Package ‘MBttest’

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Type Package
Title Multiple Beta t-Tests
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Description MBttest method was developed from beta t-test method of Baggerly et al(2003). Compared to baySeq (Hard castle and Kelly 2010), DESeq (Anders and Huber 2010) and exact test (Robinson and Smyth 2007, 2008) and the GLM of McCarthy et al(2012), MBttest is of high work efficiency, that is, it has high power, high conservativeness of FDR estimation and high stability. MBttest is suitable to transcriptomic data, tag data, SAGE data (count data) from small samples or a few replicate libraries. It can be used to identify genes, mRNA isoforms or tags differentially expressed between two conditions.
License GPL-3
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MBttest-package

Description

This package is used to perform multiple beta t-test analyses of real data and gives heatmap of
differential expressions of genes or differential splicings and MA plot. The results listing geneid
or isoformid, gene name, the other information, t-value, p-value, adjusted p-value, adjusted alpha
value, rho, and symb are saved in csv file.

Details

Package: MBttest
Type: Package
Version: 1.0
Date: 2015-01-02
License: GPL-3

Author(s)

Yuan-De Tan
Maintainer: Yuan-De Tan <tanyuande@gmail.com>

References

for normal between-library variation. Bioinformatics, 19: 1477-1483. \ Yuan-De Tan Anita M.
Large-scale Differential Transcription Analysis.Plos One,10.1371/journal.pone.0123658.

See Also

betaparametab, betaparametVP, betaparametw, betatettest, mbetatettest, maplot, myheatmap,
oddratio, pratio, simulat, smbetatettest, mtprocedure, mtpvadjust
Examples

data(jkttcell)
mbetatset(X=jkttcell[1:500,],na=3,nb=3,W=1,alpha=0.05,file="jurkat_NS_48h_tag_mbetatset.csv")

betaparametab

Estimation of Beta Parameters $a$ And $b$

Description

parameters alpha ($a$) and beta ($b$) in betat distribution are estimated by using modified Baggerly et al.(2003)'s iterative optimal method.

Usage

betaparametab(xn, w, P, V)

Arguments

xn  |  column vector, a set of library sizes.
w  |  column vector, a set of weights
P  |  proportion of counts of a gene or an isoform
V  |  variance for proportions of counts of a gene or an isoform over m replicate libraries in a condition

Value

return parameters $a$ and $b$.

Author(s)

Yuan-De Tan <tanyuande@gmail.com>

References


See Also

betaparametVP, betaparametw

Examples

p<-0.15
V=0.004
w<-c(0.3,0.3,0.3)
betaparametab(xn=XX,w=p,P=p,V=V)
# [1] 1.145868 6.493254
Description

This function is used to estimate parameters P and V by optimalizing estimation of parameters: alpha and beta.

Usage

betaparametVP(X, NX)

Arguments

X  count dataset derived from m replicate libraries in one condition.
NX vector of m library sizes. Library size is sum of counts over the whole library.

Details

Count data of RNA reads are assumed to follow binomial distribution with parameters (P) and (V), while P is assumed to follow beta distribution with parameters alpha (a) and beta(b). Parameters P and V are estimated by optimal estimation of parameters a and b. The optimal method is an iteration method driven by weighting proportion of gene or isoform in each replicate library. This is a large-scale method for estimating these parameters. Estimation of parameters P and V is core of the multiple beta t-test method because P and V will be used to calculate t-value.

Value

return a list:

P  N proportions estimated.
V  N variances estimated.

Note

betaparametVP requires functions betaparametab and betaparametw.

Author(s)

Yuan-DE Tan <tanyuande@gmail.com>

References


See Also

betaparametab, betaparametw
betaparametw

Examples

data(jkttcell)
X<-jkttcell[1:500,]
na<-3
nb<-3
cn<-length(X[1,])n<-length(X[,1])
XC<-X[,1:(cn-na-nb)]
XX<-X[,cn-na-nb+1:cn]
n<-na+nb
XA<-XX[,1:na]
SA<-apply(XA,2,sum)
PA<-betaparametw(XA,SA)

betaparametw | Estimation of Weights of Proportions

Description

Function betaparametw is used to calculate weight.

