

# Package ‘epihet’

April 15, 2020

**Title** Determining Epigenetic Heterogeneity from Bisulfite Sequencing Data

**Version** 1.2.0

**Description** epihet is an R-package that calculates the epigenetic heterogeneity between cancer cells and/or normal cells. The functions establish a pipeline that take in bisulfite sequencing data from multiple samples and use the data to track similarities and differences in epipolymorphism, proportion of discordantly methylated sequencing reads (PDR), and Shannon entropy values at epialleles that are shared between the samples. epihet can be used to perform analysis on the data by creating heatmaps, box plots, PCA plots, and t-SNE plots. MA plots can also be created by calculating the differential heterogeneity of the samples. And we construct co-epihet network and perform network analysis.

**Depends** R(>= 3.6), GenomicRanges, IRanges, S4Vectors, ggplot2, foreach, Rtsne, igraph

**License** Artistic-2.0

**biocViews** DNAMethylation, Epigenetics, MethylSeq, Sequencing, Software

**Imports** data.table, doParallel, EntropyExplorer, graphics, stats, grDevices, heatmap, utils, qvalue, WGCNA, ReactomePA

**Encoding** UTF-8

**RoxygenNote** 6.0.1

**Suggests** knitr, clusterProfiler, ggfortify, org.Hs.eg.db, rmarkdown

**VignetteBuilder** knitr

**BuildVignettes** TRUE

**URL** <https://github.com/TheJacksonLaboratory/epihet>

**BugReports** <https://github.com/TheJacksonLaboratory/epihet/issues>

**git\_url** <https://git.bioconductor.org/packages/epihet>

**git\_branch** RELEASE\_3\_10

**git\_last\_commit** b51b4f4

**git\_last\_commit\_date** 2019-10-29

**Date/Publication** 2020-04-14

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## R topics documented:

background . . . . .	2
compMatrix . . . . .	3
diffHet . . . . .	5
epiBox . . . . .	6
epiMA . . . . .	7
epiMap . . . . .	8
epiNetwork . . . . .	9
epiPathway . . . . .	10
epiPCA . . . . .	11
epiTSNE . . . . .	12
jaccard . . . . .	14
makeGR . . . . .	14
moduleAnno . . . . .	15
moduleSim . . . . .	16
moduleVisual . . . . .	17
readGR . . . . .	18
shannon . . . . .	19
splitn . . . . .	19
summarize . . . . .	20
userobj . . . . .	21

<b>Index</b>	<b>22</b>
--------------	-----------

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background	<i>example data background</i>
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## Description

background: A data frame containing 31995 elements as background used for pathway enrichment analysis

datTraits: Clinical traits containing OS,EFS,age

DEG: Differentially expressed genes compared CEBPA-sil vs.normal

DEH: DEH loci

diffhetmatrix: A differentially heterogeneity matrix

moduledm: Module information for CEBPA-dm mutation samples

modulesil: Module information for CEBPA-sil mutation samples

promoter: The promoter region annotation file

sharedmatrix: Epipolymorphism values for 6 patients on DEH loci

**Usage**

```
data(background)  
background
```

```
data(datTraits)  
datTraits
```

```
data(DEG)  
DEG
```

```
data(DEH)  
DEH
```

```
data(diffhetmatrix)  
diffhetmatrix
```

```
data(moduledm)  
moduledm
```

```
data(modulesil)  
modulesil
```

```
data(promoter)  
promoter
```

```
data(sharedmatrix)  
sharedmatrix
```

**Format**

background: A data frame with 31995 rows and 1 variables:

**gene** background gene list

**Value**

A data frame

A large GRanges object

---

compMatrix

*Make Comparison Matrix*

---

**Description**

A matrix is created for `pca/hclust/tsne` which contains read number, average methylation levels, pdr, epipolymorphism, and Shannon entropy values across multiple samples at the same loci using read number in a `GenomicRanges` object

**Usage**

```
compMatrix(eps.gr, outprefix = NULL, readNumber = 60, p = 1,  
           cores = 5, sve = FALSE)
```

**Arguments**

epi.gr	An input file containing the read number, locus, pdr, epipolymorphism, and Shannon entropy values stored in a list of GenomicRanges objects
outprefix	The prefix name of the outputted matrix file. 'sve' must be set to TRUE (default: NULL)
readNumber	The lowest number of reads required for each loci (default: 60)
p	Percentage (as decimal) of matching samples required to determine a match at a given locus, e.g. a value of 0.75 requires 75% of the samples to have an epiallele at a common loci in order to add the loci to the matrix (default: 1)
cores	The number of cores to be used for parallel execution (default: 5)
sve	A boolean to save the comparison matrix (default: FALSE)

