 0.4031318
148      CpG148    3.187753 0.0016135434    FALSE 0.4953578
238      CpG238    3.012760 0.0028504303    FALSE 0.5921086
998      CpG998   -3.008091 0.0028930386    FALSE 0.5921086
```

```
1059    CpG1059   -2.932014 0.0036749081    FALSE 0.6295151
100      CpG100    2.889847 0.0041873059    FALSE 0.6295151
1006    CpG1006   -2.831992 0.0049965867    FALSE 0.6295151
1182    CpG1182   -2.823521 0.0051263442    FALSE 0.6295151

To access results for all  1228  CpG sites use object$results
 or sort(object)$results to obtain results sorted by p-value.

0 sites were found significant by the Holm method
0 sites were found significant by BH method

The beta values were taken from: samplecpg
Other attributes are: permutation.matrix, perm.p.values, results, Holm.sig, FDR.sig ,
 info, indep, covariates, chip, coefficients.
They can be accessed using the $

>
```

cpg.qc *Performs quality control on Illumina data.*

Description

`cpg.qc` is designed to perform quality control on Illumina data prior to analysis. In addition to the matrix of beta values, this function requires as input matrices of Signal A, Signal B, and detection p-values. It will remove samples that have low intensity (mean signal intensity less than half of the overall median or 2000). It can also set to NA datapoints with detection p-values exceeding a user-specified cutoff, and can remove samples or sites that have a missing rate above a user-specified value. Finally, users can opt to compute beta values as M/(U+M) or M/(U+M+100).

Usage

cpg.qc(beta.orig,siga,sigb,pval,p.cutoff=.001,cpg.miss=NULL,sample.miss=NULL,constant100=FALSE)

Arguments

beta.orig The original beta values matrix obtained from GenomeStudio.

siga The unmethylated signals matrix obtained from GenomeStudio.

sigb The methylated signals matrix obtained from GenomeStudio.

pval A matrix of detection p-values obtained from GenomeStudio. pval should have the same dimension as the beta values and signals: one row for each site and one column for each individual.

p.cutoff The user-specified cutoff for detection p-values (default=.001).

cpg.miss Optional cutoff value. If specified, cpg.qc will remove cpg sites where the proportion of missing values exceeds this cutoff.

sample.miss	Optional cutoff value. If specified, cpg.qc will remove samples where the proportion of missing values exceeds this cutoff.
constant100	Logical. If `TRUE`, the new beta values will be calculated as M/(U+M+100); if `FALSE` (default) they will be calculated as M/(U+M).

Details

It is important that all the matrices or data frames listed above (`pval`, `siga`, `sigb`, `beta.orig`) are ordered similarly with respect to samples and CpG sites.

Value

returns a new matrix of beta values that has been subjected to the specified quality control filters. This matrix can be input directly into `cpg.assoc`.

Authors

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See Also

cpg.perm, cpg.assoc, plot.cpg scatterplot

Examples

