

Package ‘DMRcate’

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Title Methylation array and sequencing spatial analysis methods

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Description

De novo identification and extraction of differentially methylated regions (DMRs) from the human genome using Whole Genome Bisulfite Sequencing (WGBS) and Illumina Infinium Array (450K and EPIC) data. Provides functionality for filtering probes possibly confounded by SNPs and cross-hybridisation. Includes GRanges generation and plotting functions.

Depends R (>= 3.6.0), minfi, SummarizedExperiment

Imports ExperimentHub, bsseq, GenomeInfoDb, limma, edgeR, DSS, missMethyl, GenomicRanges, methods, graphics, plyr, Gviz, IRanges, stats, utils, S4Vectors

biocViews DifferentialMethylation, GeneExpression, Microarray, MethylationArray, Genetics, DifferentialExpression, GenomeAnnotation, DNAMethylation, OneChannel, TwoChannel, MultipleComparison, QualityControl, TimeCourse, Sequencing, WholeGenome, Epigenetics, Coverage, Preprocessing, DataImport

Suggests knitr, RUnit, BiocGenerics, IlluminaHumanMethylation450kanno.ilmn12.hg19, IlluminaHumanMethylationEPICanno.ilm10b4.hg19

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R topics documented:

DMRcate-package	2
changeFDR	3

cpg.annotate	4
CpGannotated-class	6
DMR.plot	7
dmrcate	8
DMResults-class	10
extractRanges	11
rmSNPandCH	12
sequencing.annotate	13
Index	16

DMRcate-package	<i>DMR calling from bisulfite sequencing and Illumina array data</i>
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Description

De novo identification and extraction of differentially methylated regions (DMRs) in the human genome using Illumina array and bisulfite sequencing data. DMRcate extracts and annotates differentially methylated regions (DMRs) using a kernel-smoothed estimate. Functions are provided for filtering probes possibly confounded by SNPs and cross-hybridisation. Includes GRanges generation and plotting functions.

Author(s)

Tim J. Peters <t.peters@garvan.org.au>

References

Peters T.J., Buckley M.J., Statham, A., Pidsley R., Samaras K., Lord R.V., Clark S.J. and Molloy P.L. *De novo* identification of differentially methylated regions in the human genome. *Epigenetics & Chromatin* 2015, **8**:6, doi:10.1186/1756-8935-8-6

Examples

```
library(ExperimentHub)
library(limma)
eh <- ExperimentHub()
FlowSorted.Blood.EPIC <- eh[["EH1136"]]
tcell <- FlowSorted.Blood.EPIC[,colData(FlowSorted.Blood.EPIC)$CD4T==100 |
                                colData(FlowSorted.Blood.EPIC)$CD8T==100]

detP <- detectionP(tcell)
remove <- apply(detP, 1, function(x) any(x > 0.01))
tcell <- tcell[!remove,]
tcell <- preprocessFunnorm(tcell)
#Subset to chr2 only
tcell <- tcell[seqnames(tcell) == "chr2",]
tcellms <- getM(tcell)
tcellms.noSNPs <- rmSNPandCH(tcellms, dist=2, mafcut=0.05)
tcell$Replicate[tcell$Replicate==""] <- tcell$Sample_Name[tcell$Replicate==""]
tcellms.noSNPs <- avearrays(tcellms.noSNPs, tcell$Replicate)
tcell <- tcell[!duplicated(tcell$Replicate)]
tcell <- tcell[rownames(tcellms.noSNPs),]
assays(tcell)[["M"]] <- tcellms.noSNPs
assays(tcell)[["Beta"]] <- ilogit2(tcellms.noSNPs)
```

```

type <- factor(tcell$CellType)
design <- model.matrix(~type)
myannotation <- cp.annotate("array", tcell, arraytype = "EPIC",
                           analysis.type="differential", design=design, coef=2)
dmrcoutput <- dmrcate(myannotation, lambda=1000, C=2)
results.ranges <- extractRanges(dmrcoutput, genome = "hg19")
groups <- c(CD8T="magenta", CD4T="forestgreen")
cols <- groups[as.character(type)]
DMR.plot(ranges=results.ranges, dmr=1, CpGs=getBeta(tcell), what="Beta",
         arraytype = "EPIC", phen.col=cols, genome="hg19")

```

changeFDR	<i>Change the individual CpG FDR thresholding for a CpGannotated object.</i>
-----------	--

Description

Takes a [CpGannotated-class](#) object and a specified FDR > 0 and < 1, and re-indexes the object in order to call DMRs at the specified rate.

