Package 'vulcan'

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Description Vulcan (VirtUaL ChIP-Seq Analysis through Networks) is a package that interrogates gene regulatory networks to infer cofactors significantly enriched in a differential binding signature coming from ChIP-Seq data.
In order to do so, our package combines strategies from different BioConductor packages: DESeq for data normalization, ChIPpeakAnno and DiffBind for annotation and definition of ChIP-Seq genomic peaks, csaw to define optimal peak width and viper for applying a regulatory network over a differential binding signature.

License LGPL-3

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`average_fragment_length` *Define the average fragment length*

Description

A function to get average fragment length from a ChIP-Seq experiment

Usage

```
average_fragment_length(bam.files, plot = TRUE, max.dist = 550)
```

Arguments

`bam.files`	a vector of BAM files locations
`plot`	logical. Should a plot be generated?
`max.dist`	numeric. Maximum fragment length accepted. Default=550

Value

nothing

Examples

```
library(vulcandata)
sheetfile<-'deleteme.csv'
vulcandata::vulcansheet(sheetfile)
a<-read.csv(sheetfile,as.is=TRUE)
bams<-a$bamReads
unlink(sheetfile)
average_fragment_length(bams,plot=TRUE)
```

---

corr2p *Convert correlation coefficient to p-value*

Description

This functions converts an R value from a correlation calculation into a p-value, using a T distribution with the provided number of samples N minus 2 degrees of freedom

Usage

```
corr2p(r, N)
```

Arguments

r	a correlation coefficient
N	a number of samples

Value

p a p-value

Examples

```
set.seed(1)
a<-rnorm(1000)
b<-a+rnorm(1000,sd=10)
r<-cor(a,b,method='pearson')
corr2p(r,N=length(a))
```

densityauc *densityauc - Calculate the AUC of a density object*

Description

This function will calculate the AUC of a density object generated by the 'density' function.

Usage

```
densityauc(dens, window)
```

Arguments

dens	a density object
window	a vector with two values, specifying the left and right borders for the AUC to be calculated

Value

a numeric value for the density AUC

Examples

```
set.seed(1)
a<-rnorm(1000)
d<-density(a)
window<-c(2,3)
da<-densityauc(d,window)

plot(d,main='')
abline(v=window,lty=2)
title(paste0('AUC between lines=',da))
```

dpareto *Probability density of Pareto distributions*

Description

Gives NA on values below the threshold

Usage

```
dpareto(x, threshold = 1, exponent, log = FALSE)
```

Arguments

x	Data vector of log probability densities
threshold	numeric value to define the start of the tail
exponent	the exponent obtained from the pareto.fit function
log	logical, should the values be log-transformed?

Value

Vector of (log) probability densities

Examples

```
set.seed(1)
x<-abs(rnorm(1000))
n<-length(x)
exponent<-1+n/sum(log(x))
dp<-dpareto(x,exponent=exponent)
plot(dp)
```

---

fisherp *Fisher integration of p-values*

Description

This function applies the Fisher integration of pvalues

Usage

```
fisherp(ps)
```

Arguments

ps	a vector of p-values

Value

p.val an integrated p-value

Examples

```
ps<-c(0.01,0.05,0.03,0.2)
fisherp(ps)
```

gsea *GSEA*

Description

This function performs Gene Set Enrichment Analysis

Usage

```
gsea(
  reflist,
  set,
  method = c("permutation", "pareto"),
  np = 1000,
  w = 1,
  gsea_null = NULL
)
```

Arguments

reflist	named vector of reference scores
set	element set
method	one of 'permutation' or 'pareto'
np	Number of permutations (Default: 1000)
w	exponent used to raise the supplied scores. Default is 1 (original scores unchanged)
gsea_null	a GSEA null distribution (Optional)

Value

A GSEA object. Basically a list of s components:

ES The enrichment score

NES The normalized enrichment socre

ledge The items in the leading edge

p.value The permutation-based p-value

Examples

```
reflist<-setNames(-sort(rnorm(1000)),paste0('gene',1:1000))
set<-paste0('gene',sample(1:200,50))
obj<-gsea(reflist,set,method='pareto',np=1000)
obj$p.value
```

kmgformat *kmgformat - Nice Formatting of Numbers*

Description

This function will convert thousand numbers to K, millions to M, billions to G, trillions to T, quadrillions to P