```
> ##See the examples in the CpGassoc tutorial.
```

cpg.work *Does the analysis between the CpG sites and phenotype of interest*

Description

Association Analysis Between Methylation Beta Values and Phenotype of Interest. This function contains the code that does the brunt of the work for `cpg.assoc` and `cpg.perm`.

Usage

cpg.work(beta.values, indep, covariates = NULL, data = NULL, logit.transform = FALSE, chip.id = NULL, subset = NULL, random = FALSE, fdr.cutoff = 0.05, callarge = FALSE, fdr.method = ”BH”, logitperm = FALSE,big.split=FALSE)

Arguments

beta.values	A vector, matrix, or data frame containing the beta values of interest (1 row per CpG site, 1 column per individual).
indep	A vector containing the main variable of interest. `cpg.work` will evaluate the association between indep and the beta values.

covariates A data frame consisting of the covariates of interest. covariates can also be a matrix if it is a model matrix minus the intercept column. It can also be a vector if there is only one covariate of interest. Can also be a formula (e.g. `cov1+cov2`).

data an optional data frame, list or environment (or object coercible by `as.data.frame` to a data frame) containing the variables in the model. If not found in data, the variables are taken from the environment from which `cpg.work` is called.

logit.transform Logical. If `TRUE`, the logit transform of the beta values log(beta.val/(1-beta.val)) will be used. Any values equal to zero or one will be set to the next smallest or next largest value respectively; values <0 or >1 will be set to NA.

chip.id An optional vector containing chip or batch identities. If specified, chip id will be included as a factor in the model.

subset an optional logical vector specifying a subset of observations to be used in the fitting process.

random Logical. If `TRUE`, the `chip.id` will be included in the model as a random effect, and a random intercept model will be fitted. If `FALSE`, `chip.id` will be included in the model as an ordinary categorical covariate, for a much faster analysis.

fdr.cutoff The threshold at which to compare the FDR values. The default setting is .05. Any FDR values less than .05 will be considered significant.

callarge Logical. Used by `cpg.assoc` when it calls `cpg.work`. If `TRUE` it means that beta.values is actually split up from a larger data set and that `memory.limit` may be a problem. This tells `cpg.work` to perform more `rm()` and `gc()` to clear up space.

fdr.method Character. Method used to calculate False Discovery Rate. Can be any of the methods listed in `p.adjust` or *qvalue* for John Storey's qvalue method (required to have *qvalue* package installed). The default method is "BH" for the Benjamini and Hochberg method.

logitperm Passes from `cpg.perm` when permutation test is performed. Stops from future checks involving the logistic transformation.

big.split Passes from `cpg.assoc`. Internal flag to inform `cpg.work` that the large data did not need to be split up.

Details

`cpg.work` does the analysis between the methylation and the phenotype of interest. It is called by `cpg.assoc` to do the brunt of the work. It can be called itself with the same input as `cpg.assoc`, it just cannot handle large data sets.

Value

`cpg.work` will return an object of class *cpg*.
The functions summary and plot can be called to get a summary of results and to create QQ plots. The output is in the same order as the original input. To sort it by p-value, use the `sort` function.

results — A data frame consisting of the statistics and P-values for each CpG site. Also has the adjusted p-value based on the fdr.method and whether the site was Holm significant.

Holm.sig — A list of sites that met criteria for Holm significance.

FDR.sig — A data.frame of the sites that were FDR significant by the fdr method.

info — A data frame consisting of the minimum P-value observed, the fdr method used, what the phenotype of interest was, and the number of covariates in the model.

indep — The main phenotype of interest.

covariates — If covariates was non `NULL`, the covariates will be included.Otherwise will be `NULL`.

chip — If chip.id was non `NULL`, the chip will be included. Otherwise will be `NULL`.

coefficients — A data frame consisting of the residual degrees of freedom, the intercept effect adjusted for possible covariates in the model, the estimated effect size, and the standard error. This item will only be returned if indep is continuous. The degrees of freedom is used in plot.cpg to compute the estimated t-statistics. If indep is a categorical variable this object will be `NULL`.

Authors

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See Also

cpg.perm, cpg.assoc, plot.cpg scatterplot, cpg.combine, manhattan, plot.cpg.perm, sort.cpg.perm, sort.cpg, cpg.qc

Examples

```
> ##See the examples listed in cpg.assoc for ways in which to use cpg.work.
> ##Just change the cpg.assoc to cpg.work.
```

---

design *Create full and reduced design matrices for the cpg.assoc function.*

Description

Designed to be used by `cpg.assoc` and `cpg.perm`. Creates a full and reduced design matrices.

Usage

design(covariates, indep, chip.id, random)

Arguments

covariates
: A data frame consisting of the covariates of interest. covariates can also be a matrix if it is a model matrix minus the intercept column. It can also be a vector if there is only one covariate of interest.If no covariates must be specified as `NULL`.

indep
: A vector containing the main variable of interest. `cpg.assoc` will evaluate the association between indep and the beta values.

chip.id
: An optional vector containing chip or batch identities. If specified, `chip.id` will be included as a factor in the model.

random
: Is the model going to be a mixed effects. If so, `chip.id` will not be included in the design matrices.

Value

Returns a list containing the full and reduced design matrices.

full
: The full design matrix

reduced
: The reduced design matrix

Author

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Note

The design function is designed to be used exclusively by the cpg.assoc and cpg.perm functions.

See Also

cpg.assoc, cpg.perm, plot.cpg, cpg.work, scatterplot, cpg.combine, manhattan, plot.cpg.perm, sort.cpg.perm, sort.cpg

examples

