minfi

March 24, 2012

IlluminaMethylationManifest-class

Class '"IlluminaMethylationManifest"'

Description

This is a class for representing an Illumina methylation microarray design, ie. the physical location and the probe sequences.

Objects from the Class

Objects can be created by calls of the form new ("IlluminaMethylationManifest", ...), but the preferred way is to use manifestNew().

Slots

data: This environment holds the design objects. See details.

annotation: A character vector giving the annotation name.

Details

The data slot contains the following objects: TypeI, TypeII and TypeControl which are all of class data.frame, describing the array design.

Methylation loci of type I are measured using two different probes, in either the red or the green channel. The columns AddressA, AddresB describes the physical location of the two probes on the array (with ProbeSeqA, ProbeSeqB giving the probe sequences), and the column Color describes which color channel is used.

Methylation loci of type II are measured using a single probe, but with two different color channels. The methylation signal is always measured in the green channel.

Methods

show: The show method.

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>.

See Also

getProbeData

Examples

```
if(require(IlluminaHumanMethylation450kmanifest)) {
    show(IlluminaHumanMethylation450kmanifest)
    head(getProbeInfo(IlluminaHumanMethylation450kmanifest@data$TypeI)
    head(IlluminaHumanMethylation450kmanifest@data$TypeI)
    head(IlluminaHumanMethylation450kmanifest@data$TypeI)
    head(IlluminaHumanMethylation450kmanifest@data$TypeI)
```

}

MethylSet-class Class MethylSet

Description

This class holds preprocessed data for Illumina methylation microarrays.

Objects from the Class

Objects can be created by calls of the form new ("MethylSet", Meth, Unmeth, ...).

Slots

- preprocessMethod: Object of class "character". This contains the preprocess method used to create the data. This is a character vector of length 3, the first component is the preprocessing method, followed by the version of **minfi** used to do the preprocessing followed by the version of the manifest package used to do the preprocessing.
- assayData: Object of class "AssayData". This has to contain the following two elements: Meth and Unmeth.
- phenoData: Object of class "AnnotatedDataFrame".
- featureData: Object of class "AnnotatedDataFrame".

experimentData: Object of class "MIAxE".

- annotation: Object of class "character". Contains the type of array for this dataset.
- protocolData: Object of class "AnnotatedDataFrame".
- .___classVersion__: Object of class "Versions".

Extends

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

Methods

show: The show method
initialize: The initialize method, see above

RGChannelSet-class

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>.

See Also

eSet for the basic class structure. Objects of this class are for example created from an RGChannelSet using preprocessRaw.

Examples

```
showClass("MethylSet")
```

RGChannelSet-class Class '"RGChannelSet"'

Description

These classes represents raw (unprocessed) data from a two color micro array; specifically an Illumina methylation array.

Objects from the Class

Objects can be created by calls of the form new("RGChannelSet", Red, Green, ...) or new("RGChannelSetExtended", Red, Green, RedSD, GreenSD, NBeads, ...).

Slots

assayData: Object of class "AssayData". A RGChannelSet contains elements with names Green and Red and a "RGChannelSetExtended" additionally adds GreenSD, RedSD and NBeads.

phenoData: Object of class "AnnotatedDataFrame".

featureData: Object of class "AnnotatedDataFrame".

experimentData: Object of class "MIAxE".

annotation: Object of class "character". Contains the array name (without "manifest".)

protocolData: Object of class "AnnotatedDataFrame".

.___classVersion__: Object of class "Versions".

Extends

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

Utilities

show: The show method.

initialize: The initialize method, see above.

getGreen: Gets the Green channel as a matrix.

getRed: Gets the Red channel as a matrix.

getManifest: Gets the manifest object associated with the array type

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>.

See Also

See eSet for the basic class that is used as a building block for "RGChannelSet (Extended)". See IlluminaMethylationManifest for a class representing the design of the array.

Examples

showClass("RGChannelSet")

controlStripPlot *Plot control probe signals.*

Description

Strip plots are produced for each control probe type specified.