**Value**

A large matrix containing values (pdr, etc.) at the same loci

**Examples**

```
p1.GR<-GRanges(seqnames = Rle(c("chr22"), c(5)),
  ranges = IRanges(c(327,821,838,755,761), end = c(364,849,858,773,781)),
  strand = Rle(strand(c("-", "+", "+", "+", "-"))),
  values.loci = c("327:350:361:364", "821:837:844:849",
  "838:845:850:858", "755:761:771:773", "761:771:773:781"),
  values.read1 = c(92,72,68,176,176), values.meth1=c(84,93,94,96,95),
  values.shannon=c(0.4,0.5,0.5,0.2,0.5), values.pdr=c(0.6,0.25,0.23,0.15,0.17),
  values.epipoly=c(0.48,0.42,0.38,0.27,0.3))
```

```
p2.GR<-GRanges(seqnames = Rle(c("chr22"), c(5)),
  ranges = IRanges(c(327,821,838,755,761), end = c(364,849,858,773,781)),
  strand = Rle(strand(c("-", "+", "+", "+", "-"))),
  values.loci = c("327:350:361:364", "821:837:844:849",
  "838:845:850:858", "755:761:771:773", "761:771:773:781"),
  values.read1 = c(107,102,102,76,76), values.meth1=c(88,66,69,71,94),
  values.shannon=c(0.12,0.25,0.54,0.23,0.25),
  values.pdr=c(0.38,1,0.97,1,0.13),
  values.epipoly=c(0.57,0.42,0.28,0.18,0.23))
```

```
N1.GR<-GRanges(seqnames = Rle(c("chr22"), c(5)),
  ranges = IRanges(c(327,821,838,755,761), end = c(364,849,858,773,781)),
  strand = Rle(strand(c("-", "+", "+", "+", "-"))),
  values.loci = c("327:350:361:364", "821:837:844:849",
  "838:845:850:858", "755:761:771:773", "761:771:773:781"),
  values.read1 = c(112,112,112,68,76), values.meth1=c(82,60,91,71,90),
  values.shannon=c(0.15,0.26,0.34,0.24,0.15),
  values.pdr=c(0.32,0.57,0.37,0.37,0.13),
  values.epipoly=c(0.57,0.42,0.28,0.38,0.23))
```

```
N2.GR<-GRanges(seqnames = Rle(c("chr22"), c(5)),
  ranges = IRanges(c(327,821,838,755,761), end = c(364,849,858,773,781)),
  strand = Rle(strand(c("-", "+", "+", "+", "-"))),
  values.loci = c("327:350:361:364", "821:837:844:849",
  "838:845:850:858", "755:761:771:773", "761:771:773:781"),
  values.read1 = c(385,78,70,96,96), values.meth1=c(96,81,87,87,93),
  values.pdr=c(0.15,0.52,0.48,0.25,0.25),
```

```

values.epipoly=c(0.26,0.58,0.58,0.37,0.37),
values.shannon=c(0.12,0.25,0.54,0.23,0.25))

GR.List<-list(p1=p1.GR,p2=p2.GR,N1=N1.GR,N2=N2.GR)
comp.Matrix <- compMatrix(epi.gr = GR.List, outprefix = NULL,
readNumber = 60, p = 1, cores = 1, sve = FALSE)

```

diffHet

*Calculate Differential Heterogeneity***Description**

From a user-inputted value and two subtype groups, calculates the mean values for both subtypes at each loci. The heterogeneity difference is calculated and the p-values and adjusted p-values are calculated if the heterogeneity difference is greater than a given cutoff

**Usage**

```

diffHet(compare.matrix, value, group1, group2, subtype,
het.dif.cutoff = 0.2, permutations = 1000, p.adjust.method = "fdr",
cores = 5)

```

**Arguments**

compare.matrix	The comparison matrix generated from the compMatrix() function
value	The value to be used in calculations. Possible values are 'read', 'pdr', 'meth', 'epipoly', and 'shannon'
group1	The first subtype group to be compared
group2	The second subtype group to be compared
subtype	A dataframe containing the subtype information for the samples in the comparison matrix. The row names should be the names of the samples and there should be one column containing the subtype information for each sample.
het.dif.cutoff	A number representing the cutoff for the heterogeneity difference. If the heterogeneity difference is greater than the cutoff value, then the p-value and adjusted p-value are calculated for the loci. If the heterogeneity difference is less than the cutoff value, then the p-value and adjusted p-value are set to NA. (default: 0.20)
permutations	The number of permutations for the EntropyExplorer function. Value must be set to 'shannon'. (default: 1000)
p.adjust.method	The method to be used as a parameter in p.adjust() function. Possible methods are 'holm', 'hochberg', 'hommel', 'bonferroni', 'BH', 'BY', 'fdr', and 'none'.(default: 'fdr')
cores	The number of cores to be used for parallel execution. Not available for 'shannon' values. (default: 5)

**Value**

A dataframe containing chromosome number, loci, mean of group1, mean of group2, heterogeneity difference, and the p-value and adjusted p-value for the loci with a heterogeneity difference greater than the cutoff

epiBox

*Make Boxplot from Comparison Matrix***Description**

From a user-inputted value, finds the mean of that value for each sample, then creates a boxplot comparing the values for each subtype.

**Usage**

```
epiBox(compare.matrix, value, type, box.colors = NULL,
        add.points = FALSE, points.colors = NULL, pdf.height = 10,
        pdf.width = 10, sve = FALSE)
```

**Arguments**

<code>compare.matrix</code>	The comparison matrix generated from the <code>compMatrix()</code> function
<code>value</code>	The value to be graphed in the boxplot. Possible values are 'read', 'pdr', 'meth', 'epipoly', and 'shannon'
<code>type</code>	A dataframe containing the type information for the samples in the comparison matrix. The row names should be the names of the samples and there should be one column containing the type information for each sample.
<code>box.colors</code>	A vector of colors to be used as the fill color for each boxplot. If not entered, the default colors of ggplot are used. (default: NULL)
<code>add.points</code>	A boolean stating if the individual points for each sample mean should be displayed over the box plots (default: FALSE)
<code>points.colors</code>	A vector of colors to be used as the color of the individual points for each sample mean. One color is used per subtype. (default: NULL)
<code>pdf.height</code>	An integer representing the height (in inches) of the outputted boxplot pdf file (default: 10)
<code>pdf.width</code>	An integer representing the width (in inches) of the outputted boxplot pdf file (default: 10)
<code>sve</code>	A boolean to save the plot (default: FALSE)