```
> library(CpGassoc)
> data(samplecpg,samplepheno,package="CpGassoc")
> #Example where there are covariates:
> covar<-data.frame(samplepheno$weight,samplepheno$Distance)
> test<-design(covar,samplepheno$SBP,samplepheno$chip,FALSE)
> dim(test$full)

[1] 258  26

> dim(test$reduced)

[1] 258  25

> test$reduced[1:5,1:5]

  (Intercept) samplepheno.weight samplepheno.Distance factor(chip.id)3
1           1           31.02998             28.49084                0
2           1           20.83885             13.10059                0
3           1           21.47078             14.76703                0
4           1           23.95091             25.54482                0
5           1           34.12922             29.45997                0
  factor(chip.id)4
1                0
2                0
3                0
4                0
5                0

> test$full[1:5,1:5]

  (Intercept)    indep samplepheno.weight samplepheno.Distance factor(chip.id)3
1           1 16.98629           31.02998             28.49084                0
2           1 34.90645           20.83885             13.10059                0
3           1 21.55838           21.47078             14.76703                0
4           1 20.90882           23.95091             25.54482                0
5           1 27.01004           34.12922             29.45997                0

> #When no covariates or chip.id:
> test2<-design(NULL,samplepheno$SBP,NULL,FALSE)
> dim(test2$full)

[1] 258   2

> dim(test2$reduced)