Usage

```
changeFDR(annot, FDR)
```

Arguments

annot	A CpGannotated-class object, created by cp.annotate or sequencing.annotate .
FDR	The desired individual CpG FDR, which will index the rate at which DMRs are called.

Details

The number of CpG sites called as significant by this function will set the post-smoothing threshold for DMR constituents in [dmrcate](#).

Value

A re-indexed [CpGannotated-class](#) object.

Author(s)

Tim Peters <t.peters@garvan.org.au>

Examples

```

library(ExperimentHub)
eh <- ExperimentHub()
bis_1072 <- eh[["EH1072"]]
pData(bis_1072) <- data.frame(replicate=gsub(".*-", "", colnames(bis_1072)),
                             tissue=substr(colnames(bis_1072), 1,
                                           nchar(colnames(bis_1072))-3),
                             row.names=colnames(bis_1072))
colData(bis_1072)$tissue <- gsub("-", "_", colData(bis_1072)$tissue)

```

```

bis_1072 <- renameSeqlevels(bis_1072, mapSeqlevels(seqlevels(bis_1072), "UCSC"))
bis_1072 <- bis_1072[seqnames(bis_1072)=="chr19",]
bis_1072 <- bis_1072[138151:138250,]
tissue <- factor(pData(bis_1072)$tissue)
tissue <- relevel(tissue, "Liver_Treg")
design <- model.matrix(~tissue)
colnames(design) <- gsub("tissue", "", colnames(design))
colnames(design)[1] <- "Intercept"
rownames(design) <- colnames(bis_1072)
methdesign <- edgeR::modelMatrixMeth(design)
cont.mat <- limma::makeContrasts(treg_vs_tcon=Lymph_N_Treg-Lymph_N_Tcon,
                               fat_vs_ln=Fat_Treg-Lymph_N_Treg,
                               skin_vs_ln=Skin_Treg-Lymph_N_Treg,
                               fat_vs_skin=Fat_Treg-Skin_Treg,
                               levels=methdesign)
seq_annot <- sequencing.annotate(bis_1072, methdesign, all.cov = TRUE,
                                contrasts = TRUE, cont.matrix = cont.mat,
                                coef = "fat_vs_skin", fdr=0.05)
seq_annot <- changeFDR(seq_annot, 0.25)

```

cpg.annotate	<i>Annotate Illumina CpGs with their chromosome position and test statistic</i>
--------------	---

Description

Annotate a matrix/GenomicRatioSet representing 450K or EPIC data with probe weights and chromosomal position.

Usage

```

cpg.annotate(datatype = c("array", "sequencing"), object, what=c("Beta", "M"),
            arraytype=c("EPIC", "450K"), analysis.type = c("differential",
            "variability", "ANOVA", "diffVar"), design, contrasts = FALSE,
            cont.matrix = NULL, fdr = 0.05, coef, ...)

```

Arguments

datatype	Character string representing the type of data being analysed.
object	Either: <ul style="list-style-type: none"> - A matrix of M-values, with unique Illumina probe IDs as rownames and unique sample IDs as column names or, - A GenomicRatioSet, appropriately annotated.
what	Does the data matrix contain Beta or M-values? Not needed if object is a GenomicRatioSet.
arraytype	Is the data matrix sourced from EPIC or 450K data? Not needed if object is a GenomicRatioSet.
analysis.type	"differential" for dmrcate() to return DMRs; "variability" to return VMRs; "ANOVA" to return "whole experiment" DMRs, incorporating all possible contrasts from the design matrix using the moderated F -statistics; "diffVar" to