**Value**

a data frame containing the mean epigenetic heterogeneity for each sample

**Examples**

```
comp.Matrix<-data.frame(
  p1=c(0.6,0.3,0.5,0.5,0.5,0.6,0.45,0.57,0.45,0.63,0.58,0.67,0.5,0.42,0.67),
  p2=c(0.62,0.63,0.55,0.75,0.84,0.58,1,0.33,1,0.97,0.57,0.68,0.73,0.72,0.82),
  p3=c(0.72,0.53,0.62,0.69,0.37,0.85,1,0.63,0.87,0.87,0.82,0.81,0.79,
  0.62,0.68),
  N1=c(0.15,0.24,0.15,0.26,0.34,0.32,0.23,0.14,0.26,0.32,0.12,0.16,0.31,
  0.24,0.32),
  N2=c(0.32,0.26,0.16,0.36,0.25,0.37,0.12,0.16,0.41,0.47,0.13,0.52,0.42,
  0.41,0.23),
  N3=c(0.21,0.16,0.32,0.16,0.36,0.27,0.24,0.26,0.11,0.27,0.39,0.5,0.4,
```

```

0.31,0.33),
type=rep(c("pdr","epipoly","shannon"),c(5,5,5)),
location=rep(c("chr22-327:350:361:364","chr22-755:761:771:773",
"chr22-761:771:773:781","chr22-821:837:844:849","chr22-838:845:850:858"),
3),stringsAsFactors =FALSE )

subtype <- data.frame(Type= c(rep('CEBPA_sil', 3), rep('Normal', 3)),
row.names <- colnames(comp.Matrix)[1:6],stringsAsFactors = FALSE)

epiBox(compare.matrix = comp.Matrix, value = 'epipoly',
type <- subtype, box.colors = NULL, add.points = FALSE,
points.colors <- NULL, pdf.height = 10, pdf.width = 10,
sve = FALSE)

```

epiMA

*Make MA Plot***Description**

Creates an MA plot from the differential heterogeneity data calculated from the `diffHet()` function. For each loci, graphs the average of both group means on the x-axis and the heterogeneity difference on the y-axis. Graphs coordinates with significant adjusted p-values in red.

**Usage**

```
epiMA(pval.matrix, padjust.cutoff = 0.05, pch = ".", sve = FALSE)
```

**Arguments**

<code>pval.matrix</code>	The data frame returned from the <code>diffHet()</code> function that contains means, p-values, adjusted p-values, and heterogeneity difference
<code>padjust.cutoff</code>	The adjusted p-value cutoff to confirm a significant value. (default: 0.05)
<code>pch</code>	The plotting character to be used in the MA plot (default: '.')
<code>sve</code>	A boolean to save the plot (default: FALSE)

**Value**

A figure

**Examples**

```

diff.het.matrix<-data.frame(chromosome=c(rep("1",10)),
loci=paste("loci",seq_len(10),sep="-"),subtype.mean=c(0.21,0.23,0.37,0.26,
0.29,0.31,0.29,0.13,0.12,0.093),Normal.mean=c(0.01,0.01,0.01,0.02,
0.02,0.01,0.01,0.79,0.73,0.79),het.dif=c(0.20,0.220,0.360,0.240,0.270,
0.300,0.280,-0.660,-0.610,-0.697),p.value=c(3.08e-03,1.43e-02,9.27e-03,
3.45e-02,2.99e-02,3.68e-02, 4.60e-02, 5.65e-10, 9.18e-10,
9.98e-11),p.adjust=c(8.84e-03,2.76e-02, 2.04e-02, 5.01e-02,
4.56e-02, 5.24e-02, 6.08e-02, 3.74e-08, 5.22e-08,
1.06e-08),type=rep("pdr",10))

epiMA(pval.matrix = diff.het.matrix, padjust.cutoff = 0.05,
pch = ".", sve = TRUE)

```

epiMap

*Make Pheatmap from Comparison Matrix***Description**

Creates a pheatmap for the top 'loci.percent' of values of max standard deviation from the comparison matrix generated by compMatrix(). The rows represent the loci of the epiallele and the columns represent the sample names. The columns can be annotated by adding annotation information as a parameter.

**Usage**

```
epiMap(compare.matrix, value, annotate,
        clustering_distance_rows = "euclidean",
        clustering_distance_cols = "euclidean",
        clustering_method = "complete", annotate.colors = NA,
        color = colorRampPalette(c("blue", "white", "red"))(1000),
        loci.percent = 0.1, show.rows = FALSE, show.columns = FALSE,
        font.size = 6, pdf.height = 10, pdf.width = 10, sve = FALSE, ...)
```

