# Package 'methylumi'

January 30, 2025

Type Package

Title Handle Illumina methylation data

Version 2.53.0 Date 2021-10-27

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**Depends** Biobase, methods, R (>= 2.13), scales, reshape2, ggplot2, matrixStats, FDb.InfiniumMethylation.hg19 (>= 2.2.0), minfi

Imports BiocGenerics, S4Vectors, IRanges, GenomeInfoDb, GenomicRanges, SummarizedExperiment, Biobase, graphics, lattice, annotate, genefilter, AnnotationDbi, minfi, stats4, illuminaio, GenomicFeatures

**Suggests** lumi, lattice, limma, xtable, SQN, MASS, matrixStats, parallel, rtracklayer, Biostrings, TCGAMethylation450k, IlluminaHumanMethylation450kanno.ilmn12.hg19, FDb.InfiniumMethylation.hg18 (>= 2.2.0), Homo.sapiens, knitr

**biocViews** DNAMethylation, TwoChannel, Preprocessing, QualityControl, CpGIsland

Maintainer Sean Davis <seandavi@gmail.com>

Description This package provides classes for holding and manipulating
Illumina methylation data. Based on eSet, it can contain MIAME
information, sample information, feature information, and
multiple matrices of data. An `intelligent" import function,
methylumiR can read the Illumina text files and create a
MethyLumiSet. methylumIDAT can directly read raw IDAT files from
HumanMethylation27 and HumanMethylation450 microarrays. Normalization,
background correction, and quality control features for GoldenGate,
Infinium, and Infinium HD arrays are also included.

Collate AllGenerics.R MethyLumiSet-class.R MethyLumiM-class.R MoreGenerics.R Methods.R bgcorr.R coercions.R detectionpval.R featureFilter.R mclapply\_replace.R methylData-class.R methylumIDAT.R methylumiCSV.R methylumiR.R normalization.R plotNegOob.R qc.probe.plot.R readIDAT2.R stripMethyLumiSet.R utilities.R varFilter.R

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VignetteBuilder knitr
BugReports https://github.com/seandavi/methylumi/issues/new
License GPL-2
Encoding UTF-8
RoxygenNote 7.1.2
git_url https://git.bioconductor.org/packages/methylumi
git_branch devel
git_last_commit 491e60e
git_last_commit_date 2024-10-29
Repository Bioconductor 3.21
Date/Publication 2025-01-29

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methylumi-package

Handle Illumina methylation data

# **Description**

This package contains a class structure for handling methylation data from Illumina as well as utility functions for loading the data from files generated by Illumina. Normalization that attempts to correct for dye bias is also included.

Important data classes include: MethyLumiSet and MethyLumiQC, both of which are subsets of the MethyLumi class, which is a subset of the eSet class.

A worked example of the use of the package can be found by typing: openVignette().

A full listing of the available documentation can be obtained by typing help.start() and selecting methylumi from the Packages link or by typing library(help="methylumi").

If you use the methylumIDAT function or its out-of-band preprocessing mechanisms in your work, a citation to the paper "Low-level processing of Illumina Infinium DNA methylation beadarrays" by TJ Triche, DJ Weisenberger, D Van Den Berg, KD Siegmund, and PW Laird, Nucleic acids research, 2013, would be appreciated.

# **Details**

Package: methylumi Type: Package License: GPL

#### Author(s)

Sean Davis <sdavis2@mail.nih.gov>

#### References

http://watson.nci.nih.gov/~sdavis/software/R

#### See Also

Biobase

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CpGs	Data frame describing loci on the 27 and 450k arrays.	

# Description

Data frame describing loci on the 27 and 450k arrays.

#### Usage

```
data(CpGs)
```

# **Examples**

```
data(CpGs)
head(CpGs)
```

_ct	Ť.	mateM

Estimate methylation M-value matrix

# **Description**

Estimate methylation M-value matrix from MethyLumiM-class object or eSet-class object, which include methylated and unmethylated probe intensities

# Usage

```
estimateM(methyLumiM, returnType=c("ExpressionSet", "matrix"), offset=100)
```

#### **Arguments**

offset

methyLumiM	MethyLumiM-class object or eSet-class object, which include methylated and
	unmethylated probe intensities
returnType	determine whether return an ExpressionSet (MethyLumiM in this case) or ma-

determine whether return an ExpressionSet (MethyLumiM in this case) or matrix object

offset added to the methylated and unmethylated probe intensities when estimat-

ing the M-value

# **Details**

M-value is the log2 ratio between Illumina methylated and unmethylated probe intensities. As variations of small intensities can cause big changes in the ratio estimation, so an offset is added to methylated and unmethylated probe intensities when estimating the M-value.

Please check the lumi package for more details of estimateM function.