[1] 258   1
```

manhattan *Create a manhattan plot*

Description

This function will produce a manhattan plot for the observed P-values from a object of class *cpg* or *cpg.perm*.

Usage

manhattan(x, cpgname, chr, pos, save.plot = NULL, file.type=”pdf”, popup.pdf = FALSE, eps.size = c(15, 5), main.title = NULL, cpg.labels = NULL, chr.list = NULL, color.list = NULL, ...)

Arguments

x Object of class *cpg* or *cpg.perm*.

cpgname A vector consisting of the labels for each CpG site.

chr A vector consisting of the chromosome number for each CpG site.

pos The map position of each CpG site within its chromosome.

save.plot Name of the file for the plot to be saved to. If not specified, plot will not be saved.

file.type Type of file to be saved. Can either be `"pdf"` or `"eps"`. Selecting `file.type="eps"` will result in publication quality editable postscript files that can be opened by Adobe Illustrator or Photoshop.

popup.pdf `TRUE` or `FALSE`. If creating a pdf file, this indicates if the plot should appear in a popup window as well. If running in a cluster-like environment, best to leave `FALSE`.

eps.size Vector indicating the size of .eps file (if creating one). Corresponds to horrizontal and height.

main.title Main title to be put on the graph. If `NULL` one based on the analysis will be used.

cpg.labels A character scalar of either `"FDR"` or `"HOLM"` which will label the significant sites on the manhattan plot.

chr.list A vector listing the chromosomes to be plotted (all available chromosomes are plotted by default). The X and Y chromosomes can be denoted by 23 and 24

color.list A vector of custom colors to be used for each chromosomes in the manhattan plot.

... Arguments to be passed to methods, such as graphical parameters.

Authors

Barfield, R.;Conneely, K.; Kilaru,V.
Maintainer: R. Barfield: richard.thomas.barfield@emory.edu

Note

`cpgname`, `chr`, and `pos` must be sorted in the same order, so that the first cpgname[1] corresponds to chr[1] and pos[1], and so on.

See Also

cpg.assoc, cpg.perm, plot.cpg, cpg.work, scatterplot, cpg.combine, design, plot.cpg.perm, sort.cpg.perm, sort.cpg

Examples

Object of class cpg	*Methods for object of class*

Usage

plot.cpg(x, save.plot = NULL, file.type="pdf", popup.pdf = FALSE, tplot = FALSE, classic = TRUE, main.title = NULL, eps.size = c(5, 5), . . .)

summary.cpg(object,. . .)

print.cpg(x,. . .)

sort.cpg(x,decreasing,. . .)

Arguments

x	Output of class *cpg* from cpg.assoc or cpg.work.
save.plot	Name of the file for the plot to be saved to. If not specified, plot will not be saved.

file.type
Type of file to be saved. Can either be `"pdf"` or `"eps"`. Selecting `file.type="eps"` will result in publication quality editable postscript files that can be opened by Adobe Illustrator or Photoshop.

popup.pdf
`TRUE` or `FALSE`. If creating a pdf file, this indicates if the plot should appear in a popup window as well. If running in a cluster-like environment, best to leave `FALSE`.

tplot
Logical. If `TRUE`, ordered t-statistics will be plotted against their expected quanties. If `FALSE` (default), -log(p) will be plotted. If indep is a class variable this option will be ignored.

classic
Logical. If `TRUE`, a classic qq-plot will be generated, with all p-values plotted against predicted values (including significant). If `FALSE` Holm-significant CpG sites will not be used to compute expected quantiles and will be plotted separately.

main.title
Main title to be put on the graph. If `NULL` one based on the analysis will be used.

eps.size
Vector indicating the size of .eps file (if creating one). Correponds to the options horizontal and height in the `postscript` function.

object
Output of class *cpg* from `cpg.assoc` or `cpg.work`.

decreasing
Logical. Should the sort be increasing or decreasing? Not available for partial sorting.

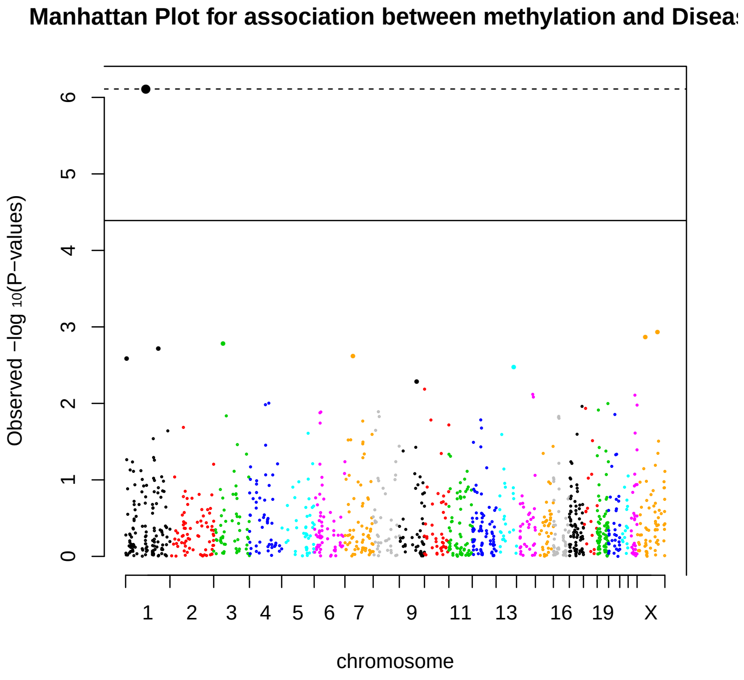
...
Arguments to be passed to methods, such as graphical parameters.

Description

Methods and extra functions for class *cpg*.

`plot.cpg` creates a QQ plot based on the association p-values or t-statistics from the function `cpg.assoc`.

