Package ‘wateRmelon’

April 5, 2014

Type Package

Title Illumina 450 methylation array normalization and metrics

Version 1.2.2

Date 2013-01-12

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Description 15 flavours of betas and three performance metrics, with methods for objects produced by methylumi, minfi and IMA packages.

License GPL-3

Depends R (>= 2.10), limma, methods, matrixStats, methylumi, lumi,IlluminaHumanMethylation450k.db, ROC

Enhances minfi, methylumi, IMA

Suggests RPMM

LazyLoad yes

biocViews DNAMethylation, Microarray, TwoChannel, Preprocessing,QualityControl

Collate adaptRefQuantiles.R as.methylumi.R beta1.R Beta2M.R betaqn.R bgeq.R bgeqot.R bgeqq2.R bgeqqn.R BMIQ_1.1.R combo.R concatenateMatrices.R coRankedMatrices.R correctI.R correctII.R dataDetectPval2NA.R db1.R detectionPval.filter.R dfs2.R dfsfit.R dmrse.R dmrse_col.R dmrse_row.R dyebuy1.R dyebuy2.R dyebuy3.R dyebuy4.R filterXY.R findAnnotationProbes.R gcoms.R gcose.R genki.R genkme.R genkus.R getMethylumiBeta.R getQuantiles.R getSamples.R getsnp.R loadMethylumi2.R lumiMethyR2.R M2Beta.R melon.R nbBeadsFilter.R normalize.quantiles2.R normalizeIlluminaMethylation.R ot.R pasteque.R peak.correction.R pfilter.R pipelineIlluminaMethylation.batch.R

preprocessIlluminaMethylation.R referenceQuantiles.R
robustQuantileNorm_Illumina450K.probeCategories.R
robustQuantileNorm_Illumina450K.R seabird.R sextest.R summits.R
swan2.R uniqueAnnotationCategory.R AllGenerics.R x_methylumi.R y_minfi.R z_IMA.R

R topics documented:

wateRmelon-package 2
adaptRefQuantiles 3
as.methylumi-methods 4
beadc 5
beadcount 6
Beta2M 6
betaqn-exprmethy450-methods 7
BMIQ 8
colnames-methods 10
combo 10
dasen 11
dasen-methods 14
dasen-minfi-methods 16
db1 18
dmrse 19
dmrse-methods 20
genki 20
genki-methods 21
genkme 22
got 23
iDMR 23
melon 24
metrics 25
pfilter 26
seabi 28
seabi-methods 29
seabird 29
sextest 30

Index **32**

`wateRmelon-package` *Illumina 450K arrays: normalization and performance metrics*

Description

Functions for calculating the index of DNA methylation proportion beta in 15 different ways, and three different ways of estimating data quality or normalization performance.

Details

Package:	wateRmelon
Type:	Package
Version:	1.0
Date:	2012-10-10
License:	GPL3

Author(s)

Leonard C Schalkwyk, Ruth Pidsley and Chloe Wong Maintainer: Who to complain to <leonard.schalkwyk@kcl.ac.uk>

References

Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

`adaptRefQuantiles` *Functions from 450-pipeline (Touleimat & Tost)*

Description

These functions are part of the 450K pipeline (Touleimat and Tost, Epigenomics 2012 4:325). For freestanding use of the normalization function, a wrapper is provided, see `tost`

Value

see `tost`

Author(s)

Nizar Touleimat, wrapper by Leonard.Schalkwyl@kcl.ac.uk

References

Touleimat N, Tost J: Complete pipeline for Infinium R Human Methylation 450K BeadChip data processing using subset quantile normalization for accurate DNA methylation estimation. Epigenomics 2012, 4:325-341

Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

as.methylumi-methods *Methods for Function* `as.methylumi`

Description

Returns a MethyLumiSet object populaed with the data provided. There are MethyLumiSet and MethylSet methods. In the default method, the data is all optional. Please note that for the results to be sane, mn, un, bn, and pv have to be in the same sample and feature order and the same size. The function does not currently do any checks!

Usage

```
# default method
as.methylumi (mn = NULL, un = NULL, bn = NULL, pv = NULL, qc = NULL, da = NULL,
fd = c("CHR", "DESIGN"), ad=NULL)
```

Arguments

`mn`	matrix of methylated signal intensities, each column representing a sample (generic) or a MethyLumiSet, RGSet, or MethylSet object. Column names are used to get Sentrix row and column by default, see '...'.
`un`	matrix of unmethylated signal intensities, each column representing a sample (default method) or NULL when mn is an object containing methylated and unmethylated values
`bn`	matrix of precalculated betas, each column representing a sample
`pv`	matrix of detection p-values, each column representing a sample
`da`	annotation data frame, such as x@featureData@data #methylumi package. If NULL (the default), the `IlluminaHumanMethylation450kmanifest` package is used. See the fd argument
`qc`	control probe intensities: list of 2 matrices, Cy3 and Cy5, with rownames, such as produced by intensitiesByChannel(QCdata(x)) (methylumi package)
`fd`	vector of items of featureData, which by default is just the chromosome and DESIGN (ie typeI or type II assay). Other data can be included using the `fd` argument, available data is listed by the function `getColumns()`
`ad`	optional assayData

Methods

`signature(mn = "MethylSet")` Coerces a MethylSet to a MethyLumiSet, and provides it with a set of featureData, which by default is just the chromosome and DESIGN (ie typeI or type II assay). Other data can be included using the `fd` argument, available data is listed by the function `getColumns()`

`signature(mn = "MethyLumiSet")` This is mainly useful for adding featureData as described under MethylSet above. MethyLumiSet objects produced by methylumiR have the full annotation, those from methylumIDAT do not, and functions such as `swan` require it

`signature(mn = "ANY") as.methylumi (mn = NULL, un = NULL, bn = NULL, pv = NULL, qc = NULL,   da = NULL, fo`

---

beadc — *Calculates the number of samples with bead count <3 for each probe in matrix of bead count values*

Description

Calculates the number of samples with bead count <3 for each probe in matrix of bead count values.

Usage

```
beadc(x)
```

Arguments

`x`	matrix of bead count values returned by the beadcount function

Value

Vector of number of samples with bead count <3 for each probe

Note

The beadc function is internal to the pfilter function

Author(s)

ruth.pidsley@kcl.ac.uk

References

[1] Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

`beadcount` *Creates matrix of beacounts from minfi data.*

Description

Creates matrix of beacounts from data read in using the minfi package. NAs represent probes with beadcount <3. An Extended RG Channel Set is required for this function to work.