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#### Value

A MethyLumiM or matrix object of methylation M-value

#### Author(s)

Pan DU

#### References

Du, P., Zhang, X, Huang, C.C., Jafari, N., Kibbe, W.A., Hou, L., and Lin, S.M., (2010) 'Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis', (under review)

extractBarcodeAndPosition

Extract the Barcode and Position Information from Sentrix ID

# Description

The sentrix IDs from an illumina sentrix array contain positional information that might be useful. This function simply extracts that information from the ID itself.

# Usage

```
extractBarcodeAndPosition(sentrixids)
```

# **Arguments**

sentrixids A character vector of sentrix IDs that look like: 1632405013\\_R001\\_C001

# Value

A data.frame with three columns:

sentrix numeric, the sentrix ID
row numeric, the sentrix row
column numeric, the sentrix column

# Author(s)

Sean Davis <sdavis2@mail.nih.gov>

#### See Also

methylumiR

# **Examples**

```
extractBarcodeAndPosition(c('12341234_R001_C001'))
```

6 featureFilter

featureFilter

Annotation-based Filtering of Features (CpG sites) in a MethyLumiSet or MethyLumiM object

#### **Description**

Features with insufficient annotation carry little value for the subsequent data analysis. The function featureFilter provides options of filtering features (CpG sites) from a MethyLumiSet (or MethyLumiM) object based on available annotation data.

# Usage

```
featureFilter(eset, require.entrez=FALSE,
    require.GOBP=FALSE, require.GOCC=FALSE,
    require.GOMF=FALSE, exclude.ChrX=FALSE,
    require.closeToTSS=FALSE, range.DistToTSS=c(-500, 300),
    require.CpGisland=FALSE, ...)
```

#### **Arguments**

eset

A MethyLumiSet or MethyLumiM object.

require.entrez If TRUE, filter out features without an Entrez Gene ID annotation.

require.GOBP, require.GOCC, require.GOMF

If TRUE, filter out features whose target genes are not annotated to at least one GO term in BP, CC and MF ontology, respectively.

exclude.ChrX If TRUE

If TRUE, filter out features in chromosome X to avoid gender effect.

require.closeToTSS

If TRUE, filter out features that are not close to transcription start site (TSS). Features without annotation of distance to TSS will also be removed. Can only used for GoldenGate platform.

range.DistToTSS

Ignored if require.colseToTSS is FALSE. A vector of numeric values of length 2, indicating the range of tolerable distance from transcription start site (TSS) in basepair (bp). If require.clostToTSS is TRUE, features whose distance to TSS falls outside this designated range will be removed. The default value is c(-500,300), where -500 represents the distance to TSS from the left and 300 the distance from the right.

require.CpGisland

If TRUE, filter out features that are not in CpG islands.

. . . Unused, but available for specializing methods.

#### Value

The function featureFilter returns a list consisting of:

eset

The filtered MethyLumiSet or MethyLumiM object.

filter.log A list giving details of how many probe sets where removed for each annotation-based filtering step performed.

#### Author(s)

Chao-Jen Wong < cwon2@fhcrc.org>

#### References

R. Bourgon, R. Gentleman, W. Huber, *Independent filtering increases power for detecting differentially expressed genes*, PNAS, vol. 107, no. 21, pp:9546-9551.

#### See Also

nsFilter

getAssayDataNameSubstitutions

Return a data.frame of AssayData name substitutions.

# Description

The Illumina methylation platforms use two distinct platforms, the "goldengate" platform and the "infinium" platform. Each of these uses different file formats as well as different assay techologies. To make the downstream data handling more straightforward and uniform between the two different systems, a simple mapping from the column names in the output files from the Illumina software is used to convert things from Red/Green or Cy5/Cy3 to unmethylated/methylated. This function simply returns that mapping.

# Usage

getAssayDataNameSubstitutions()

#### **Details**

A file in the extdata directory called "substitutions.txt" contains two columns. The function loads this file and uses the first column as a match against column names in the data file (with the "sample part" removed). If matched, the second column gives the replacement.

#### Value

A data.frame with two columns, regex and replacement.

# Author(s)

Sean Davis <seandavi@gmail.com>

#### **Examples**

getAssayDataNameSubstitutions()

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**IDATsToMatrices** 

convert multiple idats to matrices

# Description

convert multiple idats to matrices

# Usage

```
IDATsToMatrices(
  barcodes,
  fileExts = list(Cy3 = "Grn", Cy5 = "Red"),
  parallel = F,
  idatPath = "."
)
```

# Arguments

```
barcodes character()
fileExts character()
parallel logical(1)
idatPath character(1)
```

IDATtoMatrix

process a single IDAT (just the mean intensities)

# Description

```
process a single IDAT (just the mean intensities)
```

# Usage

```
IDATtoMatrix(x, fileExts = list(Cy3 = "Grn", Cy5 = "Red"), idatPath = ".")
```

# Arguments

```
x character(1)
fileExts named list
idatPath character(1)
```

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methylData-class

Class "methylData", superclass for MethyLumiSet and MethyLumiM

# **Description**

A superclass (virtual) for MethyLumiSet and MethyLumiM.