Usage

```
controlStripPlot(rgSet, controls = c("BISULFITE CONVERSION I",
    "BISULFITE CONVERSION II"), sampNames = NULL, xlim = c(5, 17))
```

Arguments

rgSet	An RGChannelSet.
controls	A vector of control probe types to plot.
sampNames	Sample names to be used for labels.
xlim	x-axis limits.

Details

This function produces the control probe signal plot component of the QC report.

Value

No return value. Plots are produced as a side-effect.

Author(s)

Martin Aryee <aryee@jhu.edu>.

See Also

qcReport,mdsPlot,densityPlot,densityBeanPlot

densityBeanPlot

Examples

```
if (require(minfiData)) {
names <- pData(RGsetEx)$Sample_Name
controlStripPlot(RGsetEx, controls=c("BISULFITE CONVERSION I"), sampNames=names)
}</pre>
```

densityBeanPlot Density bean plots of methylation Beta values.

Description

Density 'bean' plots of methylation Beta values, primarily for QC.

Usage

```
densityBeanPlot(dat, sampGroups = NULL, sampNames = NULL, main = NULL,
pal = brewer.pal(8, "Dark2"), numPositions = 10000)
```

Arguments

dat	An RGChannelSet, a MethylSet or a matrix. We either use the getBeta function to get Beta values (for the first two) or we assume the matrix contains Beta values.
sampGroups	Optional sample group labels. See details.
sampNames	Optional sample names. See details.
main	Plot title.
pal	Color palette.
numPositions	The density calculation uses ${\tt numPositions}$ randomly selected CpG positions. If ${\tt NULL}$ use all positions.

Details

This function produces the density bean plot component of the QC report. If sampGroups is specified, group-specific colors will be used. For speed reasons the plots are produced using a random subset of CpG positions. The number of positions used is specified by the numPositions option.

Value

No return value. Plots are produced as a side-effect.

Author(s)

Martin Aryee <aryee@jhu.edu>.

References

Kampstra, P. Beanplot: A boxplot alternative for visual comparison of distributions. Journal of Statistical Software 28, (2008). http://www.jstatsoft.org/v28/c01

See Also

qcReport,mdsPlot,controlStripPlot,densityPlot

Examples

```
if (require(minfiData)) {
names <- pData(RGsetEx)$Sample_Name
groups <- pData(RGsetEx)$Sample_Group
par(mar=c(5,6,4,2))
densityBeanPlot(RGsetEx, sampNames=names, sampGroups=groups)
}</pre>
```

densityPlot Density plots of methylation Beta values.

Description

Density plots of methylation Beta values, primarily for QC.

Usage

```
densityPlot(dat, sampGroups = NULL, main = "", xlab = "Beta",
    pal = brewer.pal(8, "Dark2"), xlim, ylim, add = TRUE, legend = TRUE,
    ...)
```

Arguments

dat	An RGChannelSet, a MethylSet or a matrix. We either use the getBeta function to get Beta values (for the first two) or we assume the matrix contains Beta values.
sampGroups	Optional sample group labels. See details.
main	Plot title.
xlab	x-axis label.
pal	Color palette.
xlim	x-axis limits.
ylim	y-axis limits.
add	Start a new plot?
legend	Plot legend.
	Additional options to be passed to the plot command.

Details

This function produces the density plot component of the QC report. If sampGroups is specified, group-specific colors will be used.

Value

No return value. Plots are produced as a side-effect.

detectionP

Author(s)

Martin Aryee <aryee@jhu.edu>.

See Also

qcReport, mdsPlot, controlStripPlot, densityBeanPlot

Examples

```
if (require(minfiData)) {
groups <- pData(RGsetEx)$Sample_Group
densityPlot(RGsetEx, sampGroups=groups)
}</pre>
```

detectionP

Detection p-values for all probed genomic positions.

Description

This function identifies failed positions defined as both the methylated and unmethylated channel reporting background signal levels.

Usage

```
detectionP(rgSet, type = "m+u")
```

Arguments

rgSet	An RGChannelSet.
type	How to calculate p-values. Only m+u is currently implemented (See details).

Details

A detection p-value is returned for every genomic position in every sample. Small p-values indicate a good position. Positions with non-significant p-values (typically >0.01) should not be trusted.