	return differentially variable methylated regions, using the <code>missMethyl</code> package to generate <i>t</i> -statistics. All modes are applicable when <code>datatype="array"</code> , but only "differential" is available when <code>datatype="sequencing"</code> .
<code>design</code>	Study design matrix. Identical context to differential analysis pipeline in <code>limma</code> . Must have an intercept if <code>contrasts=FALSE</code> . Applies only when <code>analysis.type %in% c("differential", "ANOVA", "diffVar")</code> . Only applicable when <code>datatype="array"</code> .
<code>contrasts</code>	Logical denoting whether a <code>limma</code> -style contrast matrix is specified. Only applicable when <code>datatype="array"</code> and <code>analysis.type %in% c("differential", "diffVar")</code> .
<code>cont.matrix</code>	<code>Limma</code> -style contrast matrix for explicit contrasting. For each call to <code>cpg.annotate</code> , only one contrast will be fit. Only applicable when <code>datatype="array"</code> and <code>analysis.type %in% c("differential", "diffVar")</code> .
<code>fdr</code>	FDR cutoff (Benjamini-Hochberg) for which CpG sites are individually called as significant. Used to index default thresholding in <code>dmrcate()</code> . Highly recommended as the primary thresholding parameter for calling DMRs. Not used when <code>analysis.type = "variability"</code> .
<code>coef</code>	The column index in <code>design</code> corresponding to the phenotype comparison. Corresponds to the comparison of interest in <code>design</code> when <code>contrasts=FALSE</code> , otherwise must be a column name in <code>cont.matrix</code> . Only applicable when <code>datatype="array"</code> and <code>analysis.type %in% c("differential", "diffVar")</code> .
<code>...</code>	Extra arguments passed to the <code>limma</code> function <code>lmFit()</code> (<code>analysis.type="differential"</code>) or <code>missMethyl</code> function <code>varFit()</code> (<code>analysis.type = "diffVar"</code>), and when <code>datatype="array"</code> .

Value

A `CpGannotated-class`.

Author(s)

Tim J. Peters <t.peters@garvan.org.au>

References

- Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). `limma` powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, **43**(7), e47.
- Feng, H., Conneely, K. N., & Wu, H. (2014). A Bayesian hierarchical model to detect differentially methylated loci from single nucleotide resolution sequencing data. *Nucleic Acids Research*, **42**(8), e69.
- Phipson, B., & Oshlack, A. (2014). `DiffVar`: a new method for detecting differential variability with application to methylation in cancer and aging. *Genome Biol*, **15**(9), 465.
- Peters T.J., Buckley M.J., Statham, A., Pidsley R., Samaras K., Lord R.V., Clark S.J. and Molloy P.L. *De novo* identification of differentially methylated regions in the human genome. *Epigenetics & Chromatin* 2015, **8**:6, doi:10.1186/1756-8935-8-6.

Examples

```
library(ExperimentHub)
library(limma)
eh <- ExperimentHub()
FlowSorted.Blood.EPIC <- eh[["EH1136"]]
tcell <- FlowSorted.Blood.EPIC[, colData(FlowSorted.Blood.EPIC)$CD4T==100 |
```

```

colData(FlowSorted.Blood.EPIC)$CD8T==100]
detP <- detectionP(tcell)
remove <- apply(detP, 1, function (x) any(x > 0.01))
tcell <- tcell[!remove,]
tcell <- preprocessFunnorm(tcell)
#Subset to chr2 only
tcell <- tcell[seqnames(tcell) == "chr2",]
tcellms <- getM(tcell)
tcellms.noSNPs <- rmSNPandCH(tcellms, dist=2, mafcut=0.05)
tcell$Replicate[tcell$Replicate==""] <- tcell$Sample_Name[tcell$Replicate==""]
tcellms.noSNPs <- avearrays(tcellms.noSNPs, tcell$Replicate)
tcell <- tcell[!duplicated(tcell$Replicate)]
tcell <- tcell[rownames(tcellms.noSNPs),]
assays(tcell)[["M"]] <- tcellms.noSNPs
assays(tcell)[["Beta"]] <- ilogit2(tcellms.noSNPs)
type <- factor(tcell$CellType)
design <- model.matrix(~type)
myannotation <- cpg.annotate("array", tcell, arraytype = "EPIC",
                             analysis.type="differential", design=design, coef=2)

```

CpGannotated-class *An object summarising individual CpG sites fitted to a given model*

Description

An S4 class that stores output from either [cpg.annotate](#) or [sequencing.annotate](#).