**Arguments**

compare.matrix	The comparison matrix generated from the compMatrix() function
value	The value to be graphed in the pheatmap. Possible values are 'read', 'pdr', 'meth', 'epipoly', and 'shannon'.
annotate	A dataframe containing the annotation information for the columns of the pheatmap. The row names must be the names of the samples. The columns (any number) are the annotations. E.g. a column called 'TET2' with factors 'Pos' and 'Neg' for each sample that is positive or negative for the TET2 gene
clustering_distance_rows	Distance measure used in clustering rows.
clustering_distance_cols	Distance measure used in clustering columns.
clustering_method	clustering method used.
annotate.colors	A list containing the colors for the annotation information. Each element in the list is a vector of colors with names that correspond to the columns of 'annotate'.
color	a vector of colors used in heatmap.
loci.percent	The top percentage of loci, as a decimal, to be displayed on the pheatmap based on standard deviation, e.g. a value of 0.20 is equivalent to the top 20% of loci (default: 0.10)
show.rows	A boolean stating if the row names should be displayed on the pheatmap (default: FALSE)
show.columns	A boolean stating if the column names should be displayed on the pheatmap (default: FALSE)
font.size	An integer representing the font size to be used for the pheatmap labels (default: 6)



pdf.height	An integer representing the height (in inches) of the pdf file for the pheatmap (default: 10)
pdf.width	An integer representing the width (in inches) of the pdf file for the pheatmap (default: 10)
sve	A boolean to save the plot (default: FALSE)
...	any arguments in the function pheatmap()

**Value**

A pheatmap object that contains the tree data for both rows and columns and the final pheatmap plot

**Examples**

```
comp.Matrix<-data.frame(
p1=c(0.6,0.3,0.5,0.5,0.5,0.6,0.45,0.57,0.45,0.63,0.58,0.67,0.5,0.42,0.67),
p2=c(0.62,0.63,0.55,0.75,0.84,0.58,1,0.33,1,0.97,0.57,0.68,0.73,0.72,0.82),
p3=c(0.72,0.53,0.62,0.69,0.37,0.85,1,0.63,0.87,0.87,0.82,0.81,0.79,
0.62,0.68),
N1=c(0.15,0.24,0.15,0.26,0.34,0.32,0.23,0.14,0.26,0.32,0.12,0.16,0.31,
0.24,0.32),
N2=c(0.32,0.26,0.16,0.36,0.25,0.37,0.12,0.16,0.41,0.47,0.13,0.52,0.42,
0.41,0.23),
N3=c(0.21,0.16,0.32,0.16,0.36,0.27,0.24,0.26,0.11,0.27,0.39,0.5,0.4,
0.31,0.33),
type=rep(c("pdr","epipoly","shannon"),c(5,5,5)),
location=rep(c("chr22-327:350:361:364","chr22-755:761:771:773",
"chr22-761:771:773:781","chr22-821:837:844:849","chr22-838:845:850:858"),
3),stringsAsFactors =FALSE )

subtype <- data.frame(Type= c(rep('CEBPA_sil', 3), rep('Normal', 3)),
row.names = colnames(comp.Matrix)[1:6],stringsAsFactors = FALSE)

pmap <- epiMap(compare.matrix = comp.Matrix,
value = 'epipoly',annotate = subtype,
clustering_distance_rows = "euclidean",
clustering_distance_cols = "euclidean",
clustering_method = "complete",annotate.colors = NA,
color= colorRampPalette(c("blue","white","red"))(1000),
loci.percent = 1, show.rows = FALSE,
show.columns = TRUE, font.size = 15,
pdf.height = 10, pdf.width = 10, sve = TRUE)
```

**Description**

Construct co-epihet network for DEH loci or for genes with genome region containing DEH loci using WGCNA and identify modules that are significantly associated with the measured clinical traits for co-epihet DEH loci network,we identify genes with genome region containing DEH loci in each module.

**Usage**

```
epiNetwork(node.type = "locus", DEH, compare.matrix, value = "pdr",
           group, subtype, datTraits = NULL, annotation.obj,
           networktype = "signed", method = "pearson", prefix = NULL,
           mergeCutHeight = 0.25, minModuleSize = 30)
```

**Arguments**

node.type	a character suggest node type in network. Possible values are 'locus','gene' (default:locus)
DEH	the dataframe containing the chromosome number, loci and strand information of DEH loci generated from diffHet() function
compare.matrix	The comparison matrix generated from the compMatrix() function.
value	A character, which is used to identify DEH loci. Possible values are 'pdr', 'epipoly',and 'shannon'(default:pdr)
group	The subtype group to be used to construct network
subtype	A dataframe containing the subtype information for the samples in the comparison matrix. The row names should be the names of the samples and there should be one column containing the subtype information for each sample
datTraits	a dataframe containing the clinical traits for all patients from the subtype group
annotation.obj	a GRanges object containing gene annotation information
networktype	network type in WGCNA (default:signed)
method	character string specifying the correlation to be used in WGCNA (default:pearson)
prefix	character string containing the file name base for files containing the consensus topological overlaps in WGCNA
mergeCutHeight	a numeric, dendrogram cut height for module merging (default: 0.25)
minModuleSize	a numeric, minimum module size for module detection in WGCNA (default: 30)

**Value**

a list, if node type is CpG site, it contains the epigenetic heterogeneity matrix for patients module information, gene.list which is a data frame containing genes with genome region containing DEH loci from one module if node type is gene,it contains the epigenetic heterogeneity matrix for patients and module information.

---

epiPathway

*pathway annotation*


---

**Description**

pathway identification significantly enriched by genes in one module.