#### **Objects from the Class**

A virtual Class: No objects may be created from it.

#### Methods

```
diagnostics signature(x = "methylData"): diagnostic plots of data
```

methylated.N signature(object = "methylData"): accessor for assayData element of the same name

methylated.N<- signature(object = "methylData", value = "matrix"): replace method for assayData element of the same name

```
plotNAs signature(object = "methylData"): ...
```

pval.detect signature(object = "methylData"): accessor for assayData element of the same
 name

pval.detect<- signature(object = "methylData", value = "numeric"): replace method for assayData element of the same name

unmethylated.N signature(object = "methylData"): accessor for assayData element of the
 same name

unmethylated.N<- signature(object = "methylData", value = "matrix"): replace method for assayData element of the same name

#### Author(s)

Tim Triche, Jr.

#### See Also

MethyLumiSet,MethyLumiM

#### **Examples**

```
showClass("methylData")
```

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MethyLumi-accessors me

methylumi accessors

# Description

These functions serve as getters and setters for information in methylumi classes.

# Usage

```
betas(object)
pvals(object)
methylated(object)
unmethylated(object)
getHistory(object)
QCdata(object)
```

# **Arguments**

object

an object of class MethyLumi or a subclass

#### **Details**

See the methods definitions in MethyLumiSet and MethyLumiQC for details.

#### Author(s)

Sean Davis <sdavis2@mail.nih.gov>

#### See Also

normalizeMethyLumiSet, MethyLumiQC, eSet

MethyLumi-class

The base class for storing Illumina Methylation data

# **Description**

This class inherits from eSet from the Biobase package and is used as a base class for the other two methylumi classes, MethyLumiSet and MethyLumiQC.

# **Objects from the Class**

The MethyLumi class is a virtual class and is not meant to be instantiated. Instead, one should instantiate a MethyLumiSet or a MethyLumiQC object.

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#### Slots

```
assayData: Object of class "AnnotatedDataFrame"

featureData: Object of class "AnnotatedDataFrame" that will hold the annotation columns from the Beadstudio output, if they are available.

experimentData: Object of class "MIAME"

annotation: Object of class "character"; note that this slot is not currently used, but may be used in the future to store the character name of the annotation package, if available.

.__classVersion__: Object of class "Versions"
```

#### **Extends**

Class "eSet", directly. Class "VersionedBiobase", by class "eSet", distance 2. Class "Versioned", by class "eSet", distance 3.

#### Methods

pvals<- signature(object = "MethyLumi", value = "matrix"): Set the assayData slot of the same name and stores the P-values from BeadStudio

pvals signature(object = "MethyLumi"): Get the assayData slot of the same name

betas<- signature(object = "MethyLumi", value = "matrix"): Set the assayData slot of the same name and represents the methylation values for the samples, analogous to exprs() in gene expression data.

**betas** signature(object = "MethyLumi"): Get the assayData slot of the same name

methylated<- signature(object = "MethyLumi", value = "matrix"): Set the assayData slot
that represents the Methylated single-channel signal</pre>

**methylated** signature(object = "MethyLumi"): Get the assayData slot that represents the Methylated single-channel signal

unmethylated<- signature(object = "MethyLumi", value = "matrix"): Set the assayData slot
that represents the Unmethylated single-channel signal</pre>

unmethylated signature(object = "MethyLumi"): Get the assayData slot that represents the
Unmethylated single-channel signal

controlTypes signature(object = "MethyLumi": Find the unique control type beeds in the QCdata slot.

**qcplot** signature(object = "MethyLumi", what,...): Plot of QC data. This plot can be useful for diagnosing the problems associated with specific samples or arrays. The value for "what" is one of the control types (which can be found by using controlTypes() on the object.

summary signature(object = "MethyLumi",...): summary method for MethyLumi objects.

#### Author(s)

Sean Davis <sdavis2@mail.nih.gov>

#### See Also

```
methylumiR, MethyLumiSet, MethyLumiQC, eSet
```

#### **Examples**

```
## The class structure
showClass("MethyLumi")
## read in some data
## Read in sample information
samps <- read.table(system.file("extdata/samples.txt",</pre>
                                package = "methylumi"),sep="\t",header=TRUE)
## Perform the actual data reading
## This is an example of reading data from a
## Sentrix Array format file (actually two files,
## one for data and one for QC probes)
mldat <- methylumiR(system.file('extdata/exampledata.samples.txt',</pre>
        package='methylumi'),
      qcfile=system.file('extdata/exampledata.controls.txt',
        package="methylumi"),
      sampleDescriptions=samps)
mldat
## Get history information
getHistory(mldat)
## Get QC data, which is another eSet-derived object
QCdata(mldat)
```

MethyLumi-strippers

Strip excessive probe-level data from MethyLumiSets

#### **Description**

450k datasets with probe-level stderrs, out-of-band intensities, and bead numbers can become huge. These functions help to manage their growth in memory, at least until preprocessing and QC is completed, whereupon the summary data can be exported to a RangedData-based object of some sort for integration.

