Package ‘vulcan’

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Type Package

Title VirtUaL ChIP-Seq data Analysis using Networks

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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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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
**corr2p**

**Value**

nothing

**Examples**

```r
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 function 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**

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

**Arguments**

- `r`: a correlation coefficient
- `N`: a number of samples

**Value**

- `p`: a p-value

**Examples**

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

```r
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 p-values

Usage

`fisherp(ps)`

Arguments

- **ps**: a vector of p-values

Value

- **p.val**: an integrated p-value

Examples

```r
ps<-c(0.01,0.05,0.03,0.2)
fisherp(ps)
```
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 5 components:

- **ES**: The enrichment score
- **NES**: The normalized enrichment score
- **ledge**: The items in the leading edge
- **p.value**: The permutation-based p-value

Examples

\[
\begin{align*}
  &\text{reflist}<-\text{setNames(-sort(rnorm(1000)), paste0('gene', 1:1000))} \\
  &\text{set}<-\text{paste0('gene', sample(1:200,50))} \\
  &\text{obj<-gsea(reflist, set, method='pareto',np=1000)} \\
  &\text{obj$p.value}
\end{align*}
\]
### 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**

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

**Arguments**

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

**Value**

A character vector of formatted number names

**Examples**

```r
# 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**

```r
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 function 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)
**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**

```r
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 population 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**

### Description

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

### Usage

```r
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

```r
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)
```

---

**slice**

### Description

This function prints a slice of a matrix.

### Usage

```r
slice(matrix)
```
stouffer

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)
Description

This function is an extension of the 'textplot' function from the 'wordcloud' package, with the extra functionality of specifying 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",
  nbbreaks = 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 - VirtUaL Chipseq data Analysis using Networks**

**Description**

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

**Usage**

```r
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

```r
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)
vulcan.annotate

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

```r
## 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**

```r
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**

```r
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)
```
Description

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

Usage

\[ z2p(z) \]

Arguments

\[ z \quad \text{a Z score} \]

Value

\[ \text{a p-value} \]

Examples

\[ \begin{align*}
  z & \leftarrow 1.96 \\
  z2p(z) & \\
\end{align*} \]
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