**Usage**

```
epiPathway(gene.list, cutoff = 0.05, prefix = NA, pdf.height = 10,
           pdf.width = 10)
```

**Arguments**

<code>gene.list</code>	a data frame generated from <code>network.construct()</code> function. The first column is gene entrez ID, the second column is module lable, the third column is module color
<code>cutoff</code>	Cutoff value of pvalue for pathway enrichment (default:0.05)
<code>prefix</code>	a prefix for PDF file name
<code>pdf.height</code>	An integer representing the height (in inches) of the outputted boxplot pdf file (default: 10)
<code>pdf.width</code>	An integer representing the width (in inches) of the outputted boxplot pdf file (default: 10)

**Value**

a data frame containing pathways that are significantly enriched by genes from one module

**Examples**

```
genelist<-data.frame(ENTREZID=c("2902", "2905", "3360", "286223", "59338",
"344018", "5144", "55001", "7410", "730051", "55743", "6804", "200634", "2802",
"2260", "651", "2104", "23432", "10505", "23194", "9855", "7101",
"389136", "124857", "1829", "3164", "3754", "8614", "9469", "3217", "9578",
"10516", "10630"),label=rep(18,33),color=rep("lightgreen",33),
stringsAsFactors = FALSE)
pathway <- epihet::epiPathway(genelist,cutoff = 0.05,
                             prefix="CEBPA_sil",pdf.height = 10,
                             pdf.width = 10)
```

epiPCA

*Make PCA Plot from Comparison Matrix***Description**

From a user-inputted value, creates a PCA plot from the sample data and colors each point by the subtype information provided.

**Usage**

```
epiPCA(compare.matrix, value, type, points.colors = NULL,
        frames = FALSE, frames.colors = NULL, probability = FALSE,
        pdf.height = 10, pdf.width = 10, sve = FALSE)
```

**Arguments**

<code>compare.matrix</code>	The comparison matrix generated from the <code>compMatrix()</code> function
<code>value</code>	The value to be graphed in the PCA plot
<code>type</code>	A dataframe containing the type information for the samples in the comparison matrix. The row names should be the names of the samples and there should be one column containing the type information for each sample.
<code>points.colors</code>	A vector to be used as the color of the individual points for each sample. One color is used per type. the names of vector is the types(default: NULL)

frames	A boolean stating if the frames should be drawn around the points for each subtype cluster. (default: False)
frames.colors	A vector of colors to be used as the color of the frames for each subtype cluster. (default: NULL)
probability	A boolean stating if the frames should be drawn as probability ellipses around the points for each subtype cluster. Both 'probability' and 'frames' must be set to TRUE to have effect. (default: False)
pdf.height	An integer representing the height (in inches) of the outputted PCA plot pdf file (default: 10)
pdf.width	An integer representing the width (in inches) of the outputted PCA plot pdf file (default: 10)
sve	A boolean to save the plot (default: FALSE)

**Value**

A PCA plot

**Examples**

```
library(ggfortify)
comp.Matrix<-data.frame(
p1 = c(0.6,0.3,0.5,0.5,0.5,0.6,0.45,0.57,0.45,0.63,0.58,0.67,0.5,0.42,0.67),
p2 = c(0.62,0.63,0.55,0.75,0.84,0.58,1,0.33,1,0.97,0.57,0.68,0.73,0.72,0.82),
p3 = c(0.72,0.53,0.62,0.69,0.37,0.85,1,0.63,0.87,0.87,0.82,0.81,0.79,
0.62,0.68),
N1=c(0.15,0.24,0.15,0.26,0.34,0.32,0.23,0.14,0.26,0.32,0.12,0.16,0.31,
0.24,0.32),
N2=c(0.32,0.26,0.16,0.36,0.25,0.37,0.12,0.16,0.41,0.47,0.13,0.52,0.42,
0.41,0.23),
N3=c(0.21,0.16,0.32,0.16,0.36,0.27,0.24,0.26,0.11,0.27,0.39,0.5,0.4,
0.31,0.33),
type=rep(c("pdr", "epipoly", "shannon"),c(5,5,5)),
location=rep(c("chr22-327:350:361:364", "chr22-755:761:771:773",
"chr22-761:771:773:781", "chr22-821:837:844:849", "chr22-838:845:850:858"),
3),stringsAsFactors =FALSE )

subtype <- data.frame(Type= c(rep('CEBPA_sil', 3), rep('Normal', 3)),
row.names = colnames(comp.Matrix)[1:6],stringsAsFactors = FALSE)

epiPCA(compare.matrix = comp.Matrix, value = 'epipoly',
        type = subtype, points.colors = NULL,
        frames = FALSE, frames.colors = NULL,
        probability = FALSE, pdf.height = 10,
        pdf.width = 10, sve = TRUE)
```

---

epiTSNE

---

*Make TSNE Plot from Comparison Matrix*


---

**Description**

From a user-inputted value, creates a TSNE plot from the sample data and colors each point by the subtype information provided.

