

Package ‘Harman’

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Type Package

Title The removal of batch effects from datasets using a PCA and constrained optimisation based technique

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Description Harman is a PCA and constrained optimisation based technique that maximises the removal of batch effects from datasets, with the constraint that the probability of overcorrection (i.e. removing genuine biological signal along with batch noise) is kept to a fraction which is set by the end-user.

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arrowPlot	<i>PCA before and after arrow plot for harman results</i>
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Description

Generates an arrow plot for an instance of `harmanresults`. The tail of the arrow is the starting point (original) in principle coordinates, while the arrow head is the new point (corrected) in principle coordinates. It can be observed that on principle components that have undergone correction (`harmanresults$stats$correction < 1.0`), the samples within a batch will be coordinately moved towards 0 on that principle component.

Usage

```
arrowPlot(  
  harmanresults,  
  pc_x = 1,  
  pc_y = 2,  
  colBy = "batch",  
  palette = "rainbow",  
  col,  
  length = 0.1,  
  legend = TRUE,  
  ...  
)
```

Arguments

harmanresults	an instance of harmanresults.
pc_x	integer, principle component for the plot x dimension.
pc_y	integer, principle component for the plot y dimension.
colBy	string, colour the points by the experimental or batch variable; legal values are expt and batch. The palette function specified in palette is used. This parameter is overridden by col.
palette	string, the function to call to create a vector of contiguous colours with the levels of factor in colBy steps.
col	colour vector for the points. This parameter overrides palette.
length	length of the arrow heads, default is 0.1.
legend	logical, whether to display a legend on the plot
...	further arguments passed to or from other methods.

Details

Generates a Principle Component plot for an instance of harmanresults. If a vector of colours is supplied via the col argument, then a legend will not be drawn.

Value

None

See Also

[harmanresults](#) [plot.harmanresults](#)

Examples

```
library(HarmanData)
data(OLF)
expt <- olf.info$Treatment
batch <- olf.info$Batch
olf.harman <- harman(olf.data, expt, batch)
arrowPlot(olf.harman, pc_x=2, pc_y=3, length=0.2)
```

callHarman

Wrapper function to call the shared C/C++ library code

Description

This wrapper should probably not be addressed directly except for debugging. Instead use [harman](#). Input of PCA scores and the experiment structure (treatments and batches) and returns a batch corrected version of the PCA scores matrix.

Usage

```
.callHarman(
  pc_data_scores,
  group,
  limit,
  numrepeats,
  randseed,
  forceRand,
  printInfo
)
```

Arguments

pc_data_scores	2D NumericMatrix of PCA scores data (from the prcomp\$x slot), rows = samples, cols = PC scores
group	The structure of the experiment, consisting of batch numbers and treatment numbers forming 2 rows or columns (HarmanMain works out which). Each entry for a sample describes what batch it came from and what treatment it was given. Has to be integer formatted data.
limit	A double precision value indicating the limit of confidence in which to stop removing a batch effect
numrepeats	The number of repeats in which to run the simulated batch mean distribution estimator. Probably should be greater than 100,000.
randseed	Random seed to pass to the random number generator (0 for use default from system time)
forceRand	Force algorithm
printInfo	Print update information to screen

Value

SEXP R list

- scores.corrected
- correction
- confidence

Note

A data matrix with samples in columns must be transposed before PCA analysis and these scores in turn are tweaked a little before handing over to `.callHarman`. See the example below.

clusterStats	<i>Compute LVR and meandiff statistics for beta values after batch correction</i>
--------------	---

Description

This function is part of a set of three functions to be run in series. [discoverClusteredMethylation](#) takes a matrix of methylation beta values (typically from the Illumina Infinium Methylation Assay) and clusters the data across a range of ks specified by the user.

Then the data is reclustered again across the the best two candidate values for k (determined by the rate of change in Bayesian information criterion), and minimum cluster size and distance filters are employed. If both clusters meet these filters, then the higher value of k is returned. This function should be run on uncorrected data that ideally has slides removed which are prone to batch effect. This will bias towards finding clusters that are driven by biological factors such as X-chromosome inactivation and allele-specific methylation.