The m+u method compares the total DNA signal (Methylated + Unmethylated) for each position to the background signal level. The background is estimated using negative control positions, assuming a normal distribution. Calculations are performed on the original (non-log) scale.

This function is different from the detection routine in Genome Studio.

Value

A matrix with detection p-values.

Author(s)

Martin Aryee <aryee@jhu.edu>.

Examples

```
if (require(minfiData)) {
  detP <- detectionP(RGsetEx)
  failed <- detP>0.01
  colMeans(failed) # Fraction of failed positions per sample
  sum(rowMeans(failed)>0.5) # How many positions failed in >50% of samples?
}
```

dmpFinder

Find differentially methylated positions

Description

Identify CpGs where methylation is associated with a continuous or categorical phenotype.

Usage

Arguments

dat	A MethylSet or a matrix.
pheno	The phenotype to be tested for association with methylation.
type	Is the phenotype 'continuous' or 'categorical'?
qCutoff	DMPs with an FDR q-value greater than this will not be returned.
shrinkVar	Should variance shrinkage be used? See details.

Details

This function tests each genomic position for association between methylation and a phenotype. Continuous phenotypes are tested with linear regression, while an F-test is used for categorical phenotypes.

Variance shrinkage (shrinkVar=TRUE) is recommended when sample sizes are small (<10). The sample variances are squeezed by computing empirical Bayes posterior means using the **limma** package.

Value

A table with one row per CpG.

Author(s)

Martin Aryee <aryee@jhu.edu>.

See Also

squeezeVar and the limma package in general.

getProbeData

Examples

```
if (require(minfiData)) {
grp <- pData(MsetEx)$Sample_Group
MsetExSmall <- MsetEx[1:1e4,] # To speed up the example
dmp <- dmpFinder(MsetExSmall, pheno=grp, type="categorical")
sum(dmp$qval < 0.05, na.rm=TRUE)
head(dmp)
}</pre>
```

getProbeData

```
Utility functions for retrieving array design information for Illumina methylation microarrays.
```

Description

A set of functions for retrieving array design information for Illumina methylation microarrays.

Usage

```
getProbeData(object)
getProbeInfo(object, type = c("I", "II", "Control", "I-Green", "I-Red"))
getManifestInfo(object, type = c("nLoci", "locusNames"))
getControlAddress(object, controlType = c("NORM_A", "NORM_C", "NORM_G", "NORM_T"
```

Arguments

object	An object of either class "RGChannelSet" or "RGChannelSetExtended" or "IlluminaMethylationManifest". In case a data object is given (the two former possibilities), the associated manifest object is retrieved and used to obtain the information.
type	A single character describing what kind of information should be returned. For getProbeInfo it represents the following subtypes of probes on the ar- ray: Type I, Type II, Controls as well as Type I (methylation measured in the Green channel) and Type II (methylation measured in the Red channel). For getManifestInfo it represents either the number of methylation loci (ap- prox. number of CpGs) on the array or the locus names.
controlType	A character vector of control types.

Value

getProbeData returns the data slot of the manifest object (mostly for internal use). getProbeInfo returns a "data.frame", getManifestInfo returns either a single number of a character vector and getControlAddress returns a vector of addresses (probe locations).

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>.

Examples

```
if (require(minfiData)) {
    info <- getProbeInfo(RGsetEx, type = c("I"))
    head(info)
    info <- getProbeInfo(RGsetEx, type = c("Control"))
    head(info)
}</pre>
```

logit2

logit in base 2.

Description

Utility functions for computing logit and inverse logit in base 2.

Usage

logit2(x) ilogit2(x)

Arguments

Х

A numeric vector.

Value

A numeric vector.

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>.

Examples

logit2(c(0.25, 0.5, 0.75))

mdsPlot	Multi-dimensional scaling plots giving an overview of similarities and
	differences between samples.

Description

Multi-dimensional scaling (MDS) plots showing a 2-d projection of distances between samples.