**Usage**

```
epiTSNE(compare.matrix, value, type, points.colors = NULL, theta = 0.5,
         curTheme = NULL, perplexity = 5, max_iter = 1000,
         pdf.height = 10, pdf.width = 10, sve = FALSE)
```

**Arguments**

<code>compare.matrix</code>	The comparison matrix generated from the <code>compMatrix()</code> function
<code>value</code>	The value to be graphed in the PCA plot
<code>type</code>	A dataframe containing the type information for the samples in the comparison matrix. The row names should be the names of the samples and there should be one column containing the type information for each sample.
<code>points.colors</code>	A vector of colors to be used as the color of the individual points for each sample. One color is used per subtype. (default: NULL)
<code>theta</code>	A decimal representing the theta parameter for the <code>Rtsne()</code> function. Represents the speed/accuracy trade-off (0.0 is exact TSNE) (default: 0.5)
<code>curTheme</code>	the theme of <code>ggplot2</code> to control control the appearance of all non-data components of the plot
<code>perplexity</code>	An integer representing the perplexity parameter for the <code>Rtsne()</code> function (default: 30)
<code>max_iter</code>	An integer representing the <code>max_iter</code> parameter for the <code>Rtsne()</code> function. Represents the number of iterations (default: 1000)
<code>pdf.height</code>	An integer representing the height (in inches) of the outputted TSNE plot pdf file (default: 10)
<code>pdf.width</code>	An integer representing the width (in inches) of the outputted TSNE plot pdf file (default: 10)
<code>sve</code>	A boolean to save the plot (default: FALSE)

**Value**

A T-SNE plot

**Examples**

```
comp.Matrix<-data.frame(
  p1=c(0.6,0.3,0.5,0.5,0.6,0.45,0.57,0.45,0.63,0.58,0.67,0.5,0.42,0.67),
  p2=c(0.62,0.63,0.55,0.75,0.84,0.58,1,0.33,1,0.97,0.57,0.68,0.73,0.72,0.82),
  p3=c(0.72,0.53,0.62,0.69,0.37,0.85,1,0.63,0.87,0.87,0.82,0.81,0.79,
    0.62,0.68),
  N1=c(0.15,0.24,0.15,0.26,0.34,0.32,0.23,0.14,0.26,0.32,0.12,0.16,0.31,
    0.24,0.32),
  N2=c(0.32,0.26,0.16,0.36,0.25,0.37,0.12,0.16,0.41,0.47,0.13,0.52,0.42,
    0.41,0.23),
  N3=c(0.21,0.16,0.32,0.16,0.36,0.27,0.24,0.26,0.11,0.27,0.39,0.5,0.4,
    0.31,0.33),
  type=rep(c("pdr","epipoly","shannon"),c(5,5,5)),
  location=rep(c("chr22-327:350:361:364","chr22-755:761:771:773",
    "chr22-761:771:773:781","chr22-821:837:844:849","chr22-838:845:850:858"),
    3),stringsAsFactors =FALSE )

subtype <- data.frame(Type= c(rep('CEBPA_sil', 3), rep('Normal', 3)),
```

```
row.names = colnames(comp.Matrix)[1:6], stringsAsFactors = FALSE)

epiTSNE(compare.matrix = comp.Matrix, value = 'epipoly',
type = subtype, points.colors = NULL, theta = 0.5,
perplexity = 1, max_iter = 1000, pdf.height = 10,
pdf.width = 10, sve = TRUE)
```

---

jaccard *Jaccard score calculation between modules from two subtypes.*

---

### Description

Jaccard score calculation based on the common genes in two modules from two subtypes.

### Usage

```
jaccard(module.subtype1, module.subtype2)
```

### Arguments

module.subtype1

a data frame generated from the epiNetwork() function. The module information of subtype1, the first column is module nodes, the second column is module label, the third column is module color.

module.subtype2

a data frame generated from the epiNetwork() function. The module information of subtype1, the first column is module nodes, the second column is module label, the third column is module color.

### Value

A matrix containing Jaccard scores.

### Examples

```
data(modulesil)
data(moduledm)
jaccard.matrix <- jaccard(modulesil, moduledm)
```

---

makeGR *Make List of GenomicRanges Object*

---

### Description

Creates a GenomicRanges object for each methclone output file

### Usage

```
makeGR(files, ids, cores = 5, sve = FALSE)
```

**Arguments**

files	A vector of input files containing methclone output files, the suffix of files should be methClone_out.gz
ids	A vector of sample ids for the files
cores	The number of cores to be used for parallel execution (default: 5)
sve	A boolean to save the GenomicRanges object (default: FALSE)

**Value**

A list, each element is a data frame of GenomicRanges objects containing pdr, epipolymorphism, and Shannon entropy values for each input file. Saves as an epi.gr.rda extension

**Examples**

```
path <- system.file('extdata', package = 'epihet')
files <- dir(path = path, pattern = 'methClone_out.gz',
            recursive = TRUE, full.names = TRUE)
ids <- basename(dirname(files))
GR.List <- epihet::makeGR(files = files, ids = ids,
                        cores = 1, sve = FALSE)
```

---

 moduleAnno

*module annotation*


---

**Description**

annotate modules using differentially expressed genes

**Usage**

```
moduleAnno(DEG, background, module.gene, cutoff = 0.05,
            adjust.method = "fdr", prefix = NA, pdf.height = 10,
            pdf.width = 10, sve = FALSE)
```

**Arguments**

DEG	a character vector containing up/down regulated genes
background	a character vector containing all genes as background in hypergeometric test
module.gene	a data frame containing genes with genome region containing DEH loci from one module, generated from epiNetwork() function. The first column is gene entrez ID, the second column is module label, the third column is module color
cutoff	Cutoff value of qvalue for gene enrichment (default: 0.05)
adjust.method	one of 'holm', 'hochberg', 'hommel', 'bonferroni', 'BH', 'BY', 'fdr', 'none' (default: fdr)
prefix	a prefix for PDF file name
pdf.height	An integer representing the height (in inches) of the outputted boxplot pdf file (default: 10)
pdf.width	An integer representing the width (in inches) of the outputted boxplot pdf file (default: 10)
sve	A boolean to save the plot (default: FALSE)