The output of this function is input for the [kClusterMethylation](#) function which extracts cluster membership and statistics on variance for a given matrix of beta values. It might be useful to discover clusters on samples less prone to clustering due to batch effect or cellular heterogeneity and then recluster all the data for set values of k via the [kClusterMethylation](#) function.

Finally, a comparison of differences of uncorrected to batch-corrected beta values can be made using [clusterStats](#). This function generates a data.frame containing log variance ratio and mean beta differences to clusters after correction.

Usage

```
clusterStats(pre_betas, post_betas, kClusters)
```

Arguments

pre_betas	a matrix of methylation beta values prior to correction.
post_betas	a matrix of methylation beta values after correction.
kClusters	a kClusters S3 object

Details

Betas values should be of type double with samples in columns and betas in rows. The betas need to be bounded between 0 and 1. The matrix is typically exported from a [GenomicRatioSet](#), [GenomicMethylSet](#) or [MethylSet](#) object via the getBeta S4 accessor method.

Value

A data.frame containing clustering stats.

See Also

[kClusterMethylation](#), [discoverClusteredMethylation](#)

Examples

```
library(HarmanData)
data(episcope)
bad_batches <- c(1, 5, 9, 17, 25)
is_bad_sample <- episcope$pd$array_num %in% bad_batches
myK <- discoverClusteredMethylation(episcope$original[, !is_bad_sample])
mykClust = kClusterMethylation(episcope$original, row_ks=myK)
res = clusterStats(pre_betas=episcope$original,
                   post_betas=episcope$harman,
                   kClusters = mykClust)
all.equal(episcope$ref_md$meandiffs_harman, res$meandiffs)
all.equal(episcope$ref_lvr$var_ratio_harman, res$log2_var_ratio)
```

detachHarman	<i>Detach the Harman package and its shared C/C++ library code</i>
--------------	--

Description

A helper function that can be called if [harman](#) had to be aborted.

Usage

```
detachHarman()
```

Value

None

discoverClusteredMethylation	<i>Discover clustered beta values</i>
------------------------------	---------------------------------------

Description

This function is part of a set of three functions to be run in series. [discoverClusteredMethylation](#) takes a matrix of methylation beta values (typically from the Illumina Infinium Methylation Assay) and clusters the data across a range of ks specified by the user.

Then the data is reclustered again across the the best two candidate values for k (determined by the rate of change in Bayesian information criterion), and minimum cluster size and distance filters are employed. If both clusters meet these filters, then the higher value of k is returned. This function should be run on uncorrected data that ideally has slides removed which are prone to batch effect. This will bias towards finding clusters that are driven by biological factors such as X-chromosome inactivation and allele-specific methylation.

The output of this function is input for the [kClusterMethylation](#) function which extracts cluster membership and statistics on variance for a given matrix of beta values. It might be useful to discover clusters on samples less prone to clustering due to batch effect or cellular heterogeneity and then recluster all the data for set values of k via the [kClusterMethylation](#) function.

Finally, a comparison of differences of uncorrected to batch-corrected beta values can be made using [clusterStats](#). This function generates a data.frame containing log variance ratio and mean beta differences to clusters after correction.

Usage

```
discoverClusteredMethylation(
  betas,
  ks = 1:10,
  min_cluster_size = 5,
  min_cluster_dist = 0.1,
  max_clusters = 4,
  cores = 1,
  printInfo = FALSE
)
```

Arguments

<code>betas</code>	a matrix of methylation beta values with samples in columns and betas in rows.
<code>ks</code>	integer, the range of k's to consider for clustering (defaults to 1:10).
<code>min_cluster_size</code>	integer, the minimum number of samples in a cluster (defaults to 5).
<code>min_cluster_dist</code>	numeric, the minimum beta difference in cluster centroids (defaults to 0.1).
<code>max_clusters</code>	integer, the maximum value of k returned (defaults to 4).
<code>cores</code>	integer, specifies the number of cores to use for computation.
<code>printInfo</code>	logical, whether to print information during computation or not.