Usage

```
beadcount(x)
```

Arguments

`x`	450K methylation data read in using minfi to create an Extended RG Channel Set

Value

A matrix of bead counts with bead counts <3 represented by NA for use in the pfilter function for quality control

Note

The beadcount function is internal to the pfilter function

Author(s)

Ruth.Pidsley@kcl.ac.uk

References

[1] Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

`Beta2M` *Internal functions for peak.correction (fuks)*

Description

Internal functions for peak.correction

Usage

```
Beta2M(B)
```

Arguments

B	a vector or matrix of beta values for conversion

Value

a vector or matrix of the same shape as the input

Author(s)

Matthieu Defrance <defrance@bigre.ulb.ac.be>

References

Dedeurwaerder S, Defrance M, Calonne E, Sotiriou C, Fuks F: Evaluation of the Infinium Methylation 450K technology . Epigenetics 2011, 3(6):771-784.

---

betaqn-exprmethy450-methods

Calculate normalized betas from exprmethy450 of Illumina 450K methylation arrays

---

Description

Quantile normalize betas from exprmethy450 objects

Arguments

bn	An exprmethy450 object.
fudge	value added to total intensity to prevent denominators close to zero when calculating betas

Details

betaqn quantile normalizes betas

Value

exprmethy450 object of the same shape and order as bn.

methods

betaqn(bn) fuks(bn)

Author(s)

Leonard.Schalkwyk@kcl.ac.uk

References

[1] Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

[2] Dedeurwaerder S, Defrance M, Calonne E, Sotiriou C, Fuks F: Evaluation of the Infinium Methylation 450K technology . Epigenetics 2011, 3(6):771-784.

[3] Touleimat N, Tost J: Complete pipeline for Infinium R Human Methylation 450K BeadChip data processing using subset quantile normalization for accurate DNA methylation estimation. Epigenomics 2012, 4:325-341

[4] Maksimovic J, Gordon L, Oshlack A: SWAN: Subset quantile Within-Array Normalization for Illumina Infinium HumanMethylation450 BeadChips. Genome biology 2012, 13(6):R44

`BMIQ` *Beta-Mixture Quantile (BMIQ) Normalisation method for Illumina 450k arrays*

Description

BMIQ is an intra-sample normalisation procedure, correcting the bias of type-2 probe values. BMIQ uses a 3-step procedure: (i) fitting of a 3-state beta mixture model, (ii) transformation of state-membership probabilities of type2 probes into quantiles of the type1 distribution, and (iii) a conformal transformation for the hemi-methylated probes. Exact details can be found in the reference below.

Usage

```
BMIQ(beta.v, design.v, nL = 3, doH = TRUE, nfit = 50000, th1.v = c(0.2, 0.75), th2.v = NULL, niter = 5, to
## S4 method for signature MethyLumiSet
BMIQ(beta.v, nL=3, doH=TRUE, nfit=5000, th1.v=c(0.2,0.75), th2.v=NULL, niter=5, tol=0.001, plots=FALSE
CheckBMIQ(beta.v, design.v, pnbeta.v)
```

Arguments

`beta.v`	vector consisting of beta-values for a given sample. NAs are passed through. Beta-values that are exactly 0 or 1 will be replaced by the minimum positive or maximum value below 1, respectively. For the MethyLumiSet method, a MethyLumiSet is the only required argument, and the function will be run on each sample.
`design.v`	corresponding vector specifying probe design type (1=type1,2=type2). This must be of the same length as beta.v and in the same order.
`nL`	number of states in beta mixture model. 3 by default. At present BMIQ only works for nL=3.
`doH`	perform normalisation for hemimethylated type2 probes. These are normalised using an empirical conformal transformation and also includes the left-tailed type2 methylated probes since these are not well described by a beta distribution. By default TRUE.

- `nfit` number of probes of a given design type to use for the fitting. Default is 50000. Smaller values (~10000) will make BMIQ run faster at the expense of a small loss in accuracy. For most applications, 5000 or 10000 is ok.
- `th1.v` thresholds used for the initialisation of the EM-algorithm, they should represent buest guesses for calling type1 probes hemi-methylated and methylated, and will be refined by the EM algorithm. Default values work well in most cases.
- `th2.v` thresholds used for the initialisation of the EM-algorithm, they should represent buest guesses for calling type2 probes hemi-methylated and methylated, and will be refined by the EM algorithm. By default this is null, and the thresholds are estimated based on th1.v and a modified PBC correction method.
- `niter` maximum number of EM iterations to do. This number should be large enough to yield good fits to the type1 distribution. By default 5.
- `tol` tolerance convergence threshold for EM algorithm. By default 0.001.
- `plots` logical specifying whether to plot the fits and normalised profiles out. By default TRUE.
- `sampleID` the ID of the sample being normalised.
- `pri` logical: print verbose progress information?
- `pnbeta.v` BMIQ normalised profile.

Details

Full details can be found in the reference below. Note: these functions require the RPMM package, not currently a dependency of the wateRmelon package.

Value

Default method: A list with following entries:

- `nbeta` the normalised beta-profile for the sample
- `class1` the assigned methylation state of type1 probes
- `class2` the assigned methylation state of type2 probes
- `av1` the mean beta-values for the nL states for type1 probes
- `av2` the mean beta-values for the nL states for type2 probes
- `hf` the estimated "Hubble" dilation factor used in the normalisation of hemi-methylated probes
- `th1` estimated thresholds for calling unmethylated and methylated type1 probes
- `th2` estimated thresholds for calling unmethylated and methylated type2 probes

MethyLumiSet method: A methyLumiSet object

Author(s)

Andrew Teschendorff, MethyLumiSet method by Leo Schalkwyk Leonard.Schalkwyk@kcl.ac.uk

References

Teschendorff AE, Marabita F, Lechner M, Bartlett T, Tegner J, Gomez-Cabrero D, Beck S. A Beta-Mixture Quantile Normalisation method for correcting probe design bias in Illumina Infinium 450k DNA methylation data. Bioinformatics. 2012 Nov 21.

Examples

```
library(RPMM)
data(melon)
BMIQ(melon,nfit=100)
```

`colnames-methods` *Methods for Function* `colnames` *in Package* **wateRmelon**

Description

Methods for function `colnames` in package **wateRmelon**.