Usage

```
kmgformat(input, roundParam = 1)
```

Arguments

input	A vector of values
roundParam	How many decimal digits you want

Value

A character vector of formatted numebr names

Examples

```
# Thousands
set.seed(1)
a<-runif(1000,0,1e4)
plot(a,yaxt='n')
kmg<-kmgformat(pretty(a))
axis(2,at=pretty(a),labels=kmg)

# Millions to Billions
set.seed(1)
a<-runif(1000,0,1e9)
plot(a,yaxt='n',pch=20,col=val2col(a))
kmg<-kmgformat(pretty(a))
axis(2,at=pretty(a),labels=kmg)
```

null_gsea *Calculate Null Distribution for GSEA*

Description

This function generates a GSEA null distribution from

Usage

```
null_gsea(set, reflist, w = 1, np = 1000)
```

Arguments

set	A vector containing gene names.
reflist	A named vector containing the weights of the entire signature
w	exponent used to raise the supplied scores. Default is 1 (original scores unchanged)
np	Number of permutations (Default: 1000)

Value

A vector of null scores appropriate for the set/reflist combination provided

Examples

```
reflist<-setNames(-sort(rnorm(26)),LETTERS)
set<-c('A','B','D','F')
nulldist<-null_gsea(set,reflist)
nulldist[1:10]
```

---

p2corr *Convert p-value to correlation coefficient*

Description

This functions converts an p-value into a the corresponding correlation coefficient, using a T distribution with the provided number of samples N minus 2 degrees of freedom

Usage

```
p2corr(p, N)
```

Arguments

p	a p-value
N	a number of samples

Value

r a correlation coefficient

Examples

```
N<-100
p<-0.05
p2corr(p,N)
```

p2z *p2z*

Description

This function gives a gaussian Z-score corresponding to the provided p-value Careful: sign is not provided

Usage

```
p2z(p)
```

Arguments

p	a p-value

Value

z a Z score

Examples

```
p<-0.05
p2z(p)
```

pareto.fit *Estimate parameters of Pareto distribution*

Description

A wrapper for functions implementing actual methods

Usage

```
pareto.fit(data, threshold)
```

Arguments

data	data vector, lower threshold (or 'find', indicating it should be found from the data), method (likelihood or regression, defaulting to former)
threshold	numeric value to define the start of the tail

Value

List indicating type of distribution ('pareto'), parameters, information about fit (depending on method), OR a warning and NA if method is not recognized

Examples

```
# Estimate the tail of a pospulation normally distributed
set.seed(1)
x<-rnorm(1000)
q95<-as.numeric(quantile(abs(x),0.95))
fit<-pareto.fit(abs(x),threshold=q95)
# We can infer the pvalue of a value very much right to the tail of the
# distribution
value<-5
pvalue<-ppareto(value, threshold=q95, exponent=fit$exponent,
lower.tail=FALSE)/20
plot(density(abs(x)),xlim=c(0,value+0.3),main='Pareto fit')
arrows(value,0.2,value,0)
text(value,0.2,labels=paste0('p=',signif(pvalue,2)))
```

---

plot_gsea *Plot GSEA results*

Description

This function generates a GSEA plot from a gsea object

Usage

```
plot_gsea(
  gsea.obj,
  twoColors = c("red", "blue"),
  plotNames = FALSE,
  colBarcode = "black",
  title = "Running Enrichment Score",
  bottomYtitle = "List Values",
  bottomYlabel = "Signature values",
  ext_nes = NULL,
  omit_middle = FALSE
)
```

Arguments

gsea.obj	GSEA object produced by the gsea function
twoColors	the two colors to use for positive[1] and negative[2] enrichment scores
plotNames	Logical. Should the set names be plotted?
colBarcode	The color of the barcode
title	String to be plotted above the Running Enrichment Score
bottomYtitle	String for the title of the bottom part of the plot
bottomYlabel	String for the label
ext_nes	Provide a NES from an external calculation
omit_middle	If TRUE, will not plot the running score (FALSE by default)

Value

Nothing, a plot is generated in the default output device

Examples

```
reflist<-setNames(-sort(rnorm(26)),LETTERS)
set<-c('A','B','D','F')
obj<-gsea(reflist,set,method='pareto')
plot_gsea(obj)
```

---

ppareto *Cumulative distribution function of the Pareto distributions ' Gives NA on values < threshold*

Description

Cumulative distribution function of the Pareto distributions ' Gives NA on values < threshold

Usage

```
ppareto(x, threshold = 1, exponent, lower.tail = TRUE)
```

Arguments

x	Data vector, lower threshold, scaling exponent, usual flags
threshold	numeric value to define the start of the tail
exponent	the exponent obtained from the pareto.fit function
lower.tail	logical. If the lower tail of the distribution should be considered. Default is TRUE