#### Usage

```
stripMethyLumiSet(object)
stripBeadNs(object)
stripBeadSDs(object)
stripOOB(object)
```

#### **Arguments**

object an object of class MethyLumi or a subclass

# Author(s)

Tim Triche, Jr. <tim.triche@gmail.com>

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|--|

# **Description**

Read a directory of methylumi idat files and return a MethylumiSet.

# Usage

```
methylumIDAT(barcodes = NULL, pdat = NULL, parallel = F, n = F, n.sd = F, oob = T, idatPath=getwd(), ...)
```

# Arguments

barcodes	A vector of barcodes to read. Either this argument or pdat must be specified.
pdat	A data frame describing the samples. A special column named "barcodes" can be used to specify the barcodes to be read.
parallel	If TRUE, an attempt will be made to process using multiple cores on a multicore machine.
n	Keep the bead numbers? (Default: no)
n.sd	Keep the bead-level SD? (Default: no)
oob	Keep the out-of-band (OOB) or opposite-channel signals? (Default: yes)
idatPath	The path to the directory containing the idat files.
	Additional arguments to be passed to sub-functions.

# **Details**

Read a set of .idat files and return a MethylumiSet object. If you use this function to any significant degree in your analysis, we would appreciate your citing the paper describing it, "Low-level processing of Illumina Infinium DNA methylation beadarrays", TJ Triche, DJ Weisenberger, D Van Den Berg, KD Siegmund, and PW Laird, Nucleic acids research, 2013.

# Value

A MethylumiSet object.

# Author(s)

Tim Triche, Jr.

#### See Also

The "methylumi450k" vignette: vignette("methylumi450k", package="methylumi")

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# **Examples**

methylumiGenerics

Generics defined in methylumi

# Description

See the individual classes for details of methods.

#### Author(s)

Sean Davis, Pan Du, and Tim Triche, Jr.

MethyLumiM-class

Class "MethyLumiM": for Illumina Methylation microarray data using logRatios

#### **Description**

MethyLumiM is a class inherited from ExpressionSet-class. It is designed for Illumina Methylation microarray data. The exprs dataMatrix included in the assayData slot of MethyLumiM object includes a matrix of M-values, which is the log2 ratio of methylated and unmethylated probe intensities. The MethyLumiM class include a boxplot function uniquely designed for two-mode histogram data. It also include a coerce function to map from MethyLumi-class, MethyLumiSet-class or other eSet-class inherited object to MethyLumiM class object.

# **Objects from the Class**

Objects can be created by calls of the form new("MethyLumiM", exprs, methylated, unmethylated, detection, methylated.N, unmethylated.N, ..., assayData). The "exprs" is a matrix of M-values, which is the log2 ratio of methylated and unmethylated probe intensities; "methylated" and "unmethylated" are intensity matrix measured by methylated and unmethylated probes of Illumina Infinium methylation microarray; "detection" is the detection p-value outputted by Illumina GenomeStudio software; "methylated.N" and "unmethylated.N" are bead numbers for methylated and unmethylated probes. "exprs", "methylated" and "unmethylated" information are required for MethyLumiM class. When creating a new MethyLumiM object, the information of "exprs", "methylated", "unmethylated" and "detection" can also be provided directly through "assayData".

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#### **Slots**

```
history: Object of class "data.frame" recording the operation history of the LumiBatch object. controlData: Object of class "MethyLumiQC" to keep the QC probe measurement information. dataType: The type of data stored in the "exprs" data matrix in "assayData". It can be "M" (Mvalue), "Beta" (Beta-value") or "Intensity" (Intensity of CpG-site) assayData: Object of class "AssayData", which includes "exprs", "methylated", "unmethylated", "detection", "methylated.N" and "unmethylated.N" data matrix phenoData: Object of class "AnnotatedDataFrame", See eSet-class featureData: Object of class "AnnotatedDataFrame", See eSet-class experimentData: Object of class "MIAME", See eSet-class annotation: Object of class "character", See eSet-class protocolData: Object of class "AnnotatedDataFrame", See eSet-class .__classVersion_: Object of class "Versions", See eSet-class
```

#### **Extends**

Class "ExpressionSet", directly. Class "eSet", by class "ExpressionSet", distance 2. Class "VersionedBiobase", by class "ExpressionSet", distance 3. Class "Versioned", by class "ExpressionSet", distance 4.

