# Package 'Harman'

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Type Package

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

**Version** 1.26.0 **Date** 2022-03-28

**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.

**NeedsCompilation** yes

**Suggests** HarmanData, BiocGenerics, BiocStyle, knitr, rmarkdown, RUnit, RColorBrewer, bladderbatch, limma, minfi, lumi, msmsEDA, affydata, minfiData, sva

**Depends** R (>= 3.6)

**Imports** Rcpp (>= 0.11.2), graphics, stats, Ckmeans.1d.dp, parallel, methods, matrixStats

LinkingTo Rcpp

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**biocViews** BatchEffect, Microarray, MultipleComparison, PrincipalComponent, Normalization, Preprocessing, DNAMethylation, Transcription, Software, StatisticalMethod

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Maintainer Jason Ross < jason.ross@csiro.au>

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arrowPlot

PCA before and after arrow plot for harman results

# 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.

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# 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

```
library(HarmanData)
data(OLF)
expt <- olf.info$Treatment
batch <- olf.info$Batch</pre>
```

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```
olf.harman <- harman(olf.data, expt, batch)
arrowPlot(olf.harman, pc_x=2, pc_y=3, length=0.2)</pre>
```

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 formated data.
limit	A double precsion 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

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#### 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	Compute LVR and meandiff statistics for beta values after batch correction

#### 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 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**

detachHarman

Detach the Harman package and its shared C/C++ library code

### **Description**

A helper function that can be called if harman had to be aborted.

#### Usage

detachHarman()

#### Value

None

discoverClusteredMethylation

Discover clustered beta values

## **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 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**

```
betas a matrix of methylation beta values with samples in columns and betas in rows.

ks integer, the range of k's to consider for clustering (defaults to 1:10).

min_cluster_size
    integer, the minimum number of samples in a cluster (defaults to 5).

min_cluster_dist
    numeric, the minimum beta difference in cluster centroids (defaults to 0.1).

max_clusters integer, the maximum value of k returned (defaults to 4).

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 named vector containing the optimal value for k.

#### See Also

kClusterMethylation, clusterStats

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### **Examples**

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**

datamatrix	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.
expt	vector or factor with the experimental variable of interest (variance to be kept).
batch	vector or factor with the batch variable (variance to be removed).
limit	numeric, confidence limit. Indicates the limit of confidence in which to stop removing a batch effect. Must be between 0 and 1.

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numrepeats integer, the number of repeats in which to run the simulated batch mean distri-

bution estimator using the random selection algorithm. (N.B. 32 bit Windows

versions may have an upper limit of 300000 before catastrophic failure)

randseed integer, the seed for random number generation.

forceRand logical, to enforce Harman to use a random selection algorithm to compute cor-

rections. 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).

printInfo 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 should 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

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#### **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)</pre>
```

harmanresults

Harman results object

### 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

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#### **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)</pre>
```

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**

Х

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 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.

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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

pcaPlot 13

# 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

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.
this	string, legal values are original or corrected.
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.
pchBy	string, point-type by the experimental or batch variable; legal values are expt and batch. This parameter is overridden by pch.
palette	string, the function to call to create a vector of contiguous colours with the levels of factor in colBy steps.
legend	logical, whether to display a legend on the plot.
col,	colour vector for the points. This parameter overrides colBy and palette.
pch,	integer vector giving the point type. This parameter overrides pchBy.
	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.

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## 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)</pre>
```

plot.harmanresults

Plot method for harman

# 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

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# Examples

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

prcompPlot

PCA plot

# 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**

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

```
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.

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## Value

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

reconstructData

Reconstruct corrected data from Harman results

# 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

```
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)</pre>
```

shiftBetas

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

# **Description**

A convienance 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)</pre>
```

summary.harmanresults Summarizing harman results.

# Description

Summary method for class harmanresults.

## Usage

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

## **Arguments**

object An object of class harmanresults.

... further parameters.

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# Value

Returns an object of class summary.harmanresults.

# See Also

harmanresults

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

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