Slots

ranges: A GRanges object, containing CpG-level information to be passed to [dmrcate](#). Mcols of this object include:

- stat: Per-CpG test statistic; t if from `limma` or Wald if from DSS if using differential mode. Variance if using variability mode, \sqrt{F} if using ANOVA mode, t if using `diffVar` mode.
- diff: Methylation difference/coefficient. In beta space for [cpg.annotate](#) output and output passed from `DSS::DMLtest()`. In logit space for when a BSseq object is passed from [sequencing.annotate](#). Not available for output passed from `DSS::DMLtest.multiFactor()`. Not applicable in variability, ANOVA or `diffVar` modes.
- ind.fdr: False discovery rate as calculated on individual CpG sites.
- is.sig: Logical determining whether a CpG site is individually significant or not. Can be adjusted using [changeFDR](#).

Methods

CpGannotated objects have a `show` method that describes the data therein.

Author(s)

Tim Peters <t.peters@garvan.org.au>

DMR.plot

*Plotting DMRs***Description**

Plots an individual DMR (in context of possibly other DMRs) as found by `dmrcate`. Heatmaps are shown as well as proximal coding regions, smoothed group means and chromosome ideogram.

Usage

```
DMR.plot(ranges, dmr, CpGs, what=c("Beta", "M"),
         arraytype=c("EPIC", "450K"), phen.col,
         genome = c("hg19", "hg38", "mm10"), ...)
```

Arguments

<code>ranges</code>	A GRanges object (ostensibly created by <code>extractRanges()</code>) describing DMR coordinates.
<code>dmr</code>	Index of ranges (one integer only) indicating which DMR to be plotted.
<code>CpGs</code>	Either: <ul style="list-style-type: none"> - A matrix of beta values for plotting, with unique Illumina probe IDs as row-names. - A GenomicRatioSet, annotated with the appropriate array and data types - A BSseq object containing per-CpG methylation and coverage counts for the samples to be plotted
<code>what</code>	Does CpGs (if a matrix) contain Beta or M-values? Not needed if object is a GenomicRatioSet or BSseq object.
<code>arraytype</code>	Is CpGs (if a matrix) sourced from EPIC or 450K data? Not needed if object is a GenomicRatioSet or BSseq object.
<code>phen.col</code>	Vector of colors denoting phenotypes of <i>all</i> samples described in CpGs. See vignette for worked example.
<code>genome</code>	Reference genome for annotating DMRs. Can be one of "hg19", "hg38" or "mm10"
<code>...</code>	Extra arguments passed to <code>Gviz:::plotTracks()</code> .

Value

A plot to the current device.

Author(s)

Aaron Statham <a.statham@garvan.org.au>, Tim J. Peters <t.peters@garvan.org.au>

Examples

```

library(ExperimentHub)
library(limma)
eh <- ExperimentHub()
FlowSorted.Blood.EPIC <- eh[["EH1136"]]
tcell <- FlowSorted.Blood.EPIC[,colData(FlowSorted.Blood.EPIC)$CD4T==100 |
                                colData(FlowSorted.Blood.EPIC)$CD8T==100]

detP <- detectionP(tcell)
remove <- apply(detP, 1, function (x) any(x > 0.01))
tcell <- tcell[!remove,]
tcell <- preprocessFunnorm(tcell)
#Subset to chr2 only
tcell <- tcell[seqnames(tcell) == "chr2",]
tcellms <- getM(tcell)
tcellms.noSNPs <- rmSNPandCH(tcellms, dist=2, mafcut=0.05)
tcell$Replicate[tcell$Replicate==""] <- tcell$Sample_Name[tcell$Replicate==""]
tcellms.noSNPs <- avearrays(tcellms.noSNPs, tcell$Replicate)
tcell <- tcell[!duplicated(tcell$Replicate)]
tcell <- tcell[rownames(tcellms.noSNPs),]
assays(tcell)[["M"]] <- tcellms.noSNPs
assays(tcell)[["Beta"]] <- ilogit2(tcellms.noSNPs)
type <- factor(tcell$CellType)
design <- model.matrix(~type)
myannotation <- cpg.annotate("array", tcell, arraytype = "EPIC",
                             analysis.type="differential", design=design, coef=2)
dmrcoutput <- dmrcate(myannotation, lambda=1000, C=2)
results.ranges <- extractRanges(dmrcoutput, genome = "hg19")
groups <- c(CD8T="magenta", CD4T="forestgreen")
cols <- groups[as.character(type)]
DMR.plot(ranges=results.ranges, dmr=1, CpGs=getBeta(tcell), what="Beta",
          arraytype = "EPIC", phen.col=cols, genome="hg19")