Details

Betas values should be of type double with samples in columns and betas in rows. The betas need to be bounded between 0 and 1. The matrix is typically exported from a [GenomicRatioSet](#), [GenomicMethylSet](#) or [MethylSet](#) object via the `getBeta` S4 accessor method.

Value

A named vector containing the optimal value for k.

See Also

[kClusterMethylation](#), [clusterStats](#)

Examples

```
library(HarmanData)
data(episcope)
bad_batches <- c(1, 5, 9, 17, 25)
is_bad_sample <- episcope$pd$array_num %in% bad_batches
myK <- discoverClusteredMethylation(episcope$original[, !is_bad_sample])
mykClust = kClusterMethylation(episcope$original, row_ks=myK)
res = clusterStats(pre_betas=episcope$original,
  post_betas=episcope$harman,
  kClusters = mykClust)
all.equal(episcope$ref_md$meandiffs_harman, res$meandiffs)
all.equal(episcope$ref_lvr$var_ratio_harman, res$log2_var_ratio)
```

harman

*Harman batch correction method***Description**

Harman is a PCA and constrained optimisation based technique that maximises the removal of batch effects from datasets, with the constraint that the probability of overcorrection (i.e. removing genuine biological signal along with batch noise) is kept to a fraction which is set by the end-user (Oytam et al, 2016; <http://dx.doi.org/10.1186/s12859-016-1212-5>).

Harman expects unbounded data, so for example, with HumanMethylation450 arrays do not use the Beta statistic (with values constrained between 0 and 1), instead use the logit transformed M-values.

Usage

```
harman(
  datamatrix,
  expt,
  batch,
  limit = 0.95,
  numrepeats = 100000L,
  randseed,
  forceRand = FALSE,
  printInfo = FALSE
)
```

Arguments

<code>datamatrix</code>	matrix or data.frame, the data values to correct with samples in columns and data values in rows. Internally, a data.frame will be coerced to a matrix. Matrices need to be of type integer or double.
<code>expt</code>	vector or factor with the experimental variable of interest (variance to be kept).
<code>batch</code>	vector or factor with the batch variable (variance to be removed).
<code>limit</code>	numeric, confidence limit. Indicates the limit of confidence in which to stop removing a batch effect. Must be between 0 and 1.
<code>numrepeats</code>	integer, the number of repeats in which to run the simulated batch mean distribution estimator using the random selection algorithm. (N.B. 32 bit Windows versions may have an upper limit of 300000 before catastrophic failure)
<code>randseed</code>	integer, the seed for random number generation.
<code>forceRand</code>	logical, to enforce Harman to use a random selection algorithm to compute corrections. Force the simulated mean code to use random selection of scores to create the simulated batch mean (rather than full explicit calculation from all permutations).
<code>printInfo</code>	logical, whether to print information during computation or not.

Details

The `datamatrix` needs to be of type integer or numeric, or alternatively a data.frame that can be coerced into one using `as.matrix`. The matrix is to be constructed with data values (typically microarray probes or sequencing counts) in rows and samples in columns, much like the ‘assayData’

slot in the canonical Bioconductor `eSet` object, or any object which inherits from it. The data should have normalisation and any other global adjustment for noise reduction (such as background correction) applied prior to using Harman.

For converge, the number of simulations, `numrepeats` parameter should probably be at least 100,000. The underlying principle of Harman rests upon PCA, which is a parametric technique. This implies Harman should be optimal when the data is normally distributed. However, PCA is known to be rather robust to very non-normal data.

The output `harmanresults` object may be presented to summary and data exploration functions such as `plot.harmanresults` and `summary.harmanresults` as well as the `reconstructData` function which creates a corrected matrix of data with the batch effect removed.