Usage

betaparametw(xn, a, b)

Arguments

xn \hspace{1cm} \text{vector of m library sizes. Library size is sum of counts over the whole library.}
a \hspace{1cm} \text{parameter alpha in beta distribution derived from output of function Bag_parametab}
b \hspace{1cm} \text{parameter beta in beta distribution derived from output of function Bag_parametab}

Details

alpha and beta are used to calculate weight. Then weight is in turn used to correct bias of estimation of alpha and beta in Bag_parametab function.

Value

return weight(W)

Author(s)

Yuan-De Tan <tanyuande@gmail.com>

References

See Also

betaparametab, betaparametVP.

Examples

a <- 1.1458
b <- 6.4932
betaparametw(xn = xx, a = a, b = b)
# [1] 0.3333333 0.3333333 0.3333333

betatetest

Beta t-test

Description

Beta t-test and degree of freedom for each gene or isoform are calculated in this function.

Usage

betatetest(X, na, nb)

Arguments

X count data of RNA reads containing N genes (or isoforms).
na number of replicate libraries in condition A
nb number of replicate libraries in condition B

Details

In beta t-test,

\[
t = \frac{(P_A - P_B)}{\sqrt{VA + VB}}
\]

where PA and PB are proportions of a gene or an isoform in conditions A and B, VA and VB are variances estimated in conditions A and B. They are outputted by Bag_parametVP.

Value

return two lists:
t t-value list.
df df list. df is degree of freedom.

Note

If pooled standard error is zero, then the t-value is not defined and set to be zero.

Author(s)

Yuan-De Tan <tanyuande@gmail.com>
References


See Also
pratio, oddratio.

Examples

data(jktttcell)
X<-jktttcell[1:1000,]
na<-3
nb<-3
cn<-ncol(X)
rn<-nrow(X)
XC<-X[,1:(cn-na-nb)]
XX<-X[,,(cn-na-nb+1):cn]
betattest<-betattest(XX,na=3,nb=3)

##

dat The Transcriptomic data and t-test results.

Description

t-value and rho are results ouputed by mbttest.

Usage
data("dat")

Format

A data frame with 13409 observations on the following 16 variables.
tagid  a numeric vector
geneid  a numeric vector
name    a string vector
chr     a string vector
strand  a character vector
pos     a numeric vector
anno    a string vector
Jurk.NS.A a numeric vector
Jurk.NS.B a numeric vector
Jurk.NS.C a numeric vector
Jurk.48h.A a numeric vector
Jurk.48h.B a numeric vector
 Jurk.48h.C  a numeric vector
 beta_t  a numeric vector
 rho  a numeric vector
 symb  a character vector

Details

t-values (beta_t) and means over all replicate libraries in two conditions are used to make MA plot. The count data of DE isoforms are selected by symb ="+" and W(omega) and used to make heatmap using myheatmap function.

Value

ID, information, count data of RNA reads, t-value and rho-value, symbol.

References


Examples

data(dat)
## maybe str(dat) ; plot(dat) ...

---

jkttcell  
*Jurkat T-cell Transcritomic Data*

Description

The data are transcriptomic count data of RNA reads generated by next generation sequencing from Jurkat T-cells.

Usage

data("jkttcell")

Format

A data frame with 13409 observations on the following 13 variables.

tagid  a numeric vector
geneid  a numeric vector
name  a string vector
chr  a string vector
strand  a character vector
pos  a numeric vector
anno  a string vector
Jurk.NS.A  a numeric vector
Jurk.NS.B  a numeric vector
Jurk.NS.C  a numeric vector
Jurk.48h.A  a numeric vector
Jurk.48h.B  a numeric vector
Jurk.48h.C  a numeric vector

Details
The data are count data generated by next generation sequencing from Jurkat T-cells. The T-cells were treated by resting and stimulating with \textit{CD3/CD28} for 48 hours. The data have 7 columns for the information of \textit{poly(A)} site: tagid, geneid, gene name, chromosome, strand, \textit{poly(A)} site position, \textit{poly(A)} site annotation and 6 columns for data: Jurk.NS.A, Jurk.NS.B, Jurk.NS.C, Jurk.48h.A, Jurk.48h.B, Jurk.48h.C. where NS means Normal state and 48h means 48 hours after \textit{CD3/CD28} stimulatuin of T-cells. 13409 RNA isoforms were detected to have alternative \textit{poly(A)} sites.