#### Methods

```
boxplot signature(x = "MethyLumiM"): plot distribution of M-value
coerce signature(from = "eSet", to = "MethyLumiM"): map from MethyLumi-class, MethyLumiSet-class
     or other eSet-class inherited object to MethyLumiM class object. MethyLumiM object will
     only keep "exprs", "methylated", "unmethylated" and "detection" data matrix in the assayData.
getHistory signature(object = "MethyLumiM"): access the operation history of MethyLumiM
     object.
initialize signature(.Object = "MethyLumiM"): class initialization
methylated signature(object = "MethyLumiM"): retrieve the data matrix measured by methy-
     lated probes
methylated <- signature (object = "MethyLumiM"): set the data matrix measured by methylated
     probes
unmethylated signature(object = "MethyLumiM"): retrieve the data matrix measured by un-
     methylated probes
unmethylated <- signature (object = "MethyLumiM"): set the data matrix measured by unmethy-
     lated probes
methylated.N signature(object = "MethyLumiM"): retrieve the data matrix keeping the number
     of beads of methylated probes
methylated.N<- signature(object = "MethyLumiM"): set the data matrix keeping the number
    of beads of methylated probes
```

unmethylated.N signature(object = "MethyLumiM"): retrieve the data matrix keeping the num-

ber of beads of unmethylated probes

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```
unmethylated.N<- signature(object = "MethyLumiM"): set the data matrix keeping the number
    of beads of unmethylated probes

detection signature(object = "MethyLumiM"): retrieve detection data matrix in AssayData-class

detection<- signature(object = "MethyLumiM"): set detection data matrix in AssayData-class

controlData signature(object = "MethyLumiM"): retrieve the controlData in MethyLumiQC-class

controlData<- signature(object = "MethyLumiM"): set controlData in MethyLumiQC-class

dataType signature(object = "MethyLumiM"): retrieve the dataType, by default it is "M", it can
    also be "Beta" or "Intensity"

dataType<- signature(object = "MethyLumiM"): set dataType in MethyLumiM-class, the value</pre>
```

#### Author(s)

Pan DU

#### References

1. Du, P., Zhang, X, Huang, C.C., Jafari, N., Kibbe, W.A., Hou, L., and Lin, S.M., (2010) 'Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis'

#### See Also

MethyLumi-class and MethyLumiSet-class

can be "M", "Beta" or "Intensity"

# Examples

showClass("MethyLumiM")

MethyLumiQC-class

Class "MethyLumiQC" for holding Illumina methylation QC data

#### **Description**

This class inherits from the MethyLumi class (and therefore, from eSet in Biobase) and is designed to hold QC data from Illumina Beadstudio output. These data can be potentially useful when determining the cause for quality problems.

# **Objects from the Class**

Objects can be created by calls of the form new("MethyLumiQC", assayData, phenoData, featureData, experimentData, annotation, betas).

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#### **Slots**

```
assayData: Object of class "AssayData"

phenoData: Object of class "AnnotatedDataFrame"

featureData: Object of class "AnnotatedDataFrame" containing the annotation columns from the Illumina Beadstudio output. In particular, the names of the probes describe the types of control probes.

experimentData: Object of class "MIAME"

annotation: Object of class "character", not currently used

.__classVersion__: Object of class "Versions"
```

#### **Extends**

Class "MethyLumi", directly. Class "eSet", by class "MethyLumi", distance 2. Class "VersionedBiobase", by class "MethyLumi", distance 3. Class "Versioned", by class "MethyLumi", distance 4.

#### Methods

```
initialize signature(.Object = "MethyLumiQC")
Cy3.N signature(object = "MethyLumiQC"): ...
Cy3<- signature(object = "MethyLumiQC", value = "matrix"): ...
Cy5.N signature(object = "MethyLumiQC"): ...
Cy5<- signature(object = "MethyLumiQC", value = "matrix"): ...
QCdata<- signature(object = "MethyLumiSet", value = "MethyLumiQC"): ...
combine signature(x = "MethyLumiQC", y = "MethyLumiQC"): ...
controlData<- signature(object = "MethyLumiSet", value = "MethyLumiQC"): ...</pre>
controlTypes signature(object = "MethyLumiQC"): determine the character vector of control
     types from the QCdata information
hist signature(x = "MethyLumiQC"): ...
intensitiesByChannel signature(object = "MethyLumiQC"): ...
methylated signature(object = "MethyLumiQC"): ...
negctls.stderr signature(object = "MethyLumiQC", channel = "character"): ...
negctls.stderr signature(object = "MethyLumiQC", channel = "missing"): ...
negctls signature(object = "MethyLumiQC", channel = "character"): ...
negctls signature(object = "MethyLumiQC", channel = "missing"): ...
negnorm signature(object = "MethyLumiQC", channel = "character"): ...
negnorm signature(object = "MethyLumiQC", channel = "missing"): ...
normctls signature(object = "MethyLumiQC"): ...
qcplot signature(object = "MethyLumiQC", what, ...): QC plots of various controltypes
unmethylated signature(object = "MethyLumiQC"): ...
```

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#### Author(s)

Sean Davis <sdavis2@mail.nih.gov>

#### See Also