```

dmrcate

DMR identification

Description

The main function of this package. Computes a kernel estimate against a null comparison to identify significantly differentially (or variable) methylated regions.

Usage

```

dmrcate(object,
         lambda = 1000,
         C=NULL,
         pcutoff = "fdr",
         consec = FALSE,
         conseclambda = 10,
         betacutoff = NULL,
         min.cpgs = 2
        )

```


Arguments

object	A <code>CpGannotated-class</code> , created from <code>cpg.annotate</code> or <code>sequencing.annotate</code> .
lambda	Gaussian kernel bandwidth for smoothed-function estimation. Also informs DMR bookend definition; gaps \geq lambda between significant CpG sites will be in separate DMRs. Support is truncated at $5*\text{lambda}$. Default is 1000 nucleotides. See details for further info.
C	Scaling factor for bandwidth. Gaussian kernel is calculated where $\text{lambda}/C = \text{sigma}$. Empirical testing shows for both Illumina and bisulfite sequencing data that, when $\text{lambda}=1000$, near-optimal prediction of sequencing-derived DMRs is obtained when C is approximately 2, i.e. 1 standard deviation of Gaussian kernel = 500 base pairs. Cannot be < 0.2 .
pcutoff	Threshold to determine DMRs. Default implies indexing at the rate of individually significant CpGs and can be set on the <code>CpGannotated-class</code> object using <code>cpg.annotate</code> , <code>sequencing.annotate</code> or <code>changeFDR</code> . Default highly recommended unless you are comfortable with the risk of Type I error. If manually specified, this value will be set on the highly permissive kernel-smoothed FDR values.
consec	Use DMRcate in consecutive mode. Treats CpG sites as equally spaced.
conseclambda	Bandwidth in <i>CpGs</i> (rather than nucleotides) to use when <code>consec=TRUE</code> . When specified the variable lambda simply becomes the minimum distance separating DMRs.
betacutoff	Optional filter; removes any region from the results where the absolute mean beta shift is less than the given value. Only available for Illumina array data and results produced from <code>DSS::DMLtest()</code> .
min.cpgs	Minimum number of consecutive CpGs constituting a DMR.

Details

The values of lambda and C should be chosen with care. For array data, we currently recommend that half a kilobase represent 1 standard deviation of support ($\text{lambda}=1000$ and $C=2$). If lambda is too small or C too large then the kernel estimator will not have enough support to significantly differentiate the weighted estimate from the null distribution. If lambda is too large then dmrcate will report very long DMRs spanning multiple gene loci, and the large amount of support will likely give Type I errors. If you are concerned about Type I errors we highly recommend using the default value of pcutoff, although this will return no DMRs if no DM CpGs are returned by limma/DSS either.

Value

A DMRResults object.

Author(s)

Tim J. Peters <t.peters@garvan.org.au>, Mike J. Buckley <Mike.Buckley@csiro.au>, Tim Triche Jr. <tim.triche@usc.edu>

References

Peters T.J., Buckley M.J., Statham, A., Pidsley R., Samaras K., Lord R.V., Clark S.J. and Molloy P.L. *De novo* identification of differentially methylated regions in the human genome. *Epigenetics & Chromatin* 2015, **8**:6, doi:10.1186/1756-8935-8-6

Wand, M.P. & Jones, M.C. (1995) *Kernel Smoothing*. Chapman & Hall.