**Value**

a data frame showing modules that were enriched by DEGs and module size, p value and q value

**Examples**

```
data(DEG,package = "epihet")
data(background,package = "epihet")
module<-data.frame(gene=c("NM_000014", "NM_000015", "NM_000017", "NM_000019",
"NM_052960", "NR_138250", "NM_000037", "NM_000038", "NM_000039", "NM_000044",
"NM_000046", "NM_015074", "NM_183416", "NM_004421", "NM_001330311",
"NM_001145210", "NM_000097", "NM_000103", "NM_000104",
"NM_000079", "NM_000083", "NM_000086", "NM_000087", "NM_000092", "NM_000094",
"NM_000095", "NM_006474"),label=rep(c(1,2),c(12,15)),
color=rep(c("purple","brown"),c(12,15)),
stringsAsFactors = FALSE)
module.annotation<-epihet::moduleAnno(DEG$refseq,background$gene,
                                         module.gene=module,
                                         cutoff=0.05,adjust.method = "fdr",
                                         prefix='epipoly',pdf.height = 10,
                                         pdf.width = 10, sve = TRUE)
```

---

moduleSim

*module comparison between two subtypes*

---

**Description**

Compare any two modules from two subtypes based on genes shared by the modules

**Usage**

```
moduleSim(module.subtype1, module.subtype2, pdf.height = 10,
           pdf.width = 10, sve = FALSE)
```

**Arguments**

module.subtype1	a data frame generated from the epiNetwork() function the module information of subtype1,the first column is module nodes,the second column is module label, the third column is module color
module.subtype2	a data frame generated from the epiNetwork() function. The module information of subtype1, the first column is module nodes, the second column is module label, the third column is module color
pdf.height	An integer representing the height (in inches) of the outputted boxplot pdf file (default: 10)
pdf.width	An integer representing the width (in inches) of the outputted boxplot pdf file (default: 10)
sve	A boolean to save the plot (default: FALSE)

**Value**

a matrix containing Jaccard scores



**Examples**

```
data(modulesil,package = "epihet")
data(moduledm,package = "epihet")
sim.score<-epihet::moduleSim(module.subtype1=modulesil,
                             module.subtype2=moduledm,
                             pdf.height = 10,pdf.width = 10,
                             sve = TRUE)
```

moduleVisual

*Modules visualization and network topology***Description**

Visualize the modules identified by epiNetwork() function, and calculate network topology

**Usage**

```
moduleVisual(TOM, value.matrix, moduleColors, mymodule, cutoff = 0.02,
             prefix = NULL, sve = FALSE)
```

**Arguments**

TOM	the topological overlap matrix in WGCNA generated from the epiNetwork() function
value.matrix	A data frame generated from the epiNetwork() function. the row name is patients in one subtype. the column name is the DEH loci the value in the matrix is epigenetic heterogeneity on one DEH loci for one patient
moduleColors	the module assignment generated from the epiNetwork() function
mymodule	a character vector containing the module colors you want to visualize
cutoff	adjacency threshold for including edges in the output (default:0.02)
prefix	a character for output filename
sve	A boolean to save the plot (default: FALSE)

**Value**

a list containing all module edge and node information for mymodule

**Examples**

```
correlation.m<-matrix(0,12,12)
correlation.m[1,c(2:10)]<-c(0.006,0.054,0.079,0.078, 0.011,0.033,0.014,
0.023,0.034)
correlation.m[2,c(3:10)]<-c(0.026,0.014,0.045,0.037, 0.026,0.011,0.034,
0.012)
correlation.m[3,c(4:10)]<-c(0.016,0.024,0.039,0.045, 0.009,0.003,0.028)
correlation.m[4,c(5:10)]<-c(0.039,0.002,0.053,0.066, 0.012,0.039)
correlation.m[5,c(6:10)]<-c(0.019,0.016,0.047,0.046, 0.013)
correlation.m[6,c(7:10)]<-c(0.017,0.057,0.029,0.056)
correlation.m[7,c(8:10)]<-c(0.071,0.018,0.001)
correlation.m[8,c(9:10)]<-c(0.046,0.014)
correlation.m[9,10]<-0.054
```

```

correlation.m[lower.tri(correlation.m)] <-
t(correlation.m)[lower.tri(correlation.m)]

matrix.v<-matrix(0.5,5,12)
matrix.v<-as.data.frame(matrix.v)
colnames(matrix.v)<-c("NM_052960", "NR_138250", "NM_015074", "NM_183416",
"NM_017891", "NM_001330306", "NM_014917", "NM_001312688", "NM_001330665",
"NM_017766", "NM_001079843", "NM_001040709")
modulecolor<-c(rep(c("yellow", "cyan"),c(10,2)))
module.topology<-epihet::moduleVisual(correlation.m,
                                     value.matrix=matrix.v,
                                     moduleColors=modulecolor,
                                     mymodule="yellow",cutoff=0.02,
                                     prefix='CEBPA_sil_epipoly',sve = TRUE)

```

---

readGR

*Make GenomicRanges Object*


---

## Description

Creates a GenomicRanges file for a singular methclone ouput file

## Usage

```
readGR(files, ids, n)
```

## Arguments

files	A vector of files containing methclone output
ids	A vector of sample ids for the files
n	The index of the file vector to be read