```
methylumiR, MethyLumiSet, MethyLumi, eSet
```

#### **Examples**

```
showClass("MethyLumiQC")
```

methylumiR

Load data from Illumina methylation platform

# **Description**

This function is useful for loading Illumina methylation data into a MethyLumiSet object. Sample information can be supplied and will then be incorporated into the resulting phenoData slot.

# Usage

```
methylumiR(filename, qcfile=NULL, sampleDescriptions = NULL, sep = NULL, ...)
```

#### **Arguments**

filename A filename of the excel-like file from BeadStudio
qcfile A filename of the excel-like file from BeadStudio

sampleDescriptions

A data frame that contains at least one column, SampleID (case insensitive). This column MUST match the part of the column headers before the .Avg\_Beta, etc. Also, if a column called SampleLabel (case insensitive), it is used for sam-

ple labels, IF the sampleLabel column contains unique identifiers

sep seperator used in the BeadStudio (or GenomeStudio) output file. If it is NULL,

the function will automatically estimate it.

... Passed into read.delim()

#### Details

Used to construct a MethyLumiSet object....

#### Value

A MethyLumiSet object

# Author(s)

Sean Davis <sdavis2@mail.nih.gov>

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#### See Also

MethyLumiSet-class, MethyLumiQC-class

#### **Examples**

MethyLumiSet-class

Class "MethyLumiSet" for containing Illumina methylation data

# **Description**

This class inherits from the MethyLumi class (and therefore, from eSet in Biobase) and is designed to hold both the intensities and the calculated betas, as well as pvalues if present.

#### **Objects from the Class**

Objects can be created by calls of the form new("MethyLumiSet", assayData, phenoData, featureData, experimentData, annotation, betas). An object of this type is the main storage class for methylation data from Illumina. Subsetting, etc., works as normal (rows represent genes, columns represent samples). There is also a rudimentary history tracking system that is modeled after that from the lumi package.

#### **Slots**

```
QC: Object of class "QCDataOrNULL", containing either the MethyLumiQC object or NULL
history: Object of class "data.frame", containing a running history of transforms to the data
contained herein
assayData: Object of class AssayData
phenoData: Object of class AnnotatedDataFrame
featureData: Object of class AnnotatedDataFrame, containing the annotation columns from the
Illumina Beadstudio output
experimentData: Object of class MIAME
annotation: Object of class "character", not currently used
.__classVersion__: Object of class "Versions"
protocolData: Object of class "AnnotatedDataFrame" that contains protocol information, in-
cluding scan date if available
```

#### **Extends**

Class "MethyLumi", directly. Class "methylData", directly. Class "eSet", by class "MethyLumi", distance 2. Class "VersionedBiobase", by class "MethyLumi", distance 3. Class "Versioned", by class "MethyLumi", distance 4.

#### Methods