Value

A `harmanresults` S3 object:

factors A `data.frame` of the `expt` and `batch` vectors

parameters The harman runtime parameters. See `harman` for details

stats Confidence intervals and the degree of correction for each principal component

center The centering vector returned by `prcomp` with `center=TRUE`

rotation The matrix of eigenvectors (by column) returned from `prcomp`

original The original PC scores returned by `prcomp`

corrected The harman corrected PC scores

References

Oytam et al (2016) BMC Bioinformatics 17:1. DOI: 10.1186/s12859-016-1212-5

See Also

`reconstructData`, `pcaPlot`, `arrowPlot`

Examples

```
library(HarmanData)
data(OLF)
expt <- olf.info$Treatment
batch <- olf.info$Batch
olf.harman <- harman(olf.data, expt, batch)
plot(olf.harman)
olf.data.corrected <- reconstructData(olf.harman)

## Reading from a csv file
datafile <- system.file("extdata", "NPM_data_first_1000_rows.csv.gz",
  package="Harman")
infofile <- system.file("extdata", "NPM_info.csv.gz", package="Harman")
datamatrix <- read.table(datafile, header=TRUE, sep=";", row.names="probeID")
batches <- read.table(infofile, header=TRUE, sep=";", row.names="Sample")
res <- harman(datamatrix, expt=batches$Treatment, batch=batches$Batch)
arrowPlot(res, 1, 3)
```

harmanresults	<i>Harman results object</i>
---------------	------------------------------

Description

The S3 object returned after running [harman](#).

Details

`harmanresults` is the S3 object used to store the results from [harman](#). This object may be presented to summary and data exploration functions such as [plot.harmanresults](#) and [summary.harmanresults](#) as well as the [reconstructData](#) function which creates a corrected matrix of data with the batch effect removed.

Slots

`factors` A data.frame of the expt and batch vectors.

`parameters` The harman runtime parameters. See [harman](#) for details.

`stats` Confidence intervals and the degree of correction for each principal component.

`center` The centering vector returned by [prcomp](#) with `center=TRUE`.

`rotation` The matrix of eigenvectors (by column) returned from [prcomp](#).

`original` The original PC scores returned by [prcomp](#).

`corrected` The harman corrected PC scores.

See Also

[harman](#), [reconstructData](#), [pcaPlot](#), [arrowPlot](#)

Examples

```
## HarmanResults
library(HarmanData)
data(OLF)
expt <- olf.info$Treatment
batch <- olf.info$Batch
olf.harman <- harman(as.matrix(olf.data), expt, batch)
plot(olf.harman)
summary(olf.harman)
pcaPlot(olf.harman, pc_x=2, pc_y=3)
pcaPlot(olf.harman, pc_x=2, pc_y=3, colBy='expt', pch=1)
olf.data.corrected <- reconstructData(olf.harman)
```

harmanScores

A Principal components prcomp function tweaked for Harman

Description

A tweaking of `stats::prcomp` such that for the `svd`, the transpose of `u` is used instead of `v` when the number of assays is less than the number of samples.

Usage

```
harmanScores(x)
```

Arguments

`x` matrix, data matrix of values to perform PCA on.

Value

scores, a `prcomp`-like object with rotation, scores and the center values. The scores are corrected, but all three are needed later to reconstruct the data.

kClusterMethylation

Cluster beta values with a set value for k

Description

This function is part of a set of three functions to be run in series. [discoverClusteredMethylation](#) takes a matrix of methylation beta values (typically from the Illumina Infinium Methylation Assay) and clusters the data across a range of `ks` specified by the user.

Then the data is reclustered again across the the best two candidate values for `k` (determined by the rate of change in Bayesian information criterion), and minimum cluster size and distance filters are employed. If both clusters meet these filters, then the higher value of `k` is returned. This function should be run on uncorrected data that ideally has slides removed which are prone to batch effect. This will bias towards finding clusters that are driven by biological factors such as X-chromosome inactivation and allele-specific methylation.

The output of this function is input for the [kClusterMethylation](#) function which extracts cluster membership and statistics on variance for a given matrix of beta values. It might be useful to discover clusters on samples less prone to clustering due to batch effect or cellular heterogeneity and then recluster all the data for set values of `k` via the [kClusterMethylation](#) function.

Finally, a comparison of differences of uncorrected to batch-corrected beta values can be made using [clusterStats](#). This function generates a `data.frame` containing log variance ratio and mean beta differences to clusters after correction.

Usage

```
kClusterMethylation(betas, row_ks, cores = 1, printInfo = FALSE)
```

Arguments

betas	a matrix of methylation beta values with samples in columns and betas in rows.
row_ks	vector, the value of k for each row in betas. The names of row_ks must match the rownames of betas.
cores	integer, specifies the number of cores to use for computation.
printInfo	logical, whether to print information during computation or not.