Usage

```
mdsPlot(dat, numPositions = 1000, sampNames = NULL, sampGroups = NULL, xlim, yli
pch = 1, pal = brewer.pal(8, "Dark2"), legendPos = "bottomleft",
legendNCol, main = NULL)
```

mdsPlot

Arguments

dat	An RGChannelSet, a MethylSet or a matrix. We either use the getBeta function to get Beta values (for the first two) or we assume the matrix contains Beta values.
numPositions	Use the numPositions genomic positions with the most methylation variability when calculating distance between samples.
sampNames	Optional sample names. See details.
sampGroups	Optional sample group labels. See details.
xlim	x-axis limits.
ylim	y-axis limits.
pch	Point type. See par for details.
pal	Color palette.
legendPos	The legend position. See legend for details.
legendNCol	The number of columns in the legend. See legend for details.
main	Plot title.

Details

Euclidean distance is calculated between samples using the numPositions most variable CpG positions. These distances are then projected into a 2-d plane using classical multidimensional scaling transformation.

Value

No return value. Plots are produced as a side-effect.

Author(s)

Martin Aryee <aryee@jhu.edu>.

References

Borg, I., Groenen, P. (2005). Modern Multidimensional Scaling: theory and applications (2nd ed.). New York: Springer-Verlag. pp. 207-212. ISBN 0387948457.

http://en.wikipedia.org/wiki/Multidimensional_scaling

See Also

qcReport, controlStripPlot, densityPlot, densityBeanPlot, par, legend

Examples

```
if (require(minfiData)) {
  names <- pData(MsetEx)$Sample_Name
  groups <- pData(MsetEx)$Sample_Group
  mdsPlot(MsetEx, sampNames=names, sampGroups=groups)</pre>
```

}

minfi-package

Description

Tools for analyzing and visualizing Illumina methylation array data. There is special focus on the 450k array; the 27k array is not supported at the moment.

Details

The package contains a (hopefully) useful vignette; this vignette contains a lengthy description of the package content and capabilities.

plotCpg

Plot methylation values at an single genomic position

Description

Plot single-position (single CpG) methylation values as a function of a categorical or continuous phenotype

Usage

```
plotCpg(dat, cpg, pheno, type = c("categorical", "continuous"),
    measure = c("beta", "M"), ylim = NULL, ylab = NULL, xlab = "",
    fitLine = TRUE, mainPrefix = NULL, mainSuffix = NULL)
```

Arguments

dat	An RGChannelSet, a MethylSet or a matrix. We either use the getBeta (or getM for measure="M") function to get Beta values (or M-values) (for the first two) or we assume the matrix contains Beta values (or M-values).
cpg	A character vector of the CpG position identifiers to be plotted.
pheno	A vector of phenotype values.
type	Is the phenotype categorical or continuous?
measure	Should Beta values or log-ratios (M) be plotted?
ylim	y-axis limits.
ylab	y-axis label.
xlab	x-axis label.
fitLine	Fit a least-squares best fit line when using a continuous phenotype.
mainPrefix	Text to prepend to the CpG name in the plot main title.
mainSuffix	Text to append to the CpG name in the plot main title.

Details

This function plots methylation values (Betas or log-ratios) at individual CpG loci as a function of a phenotype.

preprocessIllumina

Value

No return value. Plots are produced as a side-effect.

Author(s)

Martin Aryee <aryee@jhu.edu>.

Examples

```
if (require(minfiData)) {
grp <- pData(MsetEx)$Sample_Group
cpgs <- c("cg00050873", "cg00212031", "cg26684946", "cg00128718")
par(mfrow=c(2,2))
plotCpg(MsetEx, cpg=cpgs, pheno=grp, type="categorical")
}</pre>
```

preprocessIllumina Perform preprocessing as Genome Studio.

Description

These functions implements preprocessing for Illumina methylation microarrays as used in Genome Studio, the standard software provided by Illumina.

Usage

```
preprocessIllumina(rgSet, bg.correct = TRUE, normalize = c("controls", "no"),
            reference = 1)
bgcorrect.illumina(rgSet)
normalize.illumina.control(rgSet, reference = 1)
```

Arguments

rgSet	An object of class RGChannelSet.
bg.correct	logical, should background correction be performed?
normalize	logical, should (control) normalization be performed?
reference	for control normalization, which array is the reference?

