

# Package ‘vulcan’

January 18, 2025

**Type** Package

**Title** VirtUaL ChIP-Seq data Analysis using Networks

**Version** 1.29.0

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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

**LazyData** TRUE

**biocViews** SystemsBiology, NetworkEnrichment, GeneExpression, ChIPSeq

**NeedsCompilation** no

**Suggests** vulcandata

**Depends** R (>= 4.0), ChIPpeakAnno, TxDb.Hsapiens.UCSC.hg19.knownGene, zoo, GenomicRanges, S4Vectors, viper, DiffBind, locfit

**Imports** wordcloud, csaw, gplots, stats, utils, caTools, graphics, DESeq2, Biobase

**Encoding** UTF-8

**RoxygenNote** 7.1.1

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

**git\_branch** devel

**git\_last\_commit** 45481f2

**git\_last\_commit\_date** 2024-10-29

**Repository** Bioconductor 3.21

**Date/Publication** 2025-01-17

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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	<i>densityauc - Calculate the AUC of a density object</i>
------------	---

---

**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	<i>Probability density of Pareto distributions</i>
---------	--

---

**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 <code>pareto.fit</code> 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 p-values

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

**Arguments**

refflist	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 score

**ledge** The items in the leading edge

**p.value** The permutation-based p-value

**Examples**

```
refflist<-setNames(-sort(rnorm(1000)),paste0('gene',1:1000))
set<-paste0('gene',sample(1:200,50))
obj<-gsea(refflist,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**

<code>input</code>	A vector of values
<code>roundParam</code>	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, rellist, 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 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	<i>Cumulative distribution function of the Pareto distributions ' Gives NA on values &lt; threshold</i>
---------	---

---

**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 <code>pareto.fit</code> 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 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)))
```

---

rea	<i>REA: Rank Enrichment Analysis</i>
-----	--------------------------------------

---

**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	<i>Slice</i>
-------	--------------

---

**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 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	<i>Convert a numeric vector into colors</i>
---------	---

---

**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 peakrpkm (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	<p>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.</p> <p><b>sum</b> when multiple peaks are found, sum their contributions</p> <p><b>closest</b> when multiple peaks are found, keep only the closest to the TSS as the representative one</p> <p><b>strongest</b> when multiple peaks are found, keep the strongest as the representative one</p> <p><b>farthest</b> when multiple peaks are found, keep only the closest to the TSS as the representative one</p> <p><b>topvar</b> when multiple peaks are found, keep the most varying as the representative one</p> <p><b>lowvar</b> when multiple peaks are found, keep the least varying as the representative one</p>
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	<i>Function to import BAM files</i>
---------------	-------------------------------------

---

## 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

**peakrpkm** 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

**peakrpkm** 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

**rpkm** 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)
```

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z2p

z2p

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**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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