Duong T. (2013) Local significant differences from nonparametric two-sample tests. *Journal of Nonparametric Statistics*. 2013 **25**(3), 635-645.

Examples

```
library(ExperimentHub)
library(limma)
eh <- ExperimentHub()
FlowSorted.Blood.EPIC <- eh[["EH1136"]]
tcell <- FlowSorted.Blood.EPIC[,colData(FlowSorted.Blood.EPIC)$CD4T==100 |
                                colData(FlowSorted.Blood.EPIC)$CD8T==100]

detP <- detectionP(tcell)
remove <- apply(detP, 1, function (x) any(x > 0.01))
tcell <- tcell[!remove,]
tcell <- preprocessFunnorm(tcell)
#Subset to chr2 only
tcell <- tcell[seqnames(tcell) == "chr2",]
tcellms <- getM(tcell)
tcellms.noSNPs <- rmSNPandCH(tcellms, dist=2, mafcut=0.05)
tcell$Replicate[tcell$Replicate==""] <- tcell$Sample_Name[tcell$Replicate==""]
tcellms.noSNPs <- avearrays(tcellms.noSNPs, tcell$Replicate)
tcell <- tcell[!duplicated(tcell$Replicate)]
tcell <- tcell[rownames(tcellms.noSNPs),]
assays(tcell)[["M"]] <- tcellms.noSNPs
assays(tcell)[["Beta"]] <- ilogit2(tcellms.noSNPs)
type <- factor(tcell$CellType)
design <- model.matrix(~type)
myannotation <- cpg.annotate("array", tcell, arraytype = "EPIC",
                             analysis.type="differential", design=design, coef=2)
dmrcoutput <- dmrcate(myannotation, lambda=1000, C=2)
```

DMResults-class

Initial storage object for called DMRs - class

Description

An S4 class that stores DMR information as output from [dmrcate](#).

Slots

This class has eight slots, summarising DMR information to be passed to [extractRanges](#):

coord: DMR coordinates in UCSC style.

no.cpgs: Number of constituent CpG sites of DMR.

min_smoothed_fdr: Minimum FDR of the **smoothed** estimate.

Stouffer: Stouffer summary transform of the **individual** CpG FDRs.

HMFDR: Harmonic mean of the **individual** CpG FDRs.

Fisher: Fisher combined probability transform of the **individual** CpG FDRs.

maxdiff: Maximum differential/coefficient within the DMR.

meandiff: Mean differential/coefficient across the DMR.

Methods

DMResults objects have a show method describing the number of DMRs called.

Author(s)

Tim Peters <t.peters@garvan.org.au>

extractRanges	Create a GRanges object from dmrcate output.
---------------	--

Description

Takes a DMResults object and produces the corresponding GRanges object.

Usage

```
extractRanges(dmrcoutput, genome = c("hg19", "hg38", "mm10"))
```

Arguments

dmrcoutput	A DMResults object.
genome	Reference genome for annotating DMRs with promoter overlaps. Can be one of "hg19", "hg38" or "mm10". Ranges are assumed to map to the reference stated; there is no liftover.

Value

A GRanges object.

Author(s)