Methods

`signature(x = "MethyLumiSet")` returns the sample names

`combo` *Combine MethyLumiSet objects*

Description

This is a wrapper for combining different MethyLumiSet objects.

Usage

```
combo(...)
```

Arguments

`...`	Eventually, any number of MethyLumiSet objects. Currently only guaranteed for 2 objects.

Details

This is a wrapper for `methylumi::combine`, which works around a name clash with a different combine function from the `gdata` package, and also a bug in `methylumi::combine`.

Value

a `MethyLumiSet`. The `assayData`, `QCdata`, `experimentData`, `protocolData` and `phenoData` are joined on `sampleName` . `featureData` and annotation are taken from the object given in the first argument

Note

the function uses `sampleNames` and gets rid of duplicates. Numeric sampleNames cause problems (and are a Bad Idea anyway). They should be turned into names with `make.names()` first.

Author(s)

Leo Schalkwyk <leonard.schalkwyk@kcl.ac.uk>

References

[1] Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

See Also

`as.methylumi`

Examples

```
library(wateRmelon)
data(melon)
## pretend we have two different data sets
melon
pelon <- melon
sampleNames(pelon) <- gsub(^6, 7, sampleNames(pelon))
combo(melon, pelon)
```

---

dasen — *Calculate normalized betas from Illumina 450K methylation arrays*

Description

Multiple ways of calculating the index of methylation (beta) from methylated and unmethylated probe intensities used in Pidsley et al 2012. S4 methods exist where possible for MethyLumiSet, MethylSet, RGSet and exprmethy450 objects.

Usage

```
dasen ( mns, uns, onetwo, fudge = 100, ret2=FALSE, ... )
nasen ( mns, uns, onetwo, ret2=FALSE, fudge = 100 )
betaqn( bn )
naten ( mn,  un, fudge = 100, ret2=FALSE )
naten ( mn,  un, fudge = 100, ret2=FALSE )
nanet ( mn,  un, fudge = 100, ret2=FALSE )
nanes ( mns, uns, onetwo, fudge = 100, ret2=FALSE, ... )
danes ( mn,  un,  onetwo, fudge = 100, ... )
danet ( mn,  un,  onetwo, fudge = 100, ret2=FALSE, ... )
danen ( mns, uns, onetwo, fudge = 100, ret2=FALSE, ... )
daten1( mn,  un,  onetwo, fudge = 100, ret2=FALSE, ... )
daten2( mn,  un,  onetwo, fudge = 100, ret2=FALSE, ... )
tost  ( mn,  un,  da, pn )
fuks  ( data, anno)
swan  ( mn,  un,  qc, da=NULL, return.MethylSet=FALSE )
```

Arguments

`mn, mns` matrix of methylated signal intensities, each column representing a sample (generic) or a MethyLumiSet, RGSet, or MethylSet object. Column names are used to get Sentrix row and column by default, see '...'.

`un, uns` matrix of unmethylated signal intensities, each column representing a sample (default method) or NULL when mn is an object containing methylated and unmethylated values

`bn, data` matrix of precalculated betas, each column representing a sample

`onetwo` character vector or factor of length nrow(mn) indicating assay type 'I' or 'II'

`pn` matrix of detection p-values, each column representing a sample

`da, anno` annotation data frame, such as `x@featureData@data` #methylumi package. If NULL, the swan method requires the `IlluminaHumanMethylation450kmanifest` package.

`qc` control probe intensities: list of 2 matrices, Cy3 and Cy5, with rownames, such as produced by intensitiesByChannel(QCdata(x)) #methylumi package

`fudge` value added to total intensity to prevent denominators close to zero when calculating betas

`return.MethylSet` if TRUE, returns a MethylSet object instead of a naked matrix of betas.

`ret2` if TRUE, returns a list of intensities and betas instead of a naked matrix of betas.

`...` additional argument roco for dfsfit giving Sentrix rows and columns. This allows a background gradient model to be fit. This is split from data column names by default. roco=NULL disables model fitting (and speeds up processing), otherwise roco can be supplied as a character vector of strings like 'R01C01' (only 3rd and 6th characters used).

Details

dasen same as nasen but type I and type II backgrounds are equalized first. This is our recommended method

betaqn quantile normalizes betas

naten quantile normalizes methylated and unmethylated intensities separately, then calculates betas

nanet quantile normalizes methylated and unmethylated intensities together, then calculates betas. This should equalize dye bias

nanes quantile normalizes methylated and unmethylated intensities separately, except for type II probes where methylated and unmethylated are normalized together. This should equalize dye bias without affecting type I probes which are not susceptible

danes same as nanes, except type I and type II background are equalized first

danet same as nanet, except type I and type II background are equalized first

danen background equalization only, no normalization

daten1 same as naten, except type I and type II background are equalized first (smoothed only for methylated)

daten2 same as naten, except type I and type II background are equalized first (smoothed for methylated an unmethylated)

nasen same as naten but type I and typeII intensities quantile normalized separately

tost method from Touleimat and Tost 2011

fuks method from Dedeurwaerder et al 2011. Peak correction only, no normalization

swan method from Maksimovic et al 2012

Value

a matrix (default method) or object of the same shape and order as the first argument containing betas.

Author(s)

Leonard.Schalkwyk@kcl.ac.uk

References

[1] Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

[2] Dedeurwaerder S, Defrance M, Calonne E, Sotiriou C, Fuks F: Evaluation of the Infinium Methylation 450K technology . Epigenetics 2011, 3(6):771-784.

[3] Touleimat N, Tost J: Complete pipeline for Infinium R Human Methylation 450K BeadChip data processing using subset quantile normalization for accurate DNA methylation estimation. Epigenomics 2012, 4:325-341.

[4] Maksimovic J, Gordon L, Oshlack A: SWAN: Subset quantile Within-Array Normalization for Illumina Infinium HumanMethylation450 BeadChips. Genome biology 2012, 13(6):R44

See Also

pfilter, as.methylumi

Examples

```
#MethyLumiSet method
data(melon)
melon.dasen <- dasen(melon)
```

---

dasen-methods — *Calculate normalized betas from MethyLumiSets of Illumina 450K methylation arrays*

Description

Multiple ways of calculating the index of methylation (beta) from methylated and unmethylated probe intensities used in Pidsley et al 2012.