```
[ signature(x = "MethyLumiSet"): subsetting, genes as rows, samples as columns
betas<- signature(object = "MethyLumiSet", value = "matrix"): Set the assayData slot of
     the same name
betas signature(object = "MethyLumiSet"): Get the assayData slot of the same name
boxplot signature(x = "MethyLumiSet"): boxplot of all sample betas
combine signature(x = "MethyLumiSet", y = "MethyLumiSet")
corplot signature(x = "MethyLumiSet")
exprs signature(object = "MethyLumiSet"): returns m-values
getHistory signature(object = "MethyLumiSet"): returns a data.frame containing the history
     for this object
hist signature(x = "MethyLumiSet"): histogram of the betas for the data
initialize signature(.Object = "MethyLumiSet")
```

pairs signature(x = "MethyLumiSet"): pairs plot of the betas for the object. Note that pairs plots of more than a few samples are not very useful.

plotSampleIntensities signature(x = "MethyLumiSet"): The intensities as output by the Beadstudio software often show a considerable amount of dye bias. This method shows a graphical example of this dye bias. In short, for each of the Cy3 and Cy5 channels, a cutoff in beta is used to calculate which Cy3 and Cy5 values should be plotted at high-methylation and low-methylation status. Any offset between Cy3 and Cy5 when plotted in this way likely represents dye bias and will lead to biases in the estimate of beta.

QCdata<- signature(object = "MethyLumiSet", value = "MethyLumiQC"): assign QC data to the QC slot

**QCdata** signature(object = "MethyLumiSet"): retrieve the QC data.

show signature(object = "MethyLumiSet")

methylated <- signature (object = "MethyLumiSet", value = "matrix"): Set the assayData slot associated with methylated intensity

methylated signature(object = "MethyLumiSet"): Get the assayData slot associated with methylated intensity

unmethylated <- signature (object = "MethyLumiSet", value = "matrix"): Set the assayData slot associated with unmethylated intensity

unmethylated signature(object = "MethyLumiSet"): Get the assayData slot associated with unmethylated intensity

qcplot signature(object = "MethyLumiSet", what, ...): QC plots of various controltypes

controlTypes signature(object = "MethyLumiSet"): determine the character vector of control types from the QCdata information

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```
Cy3.N signature(object = "MethyLumiSet"): ...
    Cy5.N signature(object = "MethyLumiSet"): ...
    combine27k450k signature(x = "MethyLumiSet", y = "MethyLumiSet"): ...
    controlData signature(object = "MethyLumiSet"): ...
    controlData<- signature(object = "MethyLumiSet", value = "MethyLumiQC"): ...</pre>
    featureFilter signature(eset = "MethyLumiSet"): ...
    intensities.IB signature(x = "MethyLumiSet", channel = "character"): ...
    intensities.IB signature(x = "MethyLumiSet", channel = "missing"): ...
    intensities.M signature(x = "MethyLumiSet", channel = "character"): ...
    intensities.M signature(x = "MethyLumiSet", channel = "missing"): ...
    intensities.OOB.allelic signature(x = "MethyLumiSet", channel = "character", allele = "character"):
    intensities.OOB.allelic signature(x = "MethyLumiSet", channel = "missing", allele = "missing"):
   intensities.OOB signature(x = "MethyLumiSet", channel = "character"): ...
    intensities.OOB signature(x = "MethyLumiSet", channel = "missing"): ...
    intensities.U signature(x = "MethyLumiSet", channel = "character"): ...
    intensities.U signature(x = "MethyLumiSet", channel = "missing"): ...
    intensitiesByChannel signature(object = "MethyLumiSet"): ...
    negctls.stderr signature(object = "MethyLumiSet", channel = "character"): ...
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    normctls signature(object = "MethyLumiSet"): ...
    plotSampleIntensities signature(x = "MethyLumiSet"): ...
    probeNAs signature(object = "MethyLumiSet"): ...
    sampleNAs signature(object = "MethyLumiSet"): ...
    total.intensity signature(object = "MethyLumiSet"): ...
    varFilter signature(eset = "MethyLumiSet"): ...
Author(s)
    Sean Davis & Tim Triche, Jr.
See Also
    methylumiR, normalizeMethyLumiSet, methylumIDAT, MethyLumiQC, eSet
```

# Examples

```
showClass("MethyLumiSet")
```

mldat

Example SAM format Illumina methylation dataset

# **Description**

This is an example MethyLumiSet object.

# Usage

data(mldat)

#### **Examples**

data(mldat)

normalizeMethyLumiSet Normalize a MethyLumiSet, accounting for dye bias

# **Description**

The Illumina GoldenGate methylation platform uses two colors, one to represent the unmethylated state and the other to represent the methylated state. This function corrects that dye bias and recalculates the betas based on the corrected intensities.

For HumanMethylation27 data, the function does nothing.

For HumanMethylation450 data, the function delegates to normalizeViaControls() the task of scaling red and green intensities against a reference array (chip) which uses the closest-to-equal chip (i.e., which.min(abs(R.G.ratio - 1))).

The code to do this is based on code from the 'minfi' package and uses the built-in red and green normalization control probes on the hm450 arrays to scale the channels of the samples, so that a consistent degree of dye bias is maintained for Infinium II probes across an experiment or set of experiments.

# Usage

```
normalizeMethyLumiSet(x, beta.cuts = c(0.2, 0.8), mapfun = c("atan", "ratio"))
```

# Arguments

x A MethyLumiSet object

beta.cuts Two numeric values with the first less than the second and between 0 and 1,

representing the beta cutoffs that will be used when determining the median

intensities to which to correct. See details below.

mapfun Either "atan" or "ratio". See details below.

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#### **Details**

For HumanMethylation450 data, the function delegates to normalizeViaControls() the task of scaling red and green intensities against a reference array (chip) which defaults to the first chip in a set. The code to do this is based on code from the 'minfi' package and uses the built-in normalization controls to scale the channels of the samples, so that a consistent degree of dye bias is maintained for Infinium II probes across an experiment or set of experiments. The remainder of the documentation below is specific to GoldenGate data.

The Illumina GoldenGate methylation platform uses two colors, one to represent the unmethylated state and the other to represent the methylated state. This function corrects that dye bias and recalculates the betas based on the corrected intensities.