Tim Triche Jr. <tim.triche@usc.edu>, Tim Peters <t.peters@garvan.org.au>

Examples

```
library(ExperimentHub)
library(limma)
eh <- ExperimentHub()
FlowSorted.Blood.EPIC <- eh[["EH1136"]]
tcell <- FlowSorted.Blood.EPIC[,colData(FlowSorted.Blood.EPIC)$CD4T==100 |
                               colData(FlowSorted.Blood.EPIC)$CD8T==100]

detP <- detectionP(tcell)
remove <- apply(detP, 1, function (x) any(x > 0.01))
tcell <- tcell[!remove,]
tcell <- preprocessFunnorm(tcell)
#Subset to chr2 only
tcell <- tcell[seqnames(tcell) == "chr2",]
tcellms <- getM(tcell)
tcellms.noSNPs <- rmSNPandCH(tcellms, dist=2, mafcut=0.05)
tcell$Replicate[tcell$Replicate==""] <- tcell$Sample_Name[tcell$Replicate==""]
tcellms.noSNPs <- avearrays(tcellms.noSNPs, tcell$Replicate)
tcell <- tcell[,!duplicated(tcell$Replicate)]
tcell <- tcell[rownames(tcellms.noSNPs),]
```

```

assays(tcell)[["M"]] <- tcellms.noSNPs
assays(tcell)[["Beta"]] <- ilogit2(tcellms.noSNPs)
type <- factor(tcell$CellType)
design <- model.matrix(~type)
myannotation <- cpg.annotate("array", tcell, arraytype = "EPIC",
                             analysis.type="differential", design=design, coef=2)
dmrcoutput <- dmrcate(myannotation, lambda=1000, C=2)
results.ranges <- extractRanges(dmrcoutput, genome = "hg19")

```

 rmSNPandCH

Filter probes

Description

Filters a matrix of M-values (or beta values) by distance to SNP/variant. Also (optionally) removes cross-hybridising probes and sex-chromosome probes.

Usage

```
rmSNPandCH(object, dist = 2, mafcut = 0.05, and = TRUE, rmcrosshyb = TRUE, rmXY=FALSE)
```

Arguments

object	A matrix of M-values or beta values, with unique Illumina probe IDs as row-names.
dist	Maximum distance (from CpG to SNP/variant) of probes to be filtered out. See details for when Illumina occasionally lists a CpG-to-SNP distance as being < 0.
mafcut	Minimum minor allele frequency of probes to be filtered out.
and	If TRUE, the probe must have at least 1 SNP binding to it that satisfies both requirements in <code>dist</code> and <code>mafcut</code> for it to be filtered out. If FALSE, it will be filtered out if either requirement is satisfied. Default is TRUE.
rmcrosshyb	If TRUE, filters out probes found by Pidsley and Zotenko et al. (2016) for EPIC or Chen et al. (2013) for 450K to be cross-reactive with areas of the genome not at the site of interest. Many of these sites are on the X-chromosome, leading to potential confounding if the sample group is a mix of males and females. There are 63,707 probes in total in this list. Default is TRUE.
rmXY	If TRUE, filters out probe hybridising to sex chromosomes. Or-operator applies when combined with other 2 filters.

Details

Probes in `-1:dist` will be filtered out for any integer specification of `dist`. When a probe is listed as being “-1” nucleotides from a SNP (7 in total of the 153,113), that SNP is immediately adjacent to the end of the probe, and is likely to confound the measurement, in addition to those listed as 0, 1 or 2 nucleotides away. See vignette for further details.

Value

A matrix, attenuated from `object`, with rows corresponding to probes matching user input filtered out.

Author(s)

Tim J. Peters <t.peters@garvan.org.au>

References

Pidsley R, Zotenko E, Peters TJ, Lawrence MG, Risbridger GP, Molloy P, Van Dijk S, Muhlhausler B, Stirzaker C, Clark SJ. Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling. *Genome Biology*. 2016 17(1), 208.

Chen YA, Lemire M, Choufani S, Butcher DT, Grafodatskaya D, Zanke BW, Gallinger S, Hudson TJ, Weksberg R. Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. *Epigenetics*. 2013 Jan 11;8(2). http://supportres.illumina.com/documents/myillumina/88bab663-307c-444a-848e-0ed6c338ee4d/humanmethylation450_15017482_v.1.2.snpupdate.table.v3.txt

Examples

```
library(ExperimentHub)
library(limma)
eh <- ExperimentHub()
FlowSorted.Blood.EPIC <- eh[["EH1136"]]
tcell <- FlowSorted.Blood.EPIC[,colData(FlowSorted.Blood.EPIC)$CD4T==100 |
                                colData(FlowSorted.Blood.EPIC)$CD8T==100]
detP <- detectionP(tcell)
remove <- apply(detP, 1, function (x) any(x > 0.01))
tcell <- tcell[!remove,]
tcell <- preprocessFunnorm(tcell)
tcellms <- getM(tcell)
tcellms.noSNPs <- rmSNPandCH(tcellms, dist=2, mafcut=0.05)
```

sequencing.annotate *Annotate a bisulfite sequencing experiment (WGBS or RRBS) with probe weights and chromosomal position.*

Description

Either: - Annotate a BSseq object with chromosome position and test statistic, or - Parse output from `DSS::DMLtest()` or `DSS::DMLtest.multiFactor()` into a CpGannotated object.