Arguments

`mn, mns, data, bn`	A MethyLumiSet object. Sample names names are used to get Sentrix row and column by default, see '...'.
`fudge`	value added to total intensity to prevent denominators close to zero when calculating betas
`...`	additional argument roco for dfsfit giving Sentrix rows and columns. This allows a background gradient model to be fit. This is split from data column names by default. roco=NULL disables model fitting (and speeds up processing), otherwise roco can be supplied as a character vector of strings like 'R01C01' (only 3rd and 6th characters used).

Details

dasen same as nasen but type I and type II backgrounds are normalized first. This is our recommended method

betaqn quantile normalizes betas

naten quantile normalizes methylated and unmethylated intensities separately, then calculates betas

nanet quantile normalizes methylated and unmethylated intensities together, then calculates betas. This should equalize dye bias.

nanes quantile normalizes methylated and unmethylated intensities separately, except for type II probes where methylated and unmethylated are normalized together. This should equalize dye bias without affecting type I probes which are not susceptible.

danes same as nanes, except typeI and type II background are equalised first.

danet same as nanet, except typeI and type II background are equalised first.

danen background equalisation only, no normalization

daten1 same as naten, except typeI and type II background are equalised first (smoothed only for methylated)

daten2 same as naten, except typeI and type II background are equalised first (smoothed for methylated an unmethylated)

nasen same as naten but typeI and typeII intensities quantile normalized separately

tost method from Touleimat and Tost 2011

fuks method from Dedeurwaerder et al 2011. Peak correction only, no normalization

swan method from Maksimovic et al 2012

Value

a matrix (default method) or object of the same shape and order as the first argument containing betas.

methods

dasen (mns, fudge = 100, ...) nasen (mns, fudge = 100) betaqn(bn) naten (mn, fudge = 100) naten (mn, fudge = 100) nanet (mn, fudge = 100) nanes (mns,fudge = 100) danes (mn, fudge = 100, ...) danet (mn, fudge = 100, ...) danen (mns,fudge = 100, ...) daten1(mn, fudge = 100, ...) daten2(mn, fudge = 100, ...) tost (mn) fuks (data) swan (mn)

Author(s)

Leonard.Schalkwyk@kcl.ac.uk

References

[1] Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

[2] Dedeurwaerder S, Defrance M, Calonne E, Sotiriou C, Fuks F: Evaluation of the Infinium Methylation 450K technology . Epigenetics 2011, 3(6):771-784.

[3] Touleimat N, Tost J: Complete pipeline for Infinium R Human Methylation 450K BeadChip data processing using subset quantile normalization for accurate DNA methylation estimation. Epigenomics 2012, 4:325-341

[4] Maksimovic J, Gordon L, Oshlack A: SWAN: Subset quantile Within-Array Normalization for Illumina Infinium HumanMethylation450 BeadChips. Genome biology 2012, 13(6):R44

dasen-minfi-methods — *Calculate normalized betas from Illumina 450K methylation arrays*

Description

Multiple ways of calculating the index of methylation (beta) from methylated and unmethylated probe intensities used in Pidsley et al 2012.

Arguments

`mn, mns`	matrix of methylated signal intensities, each column representing a sample (default method), or an object for which a method is available. Column names are used to get Sentrix row and column by default, see '...'.
`un, uns`	matrix of unmethylated signal intensities, each column representing a sample (default method) or NULL when mn is an object containing methylated and unmethylated values
`bn, data`	matrix of precalculated betas, each column representing a sample
`onetwo`	character vector or factor of length nrow(mn) indicating assay type 'I' or 'II'
`da, anno`	annotation data frame, such as x@featureData@data #methylumi package
`qc`	control probe intensities: list of 2 matrices, Cy3 and Cy5, with rownames, such as produced by intensitiesByChannel(QCdata(x)) #methylumi package
`fudge`	value added to total intensity to prevent denominators close to zero when calculating betas
`...`	additional argument roco for dfsfit giving Sentrix rows and columns. This allows a background gradient model to be fit. This is split from data column names by default. roco=NULL disables model fitting (and speeds up processing), otherwise roco can be supplied as a character vector of strings like 'R01C01' (only 3rd and 6th characters used).

Details

dasen same as nasen but type I and type II backgrounds are normalized first. This is our recommended method

betaqn quantile normalizes betas

naten quantile normalizes methylated and unmethylated intensities separately, then calculates betas

nanet quantile normalizes methylated and unmethylated intensities together, then calculates betas. This should equalize dye bias.

nanes quantile normalizes methylated and unmethylated intensities separately, except for type II probes where methylated and unmethylated are normalized together. This should equalize dye bias without affecting type I probes which are not susceptible.

danes same as nanes, except typeI and type II background are equalised first.

danet same as nanet, except typeI and type II background are equalised first.

danen background equalisation only, no normalization

daten1 same as naten, except typeI and type II background are equalised first (smoothed only for methylated)

daten2 same as naten, except typeI and type II background are equalised first (smoothed for methylated an unmethylated)

nasen same as naten but typeI and typeII intensities quantile normalized separately

tost method from Touleimat and Tost 2011

fuks method from Dedeurwaerder et al 2011. Peak correction only, no normalization

swan method from Maksimovic et al 2012

Value

a matrix of betas is returned by the MethySet and RGChannelSet methods because they do not have a defined slot for betas.

methods

dasen (mns, uns, onetwo, fudge = 100, ...) nasen (mns, uns, onetwo, fudge = 100) betaqn(bn) naten (mn, un, fudge = 100) naten (mn, un, fudge = 100) nanet (mn, un, fudge = 100) nanes (mns, uns, onetwo, fudge = 100) danes (mn, un, onetwo, fudge = 100, ...) danet (mn, un, onetwo, fudge = 100, ...) danen (mns, uns, onetwo, fudge = 100, ...) daten1(mn, un, onetwo, fudge = 100, ...) daten2(mn, un, onetwo, fudge = 100, ...) tost (mn, un, da, pn) fuks (data, anno) swan (mn, un, qc)

Author(s)

Leonard.Schalkwyk@kcl.ac.uk

References

[1] Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

[2] Dedeurwaerder S, Defrance M, Calonne E, Sotiriou C, Fuks F: Evaluation of the Infinium Methylation 450K technology . Epigenetics 2011, 3(6):771-784.

