# 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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**Author** Yalchin Oytam [aut],
Josh Bowden [aut],
Jason Ross [aut, cre]

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

# Contents

arrowPlot . . . . . 2
callHarman . . . . . 4
clusterStats . . . . . 5
detachHarman . . . . . 6
discoverClusteredMethylation . . . . . 6
harman . . . . . 8
harmanresults . . . . . 10
harmanScores . . . . . 11
kClusterMethylation . . . . . 11
pcaPlot . . . . . 13
plot.harmanresults . . . . . 14
prcompPlot . . . . . 15
print.summary.harmanresults . . . . . 16
reconstructData . . . . . 17
shiftBetas . . . . . 18
summary.harmanresults . . . . . 18

**Index** **20**

---

`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.

## 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 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

### 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 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 *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 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

## 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

| | |
|---|---|
| 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. |

| | |
|---|---|
| numrepeats | 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) |
| randseed | integer, the seed for random number generation. |
| forceRand | 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). |
| 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

## 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 *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

## 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

| | |
|---|---|
| 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.

## 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 *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

## Examples

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

---

`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')
```

---

## `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` *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`

## 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 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)
```

## 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. |

## 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)
```

# Index


.callHarman *(*callHarman*)*, 4

arrow, *3*
arrowPlot, 2, *9, 10*
as.matrix, *9*

callHarman, 4
clusterStats, *5*, 5, *7*, *12*

detachHarman, 6
discoverClusteredMethylation, *5, 6*, 6, *11, 12*

GenomicMethylSet, *5*, *7*, *12*
GenomicRatioSet, *5*, *7*, *12*

harman, *4*, *6*, 8, *9, 10*, *17*
harmanresults, *2, 3*, 10, *13, 14*, *17–19*
harmanScores, 11

kClusterMethylation, *5–7*, 11, *12*

MethylSet, *5*, *7*, *12*

pcaPlot, *9, 10*, 13, *14*
plot.harmanresults, *3*, *9, 10*, *14*, 14
prcomp, *9, 10*, *15, 16*
prcompPlot, 15
print.summary.harmanresults, 16

rainbow, *16*
reconstructData, *9, 10*, 17

shiftBetas, 18
summary.harmanresults, *9, 10*, 18