Details

We have reverse engineered the preprocessing methods from Genome Studio, based on the documentation.

The current implementation of control normalization is equal to what Genome Studio provides (this statement is based on comparing Genome Studio output to the output of this function), with the following caveat: this kind of normalization requires the selection of a reference array. It is unclear how Genome Studio selects the reference array, but we allow for the manual specification of this parameter.

The current implementation of background correction is roughly equal to Genome Studio. Based on examining the output of 24 arrays, we are able to exactly recreate 18 out of the 24. The remaining

6 arrays had a max discrepancy in the Red and/or Green channel of 1-4 (this is on the unlogged intensity scale, so 4 is very small).

A script for doing this comparison may be found in the scripts directory (although it is of limited use without the data files).

Value

preprocessIllumina returns a MethylSet, while bgcorrect.illumina and normalize.illumina.co both return a RGChannelSet with corrected color channels.

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>.

See Also

RGChannelSet and MethylSet as well as IlluminaMethylationManifest for the basic classes involved in these functions. preprocessRaw is another basic preprocessing function.

Examples

```
if (require(minfiData)) {
  dat <- preprocessIllumina(RGsetEx, bg.correct=FALSE, normalize="controls")
  slot(name="preprocessMethod", dat)[1]
}</pre>
```

preprocessRaw Creation of a MethylSet without normalization

Description

Converts the Red/Green channel for an Illumina methylation array into methylation signal, without using any normalization.

Usage

```
preprocessRaw(rgSet)
```

Arguments

rgSet An object of class RGChannelSet.

Details

This function takes the Red and the Green channel of an Illumina methylation array, together with its associated manifest object and converts it into a MethylSet containing the methylated and unmethylated signal.

qcReport

Value

An object of class MethylSet

Author(s)

Kasper Daniel Hansen<khansen@jhsph.edu>.

See Also

RGChannelSet and MethylSet as well as IlluminaMethylationManifest.

Examples

```
if (require(minfiData)) {
  dat <- preprocessRaw(RGsetEx)
  slot(name="preprocessMethod", dat)[1]
}</pre>
```

```
qcReport
```

QC report for Illumina Infinium Human Methylation 450k arrays

Description

Produces a PDF QC report for Illumina Infinium Human Methylation 450k arrays, useful for identifying failed samples.

Usage

```
qcReport(rgSet, sampNames = NULL, sampGroups = NULL, pdf = "qcReport.pdf",
maxSamplesPerPage = 24, controls = c("BISULFITE CONVERSION I",
"BISULFITE CONVERSION II", "EXTENSION", "HYBRIDIZATION",
"NON-POLYMORPHIC", "SPECIFICITY I", "SPECIFICITY II", "TARGET REMOVAL"))
```

Arguments

rgSet	An object of class RGChannelSet.	
sampNames	Sample names to be used for labels.	
sampGroups	Sample groups to be used for labels.	
pdf	Path and name of the PDF output file.	
maxSamplesPerPage		
	Maximum number of samples to plot per page in those sections that plot each sample separately.	
controls	The control probe types to include in the report.	

Details

This function produces a QC report as a PDF file. It is a useful first step after reading in a new dataset to get an overview of quality and to flag potentially problematic samples.

Value

No return value. A PDF is produced as a side-effect.

Author(s)

Martin Aryee <aryee@jhu.edu>.

See Also

mdsPlot, controlStripPlot, densityPlot, densityBeanPlot

Examples

```
if (require(minfiData)) {
names <- pData(RGsetEx)$Sample_Name
groups <- pData(RGsetEx)$Sample_Group
## Not run:
qcReport(RGsetEx, sampNames=names, sampGroups=groups, pdf="qcReport.pdf")
## End(Not run)
}</pre>
```

read.450k

```
Parsing IDAT files from Illumina methylation arrays.
```

Description

Parsing IDAT files from Illumina methylation arrays.