[3] Touleimat N, Tost J: Complete pipeline for Infinium R Human Methylation 450K BeadChip data processing using subset quantile normalization for accurate DNA methylation estimation. Epigenomics 2012, 4:325-341)

[4] Maksimovic J, Gordon L, Oshlack A: SWAN: Subset quantile Within-Array Normalization for Illumina Infinium HumanMethylation450 BeadChips. Genome biology 2012, 13(6):R44

db1 *Internal wateRmelon functions for calculating betas*

Description

db1 is used for quantile normalizing methylated together with unmethylated (dye bias methods nanet, nanes, danes and danet. dfs* functions are used for smoothing the background equalization in methods whose names start with d (daten etc).

Usage

```
db1(mn, un)
dfsfit(mn, onetwo, roco=unlist(data.frame(strsplit(colnames(mn), "_"),
                 stringsAsFactors = FALSE)[2, ]) )

dfs2(x, onetwo)
```

Arguments

`mn, x`	matrix of methylated signal intensities, each column representing a sample (default method), or an object for which a method is available. For dfsfit and dfs2 this can also be a matrix of unmethylated intensities.
`un`	matrix of unmethylated signal intensities, each column representing a sample (default method) or NULL when mn is an object containing methylated and unmethylated values.
`onetwo`	character vector or factor of length nrow(mn) indicating assay type 'I' or 'II'
`roco`	roco for dfsfit giving Sentrix rows and columns. This allows a background gradient model to be fit. This is split from data column names by default. roco=NULL disables model fitting (and speeds up processing), otherwise roco can be supplied as a character vector of strings like 'R01C01' (3rd and 6th characters used).

Details

db1 - quantile normalizes methylated against unmethylated (basic function for dyebuy* dye bias methods). dfsfit - corrects the difference in backgrounds between type I and type II assays and fits a linear model to Sentrix rows and columns if these are available to improve precision where there is a background gradient. dfs2 - finds the difference between type I and type II assay backgrounds for one or more samples.

Value

db1 - a list of 2 matrices of intensities, methylated and unmethylated dfsfit - a matrix of adjusted intensities dfs2 - a background offset value

Author(s)

Leonard.Schalkwyk@kcl.ac.uk

References

Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

---

dmrse *Standard error of iDMR 450k array DNA methylation features*

Description

Imprinting differentially methylated regions (iDMRs) are expected to be approximately half methylated, as is observed at the 227 probes in known iDMRs. These functions calculate measures of dispersion for the beta values at these CpG sites, of which the most useful is dmrse_row, which is the between-sample standard error.

Usage

```
dmrse(betas, idmr = iDMR())
dmrse_col(betas, idmr = iDMR())
dmrse_row(betas, idmr = iDMR())
```

Arguments

`betas`	a matrix of betas (default method), a `MethyLumiSet` object (`methylumi` package), a `MethylSet` or `RGChannelSet` object (`minfi` package) or a `exprmethy450` object (`IMA` package).
`idmr`	a character vector of iDMR probe names such as returned by iDMR()

Value

return a standard error of the mean of betas for all samples and iDMR probes (dmrse) or the standard error of the mean for just the between sample component(dmrse_row) or between probe(dmrse_col) component.

Author(s)

Leonard.Schalkwyk@kcl.ac.uk

References

Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

See Also

`seabi`, a sex-difference metric, and `genki`, based on SNPs.

Examples

```
#MethyLumiSet method
   data(melon)
   dmrse(melon)

#MethyLumiSet method after normalization
   melon.dasen <- dasen(melon)
   dmrse(melon.dasen)
```

---

dmrse-methods — *Methods for Function* `dmrse` *in Package* **wateRmelon**

Description

Methods for function `dmrse`, `dmrse_row` and `dmrse_col` in package **wateRmelon**. Please see `dmrse` for details of the calculation of this standard-error performance metric.

Methods

`signature(betas = "exprmethy450")` all of the methods simply extract betas from the data object (which can be a `exprmethy450`, `MethylSet`, `MethyLumiSe`, or `RGChannelSet`) and calculate the metric.

---

genki — *SNP derived performance metrics for Illumina 450K DNA methylation arrays.*

Description

A very simple genotype calling by one-dimensional K-means clustering is performed on each SNP, and for those SNPs where there are three genotypes, the squared deviations are summed for each genotype (similar to a standard deviation for each of allele A homozygote, heterozygote and allele B homozygote). By default these are further divided by the square root of the number of samples to get a standard error-like statistic.

Usage

```
genki(bn, g = getsnp(rownames(bn)), se = TRUE)
```

Arguments

bn	a matrix of beta values(default method), a `MethyLumiSet` object (`methylumi` package), a `MethylSet` or `RGChannelSet` object (`minfi` package) or a `exprmethy450` object (`IMA` package).
g	vector of SNP names
se	TRUE or FALSE specifies whether to calculate the standard error-like statistic

Details

There are 65 well-behaved SNP genotyping probes included on the array. These each produce a distribution of betas with tight peaks for the three possible genotypes, which will be broadened by technical variation between samples. The spread of the peaks is thus usable as a performance metric.

Value

a vector of 3 values for the dispersion of the three genotype peaks (AA, AB, BB : low, medium and high beta values)

Author(s)

Leonard.Schalkwyk@kcl.ac.uk

References

Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

Examples

```
#MethyLumiSet method
   data(melon)
   genki(melon)

#MethyLumiSet method after normalization
   melon.dasen <- dasen(melon)
   genki(melon.dasen)
```

---

genki-methods *Methods for Function* `genki` *in Package* **wateRmelon**

Description

Methods for function `genki` in package **wateRmelon**. Please see `genki` for details of the calculation of this standard-error performance metric.

Methods

`signature(betas = "exprmethy450")` all of the methods simply extract betas from the data object (which can be a `exprmethy450`, `MethylSet`, `MethyLumiSe`, or `RGChannelSet`) and calculate the metric.