Value

Vector of (log) probabilities

Examples

```
# Estimate the tail of a pospulation normally distributed
set.seed(1)
x<-rnorm(1000)
q95<-as.numeric(quantile(abs(x),0.95))
fit<-pareto.fit(abs(x),threshold=q95)
# We can infer the pvalue of a value very much right to the tail of the
# distribution
value<-5
pvalue<-ppareto(value, threshold=q95, exponent=fit$exponent,
lower.tail=FALSE)/20
plot(density(abs(x)),xlim=c(0,value+0.3),main='Pareto fit')
arrows(value,0.2,value,0)
text(value,0.2,labels=paste0('p=',signif(pvalue,2)))
```

rea *REA: Rank EnrichmeNt Analysis*

Description

REA Calculates enrichment of groups of objects over a vector of values associated to a population of objects

Usage

```
rea(signatures, groups, sweights = NULL, gweights = NULL, minsize = 1)
```

Arguments

signatures	a named vector, with values as signature values (e.g. logFC) and names as object names (e.g. gene symbols)
groups	a list of vectors of objects (e.g. pathways)
sweights	weights associated to objects in the signature. If NULL (default) all objects are treated according to the signature rank
gweights	weights associated to association strength between each object and each group. If NULL (default) all associations are treated equally
minsize	integer. Minimum size of the groups to be analyzed. Default=1

Value

A numeric vector of normalized enrichment scores

Examples

```
signatures<-setNames(-sort(rnorm(1000)),paste0('gene',1:1000))
set1<-paste0('gene',sample(1:200,50))
set2<-paste0('gene',sample(1:1000,50))
groups<-list(set1=set1,set2=set2)
obj<-rea(signatures=signatures,groups=groups)
obj
```

slice *Slice*

Description

This function prints a slice of a matrix

Usage

```
slice(matrix)
```

Arguments

matrix	A matrix

Value

prints it

Examples

```
set.seed(1)
example<-matrix(rnorm(1000),nrow=100,ncol=10)
slice(example)
```

---

stouffer *Stouffer integration of Z scores*

Description

This function gives a gaussian Z-score corresponding to the provided p-value Careful: sign is not provided

Usage

```
stouffer(x)
```

Arguments

x	a vector of Z scores

Value

Z an integrated Z score

Examples

```
zs<-c(1,3,5,2,3)
stouffer(zs)
```

textplot2 *textplot2 - An x y plot of non-overlapping text*

Description

This function is an extension of the 'textplot' function from the 'wordcloud' package, with the extra functionality of specifiying the color of the points

Usage

```
textplot2(
  x,
  y,
  words,
  cex = 1,
  pch = 16,
  pointcolor = "#FFFFFF00",
  new = TRUE,
  show.lines = TRUE,
  ...
)
```

Arguments

x	x coordinates
y	y coordinates
words	the text to plot
cex	font size
pch	pch parameter for the plotted points
pointcolor	a string specifying the color of the points (default #FFFFFF00)
new	should a new plot be created
show.lines	if true, then lines are plotted between x,y and the word, for those words not covering their x,y coordinates
...	Additional parameters to be passed to wordlayout and text.

Value

nothing

Examples

```
obj_names<-apply(expand.grid(LETTERS,LETTERS),1,paste,collapse='')
a<-setNames(runif(26*26),obj_names)
b<-setNames(rnorm(26*26),obj_names)
plot(a,b,pch=20,col='grey')
top<-names(sort(-a))[1:50]
textplot2(a[top],b[top],words=top,new=FALSE,pointcolor='black')
```

`val2col` *Convert a numeric vector into colors*

Description

Convert a numeric vector into colors

Usage

```
val2col(
  z,
  col1 = "navy",
  col2 = "white",
  col3 = "red3",
  nbreaks = 100,
  center = TRUE,
  rank = FALSE
)
```

Arguments

`z`	a vector of numbers
`col1`	a color name for the min value, default 'navy'
`col2`	a color name for the middle value, default 'white'
`col3`	a color name for the max value, default 'red3'
`nbreaks`	Number of colors to be generated. Default is 30.
`center`	boolean, should the data be centered? Default is TRUE
`rank`	boolean, should the data be ranked? Default is FALSE

Value

a vector of colors

Examples

```
a<-rnorm(1000)
cols<-val2col(a)
plot(a,col=cols,pch=16)
```

vulcan *VULCAN - VirtUaL Chipseq data Analysis using Networks*

Description

This function calculates the enrichment of a gene regulatory network over a ChIP-Seq derived signature