```
> #Doing a Manhattan plot. First load the data:
>
> #Doing a Manhattan plot. First load the data:
> library(CpGassoc)
> data(samplecpg,samplepheno,annotation,package="CpGassoc")
> ###NOTE: If you are dealing with large data, do not specify large.data=FALSE. The defaul
> ##This will involve partitioning up the data and performing more gc() to clear up space
> examplemanhat<-cpg.assoc(samplecpg,samplepheno$Disease,large.data=FALSE)
> manhattan(examplemanhat,annotation$TargetID,annotation$CHR,annotation$MAPINFO)
>
```



Value

`sort.cpg` returns an item of class *cpg* that is sorted by p-value.

`summary.cpg` creates a qq-plot based on the data, and scatterplots or boxplots for the top sites.

Authurs

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Note

Plots with empirical confidence intervals based on permutation tests can be obtained from `cpg.perm`.
See plot.cpg.perm for more info

See Also

cpg.perm, cpg.work, cpg.assoc scatterplot, cpg.combine, manhattan, plot.cpg.perm, sort.cpg.perm,cpg.qc

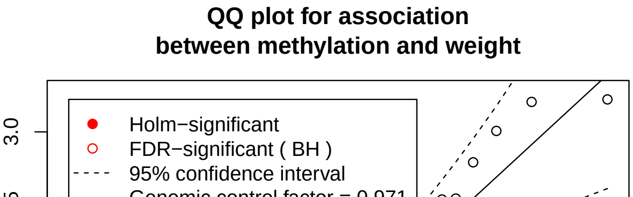
Examples

Object of class cpg.perm	*Methods for object of class cpg.perm*

Usage

plot.cpg.perm(x, save.plot = NULL, file.type=”pdf”, popup.pdf = FALSE, main.title = NULL, eps.size = c(5, 5), tplot = FALSE, perm.ci = TRUE, classic = TRUE, ...)
summary.cpg.perm(object,. . .)
print.cpg.perm(x,. . .)
sort.cpg.perm(x,decreasing,. . .)

```
> ##Using the results from the example given in cpg.assoc.
> ###NOTE: If you are dealing with large data, do not specify large.data=FALSE. The defaul
> ##This will involve partitioning up the data and performing more gc() to clear up space
> ##QQ Plot:
> library(CpGassoc)
> data(samplecpg,samplepheno,package="CpGassoc")
> test<-cpg.assoc(samplecpg,samplepheno$weight,data.frame(samplepheno$Distance,samplepheno
> plot(test)
> ##t-statistic plot:
> plot(test,tplot=TRUE)
> ##Now an example of sort
> head(sort(test)$results)

    CPG.Labels T.statistic      P.value Holm.sig       FDR
694     CpG694    3.475160 0.0006002142    FALSE 0.3833341
293     CpG293    3.464076 0.0006243226    FALSE 0.3833341
560     CpG560    3.333678 0.0009848497    FALSE 0.4031318
148     CpG148    3.187753 0.0016135434    FALSE 0.4953578
238     CpG238    3.012760 0.0028504303    FALSE 0.5921086
998     CpG998   -3.008091 0.0028930386    FALSE 0.5921086

> ##Summary
> summary(test)

The top ten CpG sites were:
     CPG.Labels T.statistic      P.value Holm.sig       FDR
694      CpG694    3.475160 0.0006002142    FALSE 0.3833341
293      CpG293    3.464076 0.0006243226    FALSE 0.3833341
560      CpG560    3.333678 0.0009848497    FALSE 0.4031318
148      CpG148    3.187753 0.0016135434    FALSE 0.4953578
238      CpG238    3.012760 0.0028504303    FALSE 0.5921086
998      CpG998   -3.008091 0.0028930386    FALSE 0.5921086
1059    CpG1059   -2.932014 0.0036749081    FALSE 0.6295151
100      CpG100    2.889847 0.0041873059    FALSE 0.6295151
1006    CpG1006   -2.831992 0.0049965867    FALSE 0.6295151
1182    CpG1182   -2.823521 0.0051263442    FALSE 0.6295151

To access results for all  1228  CpG sites use object$results
or sort(object)$results to obtain results sorted by p-value.

General info:
  Min.P.Observed Num.Cov fdr.cutoff FDR.method Phenotype chipinfo num.Holm
1   0.0006002142       2       0.05         BH    weight     NULL        0
  num.fdr
1       0

0 sites were found significant by the Holm method
0 sites were found significant by BH method