---

genkme — *Internal functions for genotype-based normalization metrics*

Description

genkme - genotype calling with 1d k-means

genkus - apply genkme to available SNPs

getsnp - grep the rs-numbered probes

gcose - calculate between-sample SNP standard error

gcoms - calculate between-sample SNP mean-squared deviation

Usage

```
genkme(y, peaks = c(0.2, 0.5, 0.8))
```

Arguments

`y`	a vector or matrix of numeric values (betas, between 0 and 1)
`peaks`	initial values for cluster positions

Details

see `genki`

Value

see `genki`

Author(s)

Leonard.Schalkwyk@kcl.ac.uk

References

Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

got — *Internal functions for Illumina i450 normalization functions*

Description

got and fot find the annotation column differentiating type I and type II assays in MethylSet (got) or MethyLumiSet (fot) objects. pop extracts columns from IlluminaHumanMethylation450k.db

Usage

```
got(obj)
fot(x)
pop(fd,rn)
```

Arguments

`x`	a MethyLumiSet
`obj`	a MethylSet
`fd`	a character vector of the desired annotation columns
`rn`	a character vector of the desired features

Details

`got` returns a character vector of 'I' and 'II', `fot` returns the index of the relevant column. `pop` returns a data frame

Author(s)

Leonard.Schalkwyk@kcl.ac.uk

References

Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

iDMR — *Imprinting differentially methylated region probes of Illumina 450 arrays*

Description

A character vector of 227 propes on the Illumina 450k methylation array

Usage

```
data(iDMR)
```

Format

The format is: chr [1:227] "cg00000029" "cg00155882" "cg00576435" "cg00702231" "cg00765653" "cg00766368" ...

Source

DMR coordinates from https://atlas.genetics.kcl.ac.uk/

References

Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

Examples

```
data(iDMR)
## maybe str(iDMR) ; plot(iDMR) ...
```

---

melon   *Small MethyLumi data set for examples and testing*

Description

This object was derived using `methylumiR` on an edited GenomeStudio file containing a small subset of features. It works with all of the wateRmelon package beta functions (see `dasen` and metrics (see `genki`, `seabi`, and `dmrse_col`) except for `swan`.

Usage

```
data(melon)
```

Format

MethyLumiSet with assayData containing 3363 features, 12 samples

Source

Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

Examples

```
library(methylumi)
data(melon)
boxplot(log(methylated(melon)), las=2)
## maybe str(melon) ; plot(melon) ...
```

metrics — *Calculate a full set of 450K normalization/performance metrics*

Description

Calculate X-chromosome, SNP and imprinting DMR metrics for a matrix of betas from an Illumina 450K Human DNA methylation array. Requires precalculated t-test p-values for sex differences, a list of X-chromosome features and of imprinting DMR features.

Usage

```
metrics(betas, pv, X, idmr = iDMR, subset = NULL)
```

Arguments

`betas`	a matrix of betas, each row representing a probe, each column a sample
`pv`	a vector of p-values such as produced by `sextest`, one per row of betas
`X`	a logical vector of the same length as `pv`, indicating whether each probe is mapped to the X-chromosome
`idmr`	a character vector of probe names known to be in imprinting DMRs. Can be obtained with `iDMR()` or `data(iDMR)`
`subset`	index or character vector giving a subset of betas to be tested

Value

`dmrse_row`	see `dmrse_row`
`dmrse_col`	see `dmrse_col`
`dmrse`	see `dmrse`
`gcoms_a`	see `genki`
`gcose_a`	see `genki`
`gcoms_b`	see `genki`
`gcose_b`	see `genki`
`gcoms_c`	see `genki`
`gcose_c`	see `genki`
`seabird`	see `seabi`

Author(s)

Leonard.Schalkwyk@kcl.ac.uk

References

Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

Examples

```
data(melon)
melon.dasen <- dasen(melon)
bn <-betas(melon.dasen)
X <- melon.dasen@featureData@data$CHR==X
data(iDMR)
sex <- pData(melon.dasen)$sex
pv <- sextest(bn,sex)
melon.metrics <- metrics(bn, pv, X, idmr = iDMR, subset = NULL)
```

`pfilter` *Basic data filtering for Illumina 450 methylation data*

Description

The pfilter function filters data sets based on bead count and detection p-values. The user can set their own thresholds or use the default pfilter settings. pfilter will take data matrices of beta values, signal intensities and annotation data, but will also take methylumi (MethyLumiSet) or minfi (RGChannelSetExtended) objects. However it has come to our attention that data read in using the various packages and input methods will give subtly variable data output as they calculate detection p-value and beta values differently, and do/don?t give information about beadcount. The pfilter function does not correct for this, but simply uses the detection p-value and bead count provided by each package.

Usage

```
pfilter(mn, un, bn, da, pn, bc, perCount=NULL, pnthresh = NULL, perc = NULL,
pthresh = NULL,logical.return=FALSE)
```

Arguments

`mn` matrix of methylated signal intensities, each column representing a sample (default method), or an object for which a method is available e.g MethyLumiSet or RGChannelSetExtended. N.B. Bead count filtering will not work unless data read in as an RGGhannelSetExtended rather than an RGChannelSet.

`un` matrix of unmethylated signal intensities, each column representing a sample (default method) or NULL when mn is a MethyLumiSet or RGChannelSetExtended object

`bn` matrix of precalculated betas, each column representing a sample, or NULL when mn is a MethyLumiSet or RGChannelSetExtended object

`da` annotation data frame, such as x@featureData@data #methylumi package, or NULL when mn is a MethyLumiSet or RGChannelSetExtended object

`pn` matrix of detection p-values, each column representing a sample, a MethyLumiSet or RGChannelSetExtended object

`bc`	matrix of arbitrary values, each column representing a sample and eeach row representing a probe, in which "NA" represents beadcount <3, or NULL when mn is a MethyLumiSet or RGChannelSetExtended object
`perCount`	remove sites having this percentage of samples with a beadcount <3, default set to 5
`pnthresh`	cutoff for detection p-value, default set to 0.05
`perc`	remove samples having this percentage of sites with a detection p-value greater than pnthresh, default set to 1
`pthresh`	remove sites having this percentage of samples with a detection p-value greater than pnthresh, default set to 1
`logical.return`	If it is TRUE, FALSE or TRUE is returned to indicate success

Value

a filtered MethyLumiSet or RGChannelSetExtended object or

a list of the filtered matrices:

mn : methylated intensities

un : unmethylated intensities

bn : betas

da : feature data

Methods

`signature(mn = "MethyLumiSet")` This is used for performing the pfilter method on MethyLumiSet objects produced by methylumiR.

`signature(mn = "RGChannelSetExtended")` This is used for performing the pfilter method on RGChannelSetExtended objects produced by minfi.

Author(s)

Ruth.Pidsley@kcl.ac.uk

References

[1] Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

Examples

```
# MethyLumiSet method
data(melon)
melon.pf <- pfilter(melon)
```

seabi — *Calculate a performance metric based on male-female differences for Illumina methylation 450K arrays*

Description

Calculates an area under ROC curve - based metric for Illumina 450K data using a t-test for male-female difference as the predictor for X-chromosome location of probes. The metric is 1-area so that small values indicate good performance, to match our other, standard error based metrics `gcose` and `dmrse`. Note that this requires both male and female samples of known sex and can be slow to compute due to running a t-test on every probe.