Usage

```
vulcan(vobj, network, contrast, annotation = NULL, minsize = 10)
```

Arguments

vobj	a list, the output of the 'vulcan.normalize' function
network	an object of class 'viper::regulon'
contrast	a vector of two fields, containing the condition names to be compared (1 vs 2)
annotation	an optional named vector to convert gene identifiers (e.g. entrez ids to gene symbols) Default (NULL) won't convert gene names.
minsize	integer indicating the minimum regulon size for the analysis to be run. Default: 10

Value

A list of components:

peakcounts A matrix of raw peak counts, peaks as rows, samples as columns

peakrpkms A matrix of peak RPKMs, peaks as rows, samples as columns

rawcounts A matrix of raw gene counts, genes as rows, samples as columns. The counts are associated to the promoter region of the gene

rpkms A matrix of RPKMs, genes as rows, samples as columns. The RPKMs are associated to the promoter region of the gene

normalized A matrix of gene abundances normalized by Variance-Stabilizing Transformation (VST), genes as rows, samples as columns. The abundances are associated to the promoter region of the gene

samples A vector of sample names and conditions

msviper a multisample virtual proteomics object from the viper package

mrs A table of master regulators for a specific signature, indicating their Normalized Enrichment Score (NES) and p-value

Examples

```
library(vulcandata)
# Get an example vulcan object (generated with vulcan.import() using the
# dummy dataset contained in the \textit{vulcandata} package)
vobj<-vulcandata::vulcanexample()
# Annotate peaks to gene names
vobj<-vulcan.annotate(vobj,lborder=-10000,rborder=10000,method='sum')
# Normalize data for VULCAN analysis
vobj<-vulcan.normalize(vobj)
# Detect which conditions are present
names(vobj$samples)

# Load an ARACNe network
# This is a regulon object as specified in the VIPER package, named 'network'
load(system.file('extdata','network.rda',package='vulcandata',mustWork=TRUE))
# Run VULCAN
# We can reduce the minimum regulon size, since in this example only one
# chromosome
# was measured, and the networks would otherwise have too few hits
vobj_analysis<-vulcan(vobj,network=network,contrast=c('t90','t0'),minsize=5)
# Visualize output using the msviper plotting function
plot(vobj_analysis$msviper,mrs=7)
```

---

vulcan.annotate *Function to annotate peaks for VULCAN analysis*

Description

This function coalesces and annotates a set of BAM files into peak-centered data. It implements the ChIPPeakANno methods, with specific choices dealing with defining the genomic area around the promoter and which peaks to include.

Usage

```
vulcan.annotate(
  vobj,
  lborder = -10000,
  rborder = 10000,
  method = c("closest", "strongest", "sum", "topvar", "farthest", "lowvar"),
  TxDb = NULL
)
```

Arguments

vobj	A list of peakcounts, samples and peakrpkms (i.e. the output of the function vulcan.import)
lborder	Boundary for peak annotation (in nucleotides) upstream of the Transcription starting site (default: -10000)

`rborder` Boundary for peak annotation (in nucleotides) downstream of the Transcription starting site (default: 10000)

`method` Method to deal with multiple peaks found within gene promoter boundaries. One of sum (default), closest, strongest, topvar, farthest or lowvar. This will affect only genes with multiple possible peaks. When a single peak can be mapped to the promoter region of the gene, that peak abundance will be considered as the gene promoter's occupancy.

sum when multiple peaks are found, sum their contributions

closest when multiple peaks are found, keep only the closest to the TSS as the representative one

strongest when multiple peaks are found, keep the strongest as the representative one

farthest when multiple peaks are found, keep only the closest to the TSS as the representative one

topvar when multiple peaks are found, keep the most varying as the representative one

lowvar when multiple peaks are found, keep the least varying as the representative one

`TxDb` TxDb annotation object containing the knownGene track. If NULL (the default), TxDb.Hsapiens.UCSC.hg19.knownGene is loaded

Value

A list of components:

peakcounts A matrix of raw peak counts, peaks as rows, samples as columns

peakrpkms A matrix of peak RPKMs, peaks as rows, samples as columns

rawcounts A matrix of raw gene counts, genes as rows, samples as columns. The counts are associated to the promoter region of the gene

rpkms A matrix of RPKMs, genes as rows, samples as columns. The RPKMs are associated to the promoter region of the gene

samples A vector of sample names and conditions

Examples

```
library(vulcandata)
vobj<-vulcandata::vulcanexample()
vobj<-vulcan.annotate(vobj,lborder=-10000,rborder=10000,method='sum')
```

vulcan.import *Function to import BAM files*

Description

This function coalesces and annotates a set of BAM files into peak-centered data

Usage

```
vulcan.import(sheetfile, intervals = NULL)
```

Arguments

sheetfile	path to a csv annotation file containing sample information and BAM location
intervals	size of the peaks. If NULL (default) it is inferred from the average fragment length observed in the dataset