The beta values were taken from: samplecpg
Effect sizes and standard error can be accessed using $coefficients
Other attributes are: results, Holm.sig, FDR.sig, info, indep, covariates, chip
 They can be accessed using the $
```



Description

Methods and extra functions for class *cpg.perm*. `plot.cpg.perm` creates a QQ plot based on the association p-values or t-statistics from the function `cpg.perm`.

Arguments

x — Output from `cpg.perm`. Of class *cpg.perm*.

save.plot — Name of the file for the plot to be saved to. If not specified, plot will not be saved.

file.type — Type of file to be saved. Can either be `"pdf"` or `"eps"`. Selecting `file.type="eps"` will result in publication quality editable postscript files that can be opened by Adobe Illustrator or Photoshop.

popup.pdf — `TRUE` or `FALSE`. If creating a pdf file, this indicates if the plot should appear in a popup window as well. If running in a cluster-like environment, best to leave `FALSE`.

main.title — Main title to be put on the graph. If `NULL` one based on the analysis will be used

eps.size — Vector indicating the size of .eps file (if creating one). Correponds to the options horizontal and height in the `postscript` function.

tplot — Logical. If `TRUE`, ordered t-statistics will be plotted against their expected quanties. If `FALSE` (default), -log(p) will be plotted. If indep is a class variable this option will be ignored.

perm.ci — Logical. If `TRUE`, the confidence intervals computed will be from the permutated values, otherwise will be based on the theoretical values.

classic — Logical. If `TRUE`, a classic qq-plot will be generated, with all p-values plotted against predicted values (including significant). If `FALSE` Holm-significant CpG sites will not be used to compute expected quantiles and will be plotted separately.

object	Output of class *cpg.perm* from *cpg.perm*.
decreasing	Logical. Should the sort be increasing or decreasing? Not available for partial sorting.
...	Arguments to be passed to methods, such as graphical parameters.

Authors

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Maintainer: R. Barfield: richard.thomas.barfield@emory.edu

Note

Empirical confidence intervals will be computed only if there are a hundred or more permutations. Otherwise the theoretical confidence intervals will be plotted.

See Also

cpg.assoc, cpg.perm, plot.cpg, cpg.work, scatterplot, cpg.combine, design, manhattan, sort.cpg

Examples

---

scatterplot — *Plot beta values of individual CpG sites against the independent variable.*

Usage

scatterplot(x, cpg.rank = NULL, cpg.name = NULL, save.plot = NULL, file.type="pdf", eps.size = c(5, 5), popup.pdf = FALSE, beta.values = NULL,main.title=NULL, ...)

Arguments

x	Object of class *cpg* or *cpg.perm*.

cpg.rank A vector listing the rank of sites to be plotted. The rank is based on the ordered p-values.

cpg.name A character vector containing the names of CpG sites to be plotted against the phenotype of interest. This option is ignored if `cpg.rank` is specified.

save.plot Prefix of the filename for the plot(s) to be saved to. If specified, plot filenames will be created by appending this prefix to either cpg.rank or cpg.name. If not specified, plot will not be saved.

file.type Type of file to be saved. Can either be `"pdf"` or `"eps"`. Selecting `file.type="eps"` will result in publication quality editable postscript files that can be opened by Adobe Illustrator or Photoshop.

eps.size Vector indicating the size of .eps file (if creating one). Correponds to horrizontal and height.

popup.pdf `TRUE` or `FALSE`. If creating a pdf file, this indicates if the plot should appear in a popup window as well. If running in a cluster-like environment, best to leave `FALSE`.

beta.values If the object has been renamed (i.e. x$info$`betainfo` is no longer in `ls(.GlobalEnv)`) then specify the new object here.

main.title Main title to be put on the graph. If `NULL` one based on the analysis will be used

... Arguments to be passed to methods, such as graphical parameters.

Details

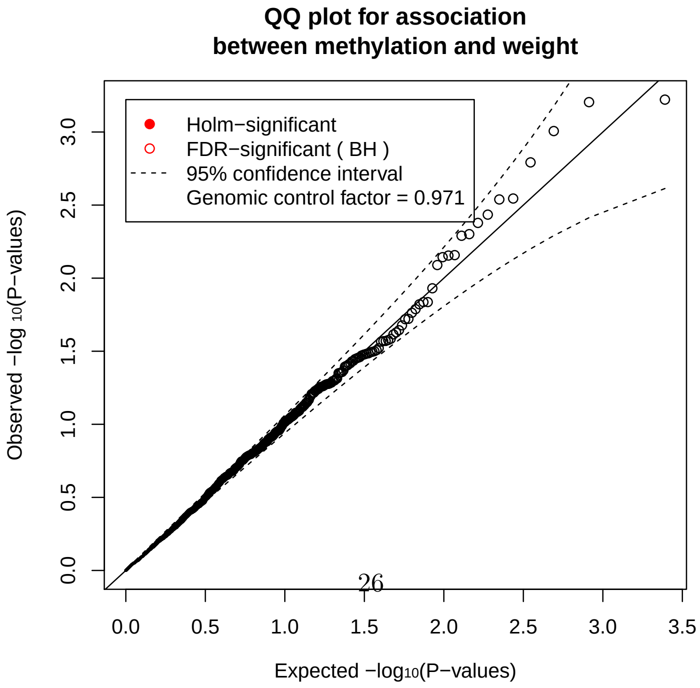
An unlimited number of CpG sites can be selected for plotting by specifying either `cpg.rank` or `cpg.name`, as shown in the Examples below. Note that only one of these options is needed; if both are entered, `cpg.rank` will be used.