Usage

```
seabi(bn, stop = 1, sex, X)
```

Arguments

`bn`	a matrix of betas (default method) or an object containing betas i.e. a `MethyLumiSet` object (`methylumi` package), a `MethylSet` or `RGChannelSet` object (`minfi` package) or a `exprmethy450` object (`IMA` package).
`stop`	partial area under curve is calculated if stop value <1 is provided
`sex`	a factor giving the sex of each sample (column)
`X`	a logical vector of length equal to the number of probes, true for features mapped to X-chromosome

Value

a value between 0 and 1. values close to zero indicate high data quality as judged by the ability to discriminate male from female X-chromosome DNA methylation.

Author(s)

leonard.schalkwyk@kcl.ac.uk

References

Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

Examples

```
library(methylumi)
data(melon)
sex  <- pData(melon)$sex
X    <- melon@featureData@data$CHR==X
seabi(betas(melon), sex=sex, X=X)
```

```
# methylumi method
   seabi(melon, sex=sex, X=X)
```

`seabi-methods` *Methods for Function* `seabi` *in Package* **wateRmelon**

Description

Methods for function `seabi` in package **wateRmelon**. Please see `seabi` for details of the calculation of this ROC AUC performance metric.

Methods

`signature(betas = "exprmethy450")` all of the methods simply extract betas from the data object (which can be a `exprmethy450`, `MethylSet`, `MethyLumiSe`, or `RGChannelSet`) and calculate the metric. All the methods also require a factor differentiating male from female samples.

`seabird` *Calculate ROC area-under-curve for X-chromosome sex differences (internal function for calculating the seabi metric)*

Description

This is a wrapper for the prediction and performance functions from the ROCR package that takes a vector of p-values and a vector of true or false for being on the X. See `seabi` function which does everything.

Usage

```
seabird(pr, stop = 1, X)
```

Arguments

`pr`	a vector of p-values, such as calculated by `seabird`
`stop`	fraction for partial area under curve. For example 0.1 gives you the area for the lowest 10% of p-values.
`X`	logical vector the same length as pv, true for features mapped to X-chromosome

Value

Returns an area value between 0 and 1, where 1 is the best possible performance.

Author(s)

Leonard C Schalkwyk 2012 Leonard.Schalkwyk@kcl.ac.uk

References

Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

`sextest` *Test Illumina methylation 450K array probes for sex difference (internal function for calculating* `seabi` *performance metric)*

Description

This is a wrapper for `lm` which does the equivalent of a Student t-test for difference in betas between males and females for each row of a matrix of betas.

Usage

```
sextest(betas, sex, ...)
```

Arguments

`betas`	a matrix of betas, each row is a probe, each column a sample
`sex`	a factor with 2 levels for male and female
`...`	additional arguments to be passed to `lm`

Value

Returns a vector of p-values of length equal to the number of rows of betas

Author(s)

Leonard.Schalkwyk@kcl.ac.uk

References

Pidsley R, Wong CCY, Volta M, Lunnon K, Mill J, Schalkwyk LC: A data-driven approach to preprocessing Illumina 450K methylation array data (submitted)

See Also

`seabi` `seabird`

Examples

```
#MethyLumiSet method
  data(melon)
  sex  <- pData(melon)$sex
  melon.sextest<-sextest(betas(melon),sex)


#MethyLumiSet method with quality control step
  data(melon)
  melon.dasen <- dasen(melon)
  sex  <- pData(melon.dasen)$sex
  melon.sextest<-sextest(betas(melon.dasen),sex)
```

Index

∗Topic **MethyLumiSet**
melon, 24
∗Topic **\textasciitilde\textasciitilde other possible keyword(s) \textasciitilde\textasciitilde**
colnames-methods, 10
dmrse-methods, 20
genki-methods, 21
∗Topic **\textasciitildekwd1**
betaqn-exprmethy450-methods, 7
BMIQ, 8
combo, 10
dasen, 11
seabi, 28
∗Topic **\textasciitildekwd2**
betaqn-exprmethy450-methods, 7
BMIQ, 8
combo, 10
dasen, 11
seabi, 28
∗Topic **datasets**
iDMR, 23
melon, 24
∗Topic **methods**
as.methylumi-methods, 4
colnames-methods, 10
dmrse-methods, 20
genki-methods, 21
seabi-methods, 29
∗Topic **package**
wateRmelon-package, 2

adaptRefQuantiles, 3
as.methylumi, *11*, *14*
as.methylumi *(*as.methylumi-methods*)*, 4
as.methylumi,ANY-method *(*as.methylumi-methods*)*, 4
as.methylumi,MethylSet-method *(*as.methylumi-methods*)*, 4
as.methylumi,MethyLumiSet-method *(*as.methylumi-methods*)*, 4
as.methylumi-methods, 4

beadc, 5
beadcount, 6
Beta2M, 6
betaqn *(*dasen*)*, 11
betaqn,exprmethy450-method *(*betaqn-exprmethy450-methods*)*, 7
betaqn,MethylSet-method *(*dasen-minfi-methods*)*, 16
betaqn,MethyLumiSet-method *(*dasen-methods*)*, 14
betaqn,RGChannelSet-method *(*dasen-minfi-methods*)*, 16
betaqn-exprmethy450-methods, 7
bgIntensitySwan.methylumi *(*adaptRefQuantiles*)*, 3
BMIQ, 8
BMIQ,ANY-method *(*BMIQ*)*, 8
BMIQ,MethyLumiSet-method *(*BMIQ*)*, 8
BMIQ-methods *(*BMIQ*)*, 8