Usage

```
sequencing.annotate(obj, methdesign, all.cov=FALSE, contrasts = FALSE,
                    cont.matrix = NULL, fdr = 0.05, coef, ...)
```

Arguments

`obj` A BSseq object or data.frame output from `DSS::DMLtest()` or `DSS::DMLtest.multiFactor()`.

`methdesign` Methylation study design matrix describing samples and groups. **Use of `edgeR::modelMatrixMeth()` to make this matrix is highly recommended**, since it transforms a regular model.matrix (as one would construct for a microarray or RNA-Seq experiment) into a “two-channel” matrix representing methylated and unmethylated reads for each sample. Only applicable when `obj` is a BSseq object.

all.cov	If TRUE, only CpG sites where all samples have > 0 coverage will be retained. If FALSE, CpG sites for which some (not all) samples have coverage=0 will be retained.
contrasts	Logical denoting whether a limma-style contrast matrix is specified. Only applicable when obj is a BSseq object.
cont.matrix	Limma-style contrast matrix for explicit contrasting. For each call to sequencing.annotate, only one contrast will be fit. Only applicable when obj is a BSseq object.
fdr	FDR cutoff (Benjamini-Hochberg) for which CpG sites are individually called as significant. Used to index default thresholding in dmrcate(). Highly recommended as the primary thresholding parameter for calling DMRs. Only applicable when obj is a BSseq object.
coef	The column index in design corresponding to the phenotype comparison. Corresponds to the comparison of interest in design when contrasts=FALSE, otherwise must be a column name in cont.matrix. Only applicable when obj is a BSseq object.
...	Extra arguments passed to the limma function lmFit(). Only applicable when obj is a BSseq object.

Value

A [CpGannotated-class](#).

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References

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, **43**(7), e47.

Chen, Y., Pal, B., Visvader, J. E., & Smyth, G. K. (2017). Differential methylation analysis of reduced representation bisulfite sequencing experiments using edgeR. *F1000Research*, **6**, 2055.

Examples

```
library(ExperimentHub)
library(SummarizedExperiment)
eh = ExperimentHub()
bis_1072 <- eh[["EH1072"]]
pData(bis_1072) <- data.frame(replicate=gsub(".*-", "", colnames(bis_1072)),
                             tissue=substr(colnames(bis_1072), 1, nchar(colnames(bis_1072))-3),
                             row.names=colnames(bis_1072))
colData(bis_1072)$tissue <- gsub("-", "_", colData(bis_1072)$tissue)
bis_1072 <- renameSeqlevels(bis_1072, mapSeqlevels(seqlevels(bis_1072), "UCSC"))
bis_1072 <- bis_1072[seqnames(bis_1072)=="chr19",]
bis_1072 <- bis_1072[240201:240300,]
tissue <- factor(pData(bis_1072)$tissue)
tissue <- relevel(tissue, "Liver_Treg")
design <- model.matrix(~tissue)
colnames(design) <- gsub("tissue", "", colnames(design))
colnames(design)[1] <- "Intercept"
rownames(design) <- colnames(bis_1072)
```


Index

*Topic **classes**

CpGannotated-class, [6](#)
DMResults-class, [10](#)

changeFDR, [3](#), [6](#), [9](#)
cpg.annotate, [3](#), [4](#), [6](#), [9](#)
CpGannotated-class, [6](#)

DMR.plot, [7](#)
DMRcate (DMRcate-package), [2](#)
dmrcate, [3](#), [6](#), [8](#), [10](#), [11](#)
DMRcate-package, [2](#)
DMResults-class, [10](#)

extractRanges, [10](#), [11](#)

plot (DMR.plot), [7](#)

rmSNPandCH, [12](#)

sequencing.annotate, [3](#), [6](#), [9](#), [13](#)