Details

Betas values should be of type double with samples in columns and betas in rows. The betas need to be bounded between 0 and 1. The matrix is typically exported from a [GenomicRatioSet](#), [GenomicMethylSet](#) or [MethylSet](#) object via the getBeta S4 accessor method.

Value

A kClusters S3 object.

See Also

[discoverClusteredMethylation](#), [clusterStats](#)

Examples

```
library(HarmanData)
data(episcope)
bad_batches <- c(1, 5, 9, 17, 25)
is_bad_sample <- episcope$pd$array_num %in% bad_batches
myK <- discoverClusteredMethylation(episcope$original[, !is_bad_sample])
mykClust = kClusterMethylation(episcope$original, row_ks=myK)
res = clusterStats(pre_betas=episcope$original,
                   post_betas=episcope$harman,
                   kClusters = mykClust)
all.equal(episcope$ref_md$meandiffs_harman, res$meandiffs)
all.equal(episcope$ref_lvr$var_ratio_harman, res$log2_var_ratio)
```

pcaPlot

PCA plot for harman results

Description

Generates a Principle Component plot for an instance of [harmanresults](#).

Usage

```
pcaPlot(
  harmanresults,
  pc_x = 1,
  pc_y = 2,
  this = "corrected",
  colBy = "batch",
  pchBy = "expt",
```

```

    palette = "rainbow",
    legend = TRUE,
    col,
    pch,
    ...
)

```

Arguments

<code>harmanresults</code>	An instance of <code>harmanresults</code> .
<code>pc_x</code>	integer, principle component for the plot x dimension.
<code>pc_y</code>	integer, principle component for the plot y dimension.
<code>this</code>	string, legal values are <code>original</code> or <code>corrected</code> .
<code>colBy</code>	string, colour the points by the experimental or batch variable; legal values are <code>expt</code> and <code>batch</code> . The palette function specified in <code>palette</code> is used. This parameter is overridden by <code>col</code> .
<code>pchBy</code>	string, point-type by the experimental or batch variable; legal values are <code>expt</code> and <code>batch</code> . This parameter is overridden by <code>pch</code> .
<code>palette</code>	string, the function to call to create a vector of contiguous colours with the levels of factor in <code>colBy</code> steps.
<code>legend</code>	logical, whether to display a legend on the plot.
<code>col</code>	colour vector for the points. This parameter overrides <code>colBy</code> and <code>palette</code> .
<code>pch</code>	integer vector giving the point type. This parameter overrides <code>pchBy</code> .
<code>...</code>	further arguments passed to or from other methods.

Details

If a vector of colours is supplied via the `col` argument, then a legend will not be drawn.

Value

None

See Also

[harmanresults](#) [plot.harmanresults](#)

Examples

```

library(HarmanData)
data(OLF)
expt <- olf.info$Treatment
batch <- olf.info$Batch
olf.harman <- harman(as.matrix(olf.data), expt, batch)
pcaPlot(olf.harman)
pcaPlot(olf.harman, colBy='expt')
pcaPlot(olf.harman, pc_x=2, pc_y=3, this='original', pch=17)

```

plot.harmanresults	<i>Plot method for harman</i>
--------------------	-------------------------------

Description

Plot method for instances of [harmanresults](#).

Usage

```
## S3 method for class 'harmanresults'
plot(x, ...)
```

Arguments

x	An instance of harmanresults.
...	further plotting parameters.

Value

None

See Also

[harmanresults.pcaPlot](#)

Examples

```
library(HarmanData)
data(OLF)
expt <- olf.info$Treatment
batch <- olf.info$Batch
olf.harman <- harman(olf.data, expt, batch)
plot(olf.harman)
```

prcompPlot	<i>PCA plot</i>
------------	-----------------

Description

Generates a Principle Component plot for data.frames, matrices, or a pre-made [prcomp](#) object.