CheckBMIQ *(*BMIQ*)*, 8
colnames,MethyLumiSet-method *(*colnames-methods*)*, 10
colnames-methods, 10
combo, 10
concatenateMatrices *(*adaptRefQuantiles*)*, 3
coRankedMatrices *(*adaptRefQuantiles*)*, 3
correctI *(*Beta2M*)*, 6
correctII *(*Beta2M*)*, 6

danen *(*dasen*)*, 11
danen,MethylSet-method *(*dasen-minfi-methods*)*, 16

danen,MethyLumiSet-method (dasen-methods), 14
danen,RGChannelSet-method (dasen-minfi-methods), 16
danes (dasen), 11
danes,MethylSet-method (dasen-minfi-methods), 16
danes,MethyLumiSet-method (dasen-methods), 14
danes,RGChannelSet-method (dasen-minfi-methods), 16
danet (dasen), 11
danet,MethylSet-method (dasen-minfi-methods), 16
danet,MethyLumiSet-method (dasen-methods), 14
danet,RGChannelSet-method (dasen-minfi-methods), 16
dasen, 11, *24*
dasen,MethylSet-method (dasen-minfi-methods), 16
dasen,MethyLumiSet-method (dasen-methods), 14
dasen,RGChannelSet-method (dasen-minfi-methods), 16
dasen-methods, 14
dasen-minfi-methods, 16
dataDetectPval2NA (adaptRefQuantiles), 3
daten1 (dasen), 11
daten1,MethylSet-method (dasen-minfi-methods), 16
daten1,MethyLumiSet-method (dasen-methods), 14
daten1,RGChannelSet-method (dasen-minfi-methods), 16
daten2 (dasen), 11
daten2,MethylSet-method (dasen-minfi-methods), 16
daten2,MethyLumiSet-method (dasen-methods), 14
daten2,RGChannelSet-method (dasen-minfi-methods), 16
db1, 18
detectionPval.filter (adaptRefQuantiles), 3
dfs2 (db1), 18
dfsfit (db1), 18
dmrse, 19, *20*, *28*
dmrse,exprmethy450-method (dmrse-methods), 20
dmrse,MethylSet-method (dmrse-methods), 20
dmrse,MethyLumiSet-method (dmrse-methods), 20
dmrse,RGChannelSet-method (dmrse-methods), 20
dmrse-methods, 20
dmrse_col, *24*
dmrse_col (dmrse), 19
dmrse_col,exprmethy450-method (dmrse-methods), 20
dmrse_col,MethylSet-method (dmrse-methods), 20
dmrse_col,MethyLumiSet-method (dmrse-methods), 20
dmrse_col,RGChannelSet-method (dmrse-methods), 20
dmrse_col-methods (dmrse-methods), 20
dmrse_row (dmrse), 19
dmrse_row,exprmethy450-method (dmrse-methods), 20
dmrse_row,MethylSet-method (dmrse-methods), 20
dmrse_row,MethyLumiSet-method (dmrse-methods), 20
dmrse_row,RGChannelSet-method (dmrse-methods), 20
dmrse_row-methods (dmrse-methods), 20

filterXY (adaptRefQuantiles), 3
findAnnotationProbes (adaptRefQuantiles), 3
fot (got), 23
fuks (dasen), 11
fuks,exprmethy450-method (betaqn-exprmethy450-methods), 7
fuks,MethylSet-method (dasen-minfi-methods), 16
fuks,MethyLumiSet-method (dasen-methods), 14
fuks,RGChannelSet-method (dasen-minfi-methods), 16

gcoms (genkme), 22
gcose, *28*
gcose (genkme), 22

genki, 20, *20–22*, *24*
genki,exprmethy450-method (genki-methods), 21
genki,MethylSet-method (genki-methods), 21
genki,MethyLumiSet-method (genki-methods), 21
genki,RGChannelSet-method (genki-methods), 21
genki-methods, 21
genkme, 22
genkus (genkme), 22
getColumns (as.methylumi-methods), 4
getMethylumiBeta (adaptRefQuantiles), 3
getQuantiles (adaptRefQuantiles), 3
getSamples (adaptRefQuantiles), 3
getsnp (genkme), 22
got, 23

iDMR, 23

loadMethylumi2 (adaptRefQuantiles), 3
lumiMethyR2 (adaptRefQuantiles), 3

M2Beta (Beta2M), 6
melon, 24
methylumiR, *24*
metrics, 25

nanes (dasen), 11
nanes,MethylSet-method (dasen-minfi-methods), 16
nanes,MethyLumiSet-method (dasen-methods), 14
nanes,RGChannelSet-method (dasen-minfi-methods), 16
nanet (dasen), 11
nanet,MethylSet-method (dasen-minfi-methods), 16
nanet,MethyLumiSet-method (dasen-methods), 14
nanet,RGChannelSet-method (dasen-minfi-methods), 16
nasen (dasen), 11
nasen,MethylSet-method (dasen-minfi-methods), 16
nasen,MethyLumiSet-method (dasen-methods), 14
nasen,RGChannelSet-method (dasen-minfi-methods), 16
naten (dasen), 11
naten,MethylSet-method (dasen-minfi-methods), 16
naten,MethyLumiSet-method (dasen-methods), 14
naten,RGChannelSet-method (dasen-minfi-methods), 16
nbBeadsFilter (adaptRefQuantiles), 3
normalize.quantiles2 (adaptRefQuantiles), 3
normalizeIlluminaMethylation (adaptRefQuantiles), 3

pfilter, *14*, 26
pfilter,MethyLumiSet-method (pfilter), 26
pfilter,RGChannelSetExtended-method (pfilter), 26
pfilter-methods (pfilter), 26
pipelineIlluminaMethylation.batch (adaptRefQuantiles), 3
pop (got), 23
preprocessIlluminaMethylation (adaptRefQuantiles), 3

referenceQuantiles (adaptRefQuantiles), 3
robustQuantileNorm_Illumina450K (adaptRefQuantiles), 3

seabi, *20*, *24*, 28, *29*, *30*
seabi,exprmethy450-method (seabi-methods), 29
seabi,MethylSet-method (seabi-methods), 29
seabi,MethyLumiSet-method (seabi-methods), 29
seabi,RGChannelSet-method (seabi-methods), 29
seabi-methods, 29
seabird, 29, *30*
sextest, 30
summits (Beta2M), 6
swan, *5*, *24*
swan (dasen), 11
swan,MethylSet-method (dasen-minfi-methods), 16
swan,MethyLumiSet-method (dasen-methods), 14

swan,RGChannelSet-method
(dasen-minfi-methods), 16

tost, *3*
tost (dasen), 11
tost,MethylSet-method
(dasen-minfi-methods), 16
tost,MethyLumiSet-method
(dasen-methods), 14
tost,RGChannelSet-method
(dasen-minfi-methods), 16

uniqueAnnotationCategory
(adaptRefQuantiles), 3

wateRmelon (wateRmelon-package), 2
wateRmelon-package, 2