Usage

```
read.450k(basenames, extended = FALSE, verbose = FALSE)
```

Arguments

basenames	The basenames or filenames of the IDAT files. By basenames we mean the
	filename without the ending _Grn.idat or _Red.idat (such that each sam-
	<pre>ple occur once). By filenames we mean filenames including _Grn.idat on _Red.idat (but only one of the colors)</pre>
extended	Should a RGChannelSet or a RGChannelSetExtended be returned.
verbose	Should the function be verbose?

Value

An object of class RGChannelSet or RGChannelSetExtended.

Author(s)

Kasper Daniel Hansen<khansen@jhsph.edu>.

read.450k.exp

See Also

read.450k.exp for a convenience function for reading an experiment, read.450k.sheet for reading a sample sheet and RGChannelSet for the output class.

Examples

read. 450k.exp Reads an entire 450k experiment using a sample sheet

Description

Reads an entire 450k experiment using a sample sheet or (optionally) a target like data.frame.

Usage

```
read.450k.exp(base, targets = NULL, extended = FALSE,
recursive = FALSE, verbose = FALSE)
```

Arguments

base	The base directory.
targets	A targets data.frame, see details
extended	Should the output of the function be a "RGChannelSetExtended" (default is "RGChannelSet").
recursive	Should the search be recursive (see details)
verbose	Should the function be verbose?

Details

If the targets argument is NULL, the function finds all two-color IDAT files in the directory given by base. If recursive is TRUE, the function searches base and all subdirectories. A two-color IDAT files are pair of files with names ending in _Red.idat or _Grn.idat.

If the targets argument is not NULL it is assumed it has a columned named Basename, and this is assumed to be pointing to the base name of a two color IDAT file, ie. a name that can be made into a real IDAT file by appending either _Red.idat or _Grn.idat.

Value

An object of class "RGChannelSet" or "RGChannelSetExtended".

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>.

See Also

read.450k for the workhorse function, read.450k.sheet for reading a sample sheet and RGChannelSet for the output class.

Examples

```
if(require(minfiData)) {
baseDir <- system.file("extdata", package = "minfiData")
RGset <- read.450k.exp(file.path(baseDir, "5723646052"))
}</pre>
```

read.450k.sheet Reading an Illumina methylation sample sheet

Description

Reading an Illumina methylation sample sheet, containing pheno-data information for the samples in an experiment.

Usage

```
read.450k.sheet(base, pattern = "csv$", ignore.case = TRUE,
recursive = TRUE, verbose = TRUE)
```

Arguments

base	The base directory from which the search is started.
pattern	What pattern is used to identify a sample sheet file, see list.files
ignore.case	Should the file search be case sensitive?
recursive	Should the file search be recursive, see list.files?
verbose	Should the function be verbose?

Details

This function search the directory base (possibly including subdirectories depending on the argument recursive for "sample sheet" files (see below). These files are identified solely on the base of their filename given by the arguments pattern and ignore.case (note the use of a dollarsign to mean end of file name).

In case multiple sheet files are found, they are all read and the return object will contain the concatenation of the files.

A sample sheet file is essentially a CSV (comma-separated) file containing one line per sample, with a number of columns describing pheno-data or other important information about the sample. The file may contain a header, in which case it is assumed that all lines up to and including a line starting with $\[Data]\]$ should be dropped. This is modelled after a sample sheet file Illumina provides. It is also very similar to the targets file made used by the popular limma package (see the extensive package vignette).

An attempt at guessing the file path to the IDAT files represented in the sheet is made. This should be doublechecked and might need to manually changed.

read.450k.sheet

Value

A data.frame containing the columns of all the sample sheets. As described in details, a column named Sentrix_Position is renamed to Array and Sentrix_ID is renamed to Slide. In addition the data.frame will contain a column named Basename.

Author(s)

Kasper Daniel Hansen<khansen@jhsph.edu>.

See Also

read.450k.exp and read.450k for functions reading IDAT files. list.files for help on the arguments recursive and ignore.case.

Examples

```
if(require(minfiData)) {
   baseDir <- system.file("extdata", package = "minfiData")
   sheet <- read.450k.sheet(baseDir)</pre>
```

}

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