Value

A list of components:

peakcounts A matrix of raw peak counts, peaks as rows, samples as columns

peakrpkms A matrix of peak RPKMs, peaks as rows, samples as columns

samples A vector of sample names and conditions

Examples

```
library(vulcandata)
# Generate an annotation file from the dummy ChIP-Seq dataset
vfile<-tempfile()
vulcandata::vulcansheet(vfile)
# Import BAM and BED information into a list object
# vobj<-vulcan.import(vfile)
# This vobj is identical to the object returned by
# vulcandata::vulcanexample()
unlink(vfile)
```

`vulcan.normalize` *Function to normalize promoter peak data*

Description

This function normalizes gene-centered ChIP-Seq data using VST

Usage

```
vulcan.normalize(vobj)
```

Arguments

`vobj`	a list, the output of the `'vulcan.annotate'` function

Value

A list of components:

peakcounts A matrix of raw peak counts, peaks as rows, samples as columns

peakrpkms A matrix of peak RPKMs, peaks as rows, samples as columns

rawcounts A matrix of raw gene counts, genes as rows, samples as columns. The counts are associated to the promoter region of the gene

rpkms A matrix of RPKMs, genes as rows, samples as columns. The RPKMs are associated to the promoter region of the gene

normalized A matrix of gene abundances normalized by Variance-Stabilizing Transformation (VST), genes as rows, samples as columns. The abundances are associated to the promoter region of the gene

samples A vector of sample names and conditions

Examples

```
## Not run:
library(vulcandata)
vobj<-vulcandata::vulcanexample()
vobj<-vulcan.annotate(vobj,lborder=-10000,rborder=10000,method='sum')
vobj<-vulcan.normalize(vobj)

## End(Not run)
```

vulcan.pathways *Function to calculate pathway enrichment over a ChIP-Seq profile*

Description

This function applies Gene Set Enrichment Analysis or Rank Enrichment Analysis over a ChIP-Seq signature contained in a vulcan package object

Usage

```
vulcan.pathways(
  vobj,
  pathways,
  contrast = NULL,
  method = c("GSEA", "REA"),
  np = 1000
)
```

Arguments

vobj	a list, the output of the 'vulcan.annotate' function
pathways	a list of vectors, one vector of gene identifiers per pathway
contrast	a vector with the name of the two conditions to compare. If method=='REA', contrast can be set to 'all', and Rank Enrichment Analysis will be performed for every sample independently, compared to the mean of the dataset.
method	either 'REA' for Rank Enrichment Analysis or 'GSEA' for Gene Set Enrichment Analysis
np	numeric, only for GSEA, the number of permutations to build the null distribution. Default is 1000

Value

if method=='GSEA', a named vector, with pathway names as names, and the normalized enrichment score of either the GSEA as value. If method=='REA', a matrix, with pathway names as rows and specific contrasts as columns (the method 'REA' allows for multiple contrasts to be calculated at the same time)

Examples

```
library(vulcandata)
vfile<-tempfile()
vulcandata::vulcansheet(vfile)
#vobj<-vulcan.import(vfile)
vobj<-vulcandata::vulcanexample()
unlink(vfile)
vobj<-vulcan.annotate(vobj,lborder=-10000,rborder=10000,method='sum')
vobj<-vulcan.normalize(vobj)
```

```
# Create a dummy pathway list (in entrez ids)
pathways<-list(
   pathwayA=rownames(vobj$normalized)[1:20],
   pathwayB=rownames(vobj$normalized)[21:50]
)
# Which contrast groups can be queried
names(vobj$samples)
results_gsea<-vulcan.pathways(vobj,pathways,contrast=c('t90','t0'),
method='GSEA')
results_rea<-vulcan.pathways(vobj,pathways,contrast=c('all'),method='REA')
```

`wstouffer` *Weighted Stouffer integration of Z scores*

Description

This function gives a gaussian Z-score corresponding to the provided p-value Careful: sign is not provided

Usage

```
wstouffer(x, w)
```

Arguments

`x`	a vector of Z scores
`w`	weight for each Z score

Value

Z an integrated Z score

Examples

```
zs<-c(1,-3,5,2,3)
ws<-c(1,10,1,2,1)
wstouffer(zs,ws)
```

z2p *z2p*

Description

This function gives a gaussian p-value corresponding to the provided Z-score

Usage

```
z2p(z)
```

Arguments

z	a Z score

Value

a p-value

Examples

```
z<-1.96
z2p(z)
```

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