As a first step, the medians for each of Cy3 and Cy5 are calculated at high and low betas, representing the (nearly) fully methylated state and the (nearly) fully unmethylated states. Values of Cy3 and Cy5 that are negative are set to zero for this process. Then, the Cy5 medians are adjusted to match those of the Cy3 channel, thereby correcting the dye bias.

To map the new intensities back to betas, one of two map functions can be used. The default is the atan(Cy3/Cy5). The ratio maps using the function (Cy3/Cy3+Cy5). The differences should be very small, but we feel that the atan map function is probably the mathematically appropriate way of doing this.

#### Value

A new "MethyLumiSet" that contains the corrected betas and the adjusted intensities.

#### Author(s)

Sean Davis <sdavis2@mail.nih.gov>

# **Examples**

 ${\tt plotSampleIntensities} \ \ {\it Plot the sample intensities}.$ 

#### **Description**

The Illumina methylation platforms all show a significant dye bias. The plotSampleIntensities method shows the density plots for the two channels allowing direct visualization of the effect.

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#### Usage

```
plotSampleIntensities(x,beta.cuts,s)
```

# **Arguments**

x an object of class MethyLumi or a subclass

beta.cuts cutoffs for low and high beta values

s sample number to plot

# **Examples**

```
data(mldat)
plotSampleIntensities(mldat,s=1)
```

qcplot

Methods for dealing with control data for Illumina methylation data.

# **Description**

The qcplot function simply generates a plot of the control probe information for a given control Type.

# Usage

```
qcplot(object,controltype,...)
controlTypes(object,...)
```

# Arguments

object An object of class MethyLumiSet or MethyLumiQC

control type A single character value representing the bead type to plot from the quality con-

trol data. The available types are accessible via the controlTypes method.

... passed to plot function

#### **Details**

The descriptions of the various control types can be obtained from the Illumina methylation user's guides.

# Author(s)

Sean Davis <sdavis2@mail.nih.gov>

#### See Also

MethyLumiSet, MethyLumiQC

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#### **Examples**

```
data(mldat)
controlTypes(mldat)
qcplot(mldat,controlTypes(mldat)[3])
```

tcgaPipeline

Total convenience function for processing IDATs like tcga

# **Description**

Total convenience function for processing IDATs like tcga

# Usage

```
tcgaPipeline(IDATs)
```

# **Arguments**

**IDATs** 

character() of idat files

varFilter

Variation-based Filtering of Features (CpG sites) in a MethyLumiSet or MethyLumiM object

# **Description**

The function varFilter removes features exhibiting little variation across samples. Such non-specific filtering can be advantageous for downstream data analysis.

# Usage

```
varFilter(eset, var.func=IQR, var.cutoff=0.5, filterByQuantile=TRUE, ...)
```

# **Arguments**

eset An MethyLumiSet or MethyLumiM object.

var. func The function used as the per-feature filtering statistics.

var.cutoff A numeric value indicating the cutoff value for variation. If filterByQuantile

is TRUE, features whose value of var.func is less than var.cutoff-quantile of all var.func value will be removed. It FALSE, features whose values are less

than var.cutoff will be removed.

filterByQuantile

A logical indicating whether var.cutoff is to be interprested as a quantile of

all var. func (the default), or as an absolute value.

. . . Unused, but available for specializing methods.

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#### **Details**

This function is a counterpart of functions nsFilter and varFilter available from the genefilter package. See R. Bourgon et. al. (2010) and nsFilter for detail.

It is proven that non-specific filtering, for which the criteria does not depend on sample class, can increase the number of discoverie. Inappropriate choice of test statistics, however, might have adverse effect. 1 imma's moderated t-statistics, for example, is based on empirical Bayes approach which models the conjugate prior of gene-level variance with an inverse of  $\chi^2$  distribution scaled by observed global variance. As the variance-based filtering removes the set of genes with low variance, the scaled inverse  $\chi^2$  no longer provides a good fit to the data passing the filter, causing the 1 imma algorithm to produce a posterior degree-of-freedom of infinity (Bourgon 2010). This leads to two consequences: (i) gene-level variance estimate will be ignore, and (ii) the p-value will be overly optimistic (Bourgon 2010).

#### Value

The function featureFilter returns a list consisting of:

eset The filtered MethyLumiSet or MethyLumiM object. filter.log Shows many low-variant features are removed.

#### Author(s)

Chao-Jen Wong < cwon2@fhcrc.org>

#### References

R. Bourgon, R. Gentleman, W. Huber, *Independent filtering increases power for detecting differentially expressed genes*, PNAS, vol. 107, no. 21, pp:9546-9551, 2010.

# See Also

```
nsFilter
```

# **Examples**

```
data(mldat)
## keep top 75 percent
filt <- varFilter(mldat, var.cutoff=0.25)
filt$filter.log
dim(filt$eset)</pre>
```

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