## Value

A GenomicRanges object containing pdr, epipolymorphism, and Shannon entropy values for the nth file

## Examples

```

files <- c(system.file("extdata", "D-2238.chr22.region.methClone_out.gz", package = "epihet"),
system.file("extdata", "D-2668.chr22.region.methClone_out.gz", package = "epihet"),
system.file("extdata", "N-1.chr22.region.methClone_out.gz", package = "epihet"),
system.file("extdata", "N-2.chr22.region.methClone_out.gz", package = "epihet"))
ids <- epihet::splitn(basename(files), "[.]", 1)
GR.Object <- epihet::readGR(files = files, ids = ids, n = 3)

```

---

shannon	<i>Shannon Entropy</i>
---------	------------------------

---

**Description**

Calculates the Shannon entropy value

**Usage**

```
shannon(p)
```

**Arguments**

p                    A vector of epiallele probabilities

**Value**

The Shannon entropy value

**Examples**

```
a<-c(rep(0,13),60.86960,0.00000,39.1304)
shannon(a)
```

---

splitn	<i>The subtring extraction of a character vector</i>
--------	--

---

**Description**

Extract the substrings of a character vector according to the matches to substring split within them.

**Usage**

```
splitn(strings, field, n)
```

**Arguments**

strings            A GenomicRanges object to be compared  
field                A GenomicRanges object to be compared  
n                    The value of gr1 to be compared

**Value**

A data frame containing a summary of the GenomicRanges object

**Examples**

```
x<-'chr1:10000-10005'
splitn(x, ':', 1)
```

summarize

*Summarize Data***Description**

Summarizes pdr, epipolymorphism, and shannon values over the annotation regions

**Usage**

```
summarize(gr1, gr2, value1, value2, cutoff1 = 10, cutoff2 = 60)
```

**Arguments**

gr1	A GenomicRanges object to be compared
gr2	A GenomicRanges object to be compared
value1	The value of gr1 to be compared
value2	The value of gr2 be compared
cutoff1	The first cutoff value for the number of reads (default:10)
cutoff2	The second cutoff value for the number of reads (default:60)

**Value**

A data frame containing a summary of the GenomicRanges object

**Examples**

```
p1.GR<-GRanges(seqnames = Rle(c("chr22"), c(5)),
  ranges = IRanges(c(327,821,838,755,761), end = c(364,849,858,773,781)),
  strand = Rle(strand(c("-", "+", "+", "+", "-"))),
  values.loci = c("327:350:361:364", "821:837:844:849",
    "838:845:850:858", "755:761:771:773", "761:771:773:781"),
  values.read1 = c(92,72,68,176,176), values.meth1=c(84,93,94,96,95),
  values.shannon=c(0.4,0.5,0.5,0.2,0.5), values.pdr=c(0.6,0.25,0.23,0.15,0.17),
  values.epipoly=c(0.48,0.42,0.38,0.27,0.3))
```

```
p2.GR<-GRanges(seqnames = Rle(c("chr22"), c(5)),
  ranges = IRanges(c(327,821,838,755,761), end = c(364,849,858,773,781)),
  strand = Rle(strand(c("-", "+", "+", "+", "-"))),
  values.loci = c("327:350:361:364", "821:837:844:849",
    "838:845:850:858", "755:761:771:773", "761:771:773:781"),
  values.read1 = c(107,102,102,76,76), values.meth1=c(88,66,69,71,94),
  values.shannon=c(0.12,0.25,0.54,0.23,0.25),
  values.pdr=c(0.38,1,0.97,1,0.13),
  values.epipoly=c(0.57,0.42,0.28,0.18,0.23))
```

```
GR.List<-list(p1=p1.GR,p2=p2.GR)
summary <- summarize(gr1 = GR.List[[1]], gr2 = GR.List[[2]],
  value1 = 'pdr', value2 = 'epipoly',
  cutoff1 = 10, cutoff2 = 60)
```

---

userobj	<i>GenomicRanges object generation</i>
---------	--

---

**Description**

generate GenomicRanges object for DEH loci

**Usage**

```
userobj(data)
```

**Arguments**

data	a data frame containing the chromosome number, loci and strand information of DEH loci generated from diffHet() function.
------	---

**Value**

A GenomicRanges object

**Examples**

```
data<-data.frame(chromosome=c('chr1','chr1','chr1'),
  loci=c('6480531:6480554:6480561:6480565','6647655:6647696:6647701:6647705',
  '7130155:7130172:7130179:7130188'),
  strand=c('+','-','+'),stringsAsFactors = FALSE)
userobj(data)
```

# Index

## \*Topic **datasets**

background, [2](#)

background, [2](#)

compMatrix, [3](#)

datTraits (background), [2](#)

DEG (background), [2](#)

DEH (background), [2](#)

diffHet, [5](#)

diffhetmatrix (background), [2](#)

epiBox, [6](#)

epiMA, [7](#)

epiMap, [8](#)

epiNetwork, [9](#)

epiPathway, [10](#)

epiPCA, [11](#)

epiTSNE, [12](#)

jaccard, [14](#)

makeGR, [14](#)

moduleAnno, [15](#)

moduledm (background), [2](#)

modulesil (background), [2](#)

moduleSim, [16](#)

moduleVisual, [17](#)

promoter (background), [2](#)

readGR, [18](#)

shannon, [19](#)

sharedmatrix (background), [2](#)

splitn, [19](#)

summarize, [20](#)

userobj, [21](#)