```
> library(CpGassoc)
> data(samplecpg,samplepheno,package="CpGassoc")
> ##We will do the analysis on a subset to save time
> ###NOTE: If you are dealing with large data, do not specify large.data=FALSE. The defaul
> ##This will involve partitioning up the data and performing more gc() to clear up space
> #The qq plot:
> Testperm<-cpg.perm(samplecpg,samplepheno$weight,data.frame(samplepheno$Dose,samplepheno$
+                 seed=2314,nperm=10,large.data=FALSE)
> plot(Testperm)
> #The t-statistic plot from cpg.perm has confidence intervals since we were allowed to pe
> plot(Testperm,tplot=TRUE)
> #If there was 100 or more permutations, there would be emperical confidence intervals.
>
> ###Now for Sort
> head(sort(Testperm)$results)

    CPG.Labels T.statistic      P.value Holm.sig       FDR
694     CpG694    3.475160 0.0006002142    FALSE 0.3833341
293     CpG293    3.464076 0.0006243226    FALSE 0.3833341
560     CpG560    3.333678 0.0009848497    FALSE 0.4031318
148     CpG148    3.187753 0.0016135434    FALSE 0.4953578
238     CpG238    3.012760 0.0028504303    FALSE 0.5921086
998     CpG998   -3.008091 0.0028930386    FALSE 0.5921086

> head(Testperm$results)

  CPG.Labels T.statistic    P.value Holm.sig       FDR
1       CpG1 -1.63736663 0.10279215    FALSE 0.9439499
2       CpG2 -0.09076561 0.92775038    FALSE 0.9927071
3       CpG3 -2.36081337 0.01899094    FALSE 0.9057057
4       CpG4  1.28326656 0.20056830    FALSE 0.9530109
5       CpG5 -1.29476076 0.19657851    FALSE 0.9530109
6       CpG6 -0.94975324 0.34314045    FALSE 0.9911946
```



```
> #Load the data:
> data(samplecpg,samplepheno,package="CpGassoc")
> library(CpGassoc)
> ###NOTE: If you are dealing with large data, do not specify large.data=FALSE. The defaul
> ##This will involve partitioning up the data and performing more gc() to clear up space
> test<-cpg.assoc(samplecpg,samplepheno$weight,large.data=FALSE)
> ##Using rank, will plot the top three sites in order of significance:
> scatterplot(test,c(1:3))

Press enter to continue

Press enter to continue

Press enter to continue

All 3 sites plotted

> ##Using name, specify three sites:
> scatterplot(test,cpg.name=c("CpG1182","CpG1000","CpG42"))

Press enter to continue

Press enter to continue

Press enter to continue

All 3 sites plotted

> ##Plotting something that is categorical in nature:
> test2<-cpg.assoc(samplecpg,factor(samplepheno$Disease),large.data=FALSE)
> scatterplot(test2,c(2))

Press enter to continue

All 1 sites plotted
```

Authors

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See Also

cpg.assoc, cpg.perm, manhattan, cpg.work, plot.cpg.perm, cpg.combine, design, plot.cpg, sort.cpg.perm, sort.cpg

Examples