Usage

```
prcompPlot(
  object,
  pc_x = 1,
  pc_y = 2,
  scale = FALSE,
  colFactor = NULL,
  pchFactor = NULL,
```

```

    palette = "rainbow",
    legend = TRUE,
    ...
)

```

Arguments

<code>object</code>	data.frame, matrix or prcomp object.
<code>pc_x</code>	integer, principle component for the plot x dimension.
<code>pc_y</code>	integer, principle component for the plot y dimension.
<code>scale</code>	logical, whether to scale to unit variance before PCA.
<code>colFactor</code>	factor or vector, colour the points by this factor, default is NULL.
<code>pchFactor</code>	factor or vector, point-type by this factor, default is NULL.
<code>palette</code>	string, the function to call to create a vector of contiguous colours with <code>levels(colFactor)</code> steps.
<code>legend</code>	logical, whether to display a legend on the plot.
<code>...</code>	further arguments passed to or from other methods.

Details

A data.frame object will be coerced internally to a matrix. Matrices must be of type double or integer. The `prcompPlot` function will then perform a principle component analysis on the data prior to plotting. The function is call is `prcomp(t(object), retx=TRUE, center=TRUE, scale.=scale)`. Instead of specifying a data.frame or matrix, a pre-made prcomp object can be given to `prcompPlot`. In this case, care should be taken in setting the appropriate value of `scale..` If a vector is given to `colFactor` or `pchFactor`, they will be coerced internally to factors.

For the default NULL values of `colFactor` and `pchFactor`, all colours will be black and circles the point type, respectively.

Value

None

See Also

[prcomp rainbow](#)

Examples

```

library(HarmanData)
data(IMR90)
expt <- imr90.info$Treatment
batch <- imr90.info$Batch
prcompPlot(imr90.data, colFactor=expt)
pca <- prcomp(t(imr90.data), scale.=TRUE)
prcompPlot(pca, 1, 3, colFactor=batch, pchFactor=expt, palette='topo.colors',
main='IMR90 PCA plot of Dim 1 and 3')

```

```
print.summary.harmanresults
```

Printing Harmanresults summaries.

Description

Print method for summary.harmanresults.

Usage

```
## S3 method for class 'summary.harmanresults'
print(x, ...)
```

Arguments

x	an object of class summary.harmanresults, usually, a result of a call to summary.harmanresults.
...	further parameters.

Value

Prints summary information from an object of class summary.harmanresults.

reconstructData	<i>Reconstruct corrected data from Harman results</i>
-----------------	---

Description

Method which reverts the PCA factorisation for instances of [harmanresults](#). This allows the original or corrected data to be returned back from the PCA domain into the original data domain.

Usage

```
reconstructData(object, this = "corrected")
```

Arguments

object	An instance of harmanresults.
this	string, legal values are original or corrected.

Value

matrix of data

See Also

[harman](#) [harmanresults](#)

Examples

```
library(HarmanData)
data(OLF)
expt <- olf.info$Treatment
batch <- olf.info$Batch
olf.harman <- harman(olf.data, expt, batch)
olf.data.corrected <- reconstructData(olf.harman)
```

shiftBetas

Shift beta values from 0 and 1 to avoid infinite M values

Description

A convenience function for methylation data.

Usage

```
shiftBetas(betas, shiftBy = 1e-04)
```

Arguments

betas matrix, beta values.
 shiftBy numeric, the amount to shift values of 0 and 1 by.

Value

None

Examples

```
betas <- seq(0, 1, by=0.05)
range(betas)
newBetas <- shiftBetas(betas, shiftBy=1e-4)
newBetas
range(newBetas)
```

summary.harmanresults *Summarizing harman results.*

Description

Summary method for class [harmanresults](#).

Usage

```
## S3 method for class 'harmanresults'
summary(object, ...)
```

Arguments

<code>object</code>	An object of class <code>harmanresults</code> .
<code>...</code>	further parameters.

Value

Returns an object of class `summary.harmanresults`.

See Also

[harmanresults](#)

Examples

```
library(HarmanData)
data(OLF)
expt <- olf.info$Treatment
batch <- olf.info$Batch
olf.harman <- harman(olf.data, expt, batch)
summary(olf.harman)
```

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