Value
ID, information, count data of RNA reads

Source
Real transcriptomic count data

References

Examples
data(jkttcell)
## maybe str(jkttcell) ; plot(jkttcell) ...

maplot MA plot of t-values Against Log Mean

Description
This function is to display MA plot of t-value against log mean.

Usage
maplot(dat, r1, r2, TT, matitle)

Arguments
dat object outputted by mbetattest containing data ordered by absolution of t-value and rho.

r1 number of replicate libraries in condition 1.

r2 number of replicate libraries in condition 2.

TT a numeric parameter that gives truncate value of t-values.

matitle string for MA plot title.
Details
In MA plot, t-value is in y-axis and log mean in x-axis; Black points gathered nearby zero along log mean are genes without differential expressions or differential splicings while red points scattered out of black points are those of being differentially expressed or differentially spliced.

Value
no return value

Author(s)
Yuan-De Tan <tanyuande@gmail.com>

Examples
data(dat)
maplot(dat=dat,r1=3,r2=3,TT=350,matitle="MA plot")
maplot(dat=dat,r1=3,r2=3,TT=50,matitle="MA plot")

mbetattest
Performance of multiple beta t-test on simulated data

Description
This function is to perform multiple beta t-test method on real data. The result lists geneid or isoformid, gene name, the other information, t-value, p-value, adjusted p-value, adjusted alpha value, rho, and symb. All these lists are ordered by absolute of t-values.

Usage
mbetattest(X, na, nb, W, alpha, file)

Arguments
X count data of RNA reads with na replicates in condition A ans nb replicates in condition B.
na number of replicate libraries in condition A.
nb number of replicate libraries in condition B.
W numeric parameter, called omega that is a constant,determined by null simulation.
alpha the probabilistic threshold. User can set alpha=0.05 or 0.01 or the other values. Defalt value is 0.05
file a csv file. User needs to give file name and specify direction path. But if user uses setwd function, drive is not necessarily specified in file.
Details

t-statistic is defined as t-statistic multiplied by (rho/omega), that is,

\[ T = t \times \frac{\rho}{\omega} \]

where

\[ t = \frac{(PA - PB)}{\sqrt{VA + VB}} \]

\[ \rho = \sqrt{\psi \cdot \zeta} \]

where

\[ \psi = \max\left(\frac{\min(XA)}{\max(XB)}, \frac{\min(XB)}{\max(XA)}\right) \]

\[ \zeta = \log(1 + \frac{(\text{mean}(XA, XB) \cdot \text{var}(XA, XB))}{(\text{mean}(XA) \cdot \text{var}(XA) + \text{mean}(XB) \cdot \text{var}(XB))}) \]

omega is a constant as threshold.

Value

return a dat list: the data ordered by abs(t) contain information columns, data columns, t-values, rho and symb that are used to make heatmap and MAplot.

Author(s)

Yuan-De Tan <tanyuande@gmail.com>

References


See Also

smbetattest.

Examples

data(jkttcell)

dat<-mbetattest(X=jkttcell[1:1000,],na=3,nb=3,W=1,alpha=0.05,file="jurkat_NS_48h_tag_mbetattest.csv")
mtprocedure

Multiple-Test Procedures

Description

Similar to Benjamini-Hochberg multiple-test procedure, alpha is adjusted to be a set of values.

Usage

mtprocedure(alpha, N, C)

Arguments

alpha  probabilistic threshold. alpha is usually set to be 0.05 or 0.01. Default value is 0.05
N   numeric constant, number of genes to be detected in microarray or transcriptome.
C   numeric constant, it can be taken from 0 to N. C is used to choose multiple-test procedure. Default value is 0.01. This procedure is single test with C=0, Benjamini-Hochberg procedure with C=1.22 and Bonferroni procedure with C=N.

Details

This is a multiple-test procedure family including Benjamini-Hochberg procedure, Bonferroni procedure and single-test procedure. By choosing C-value, it can generate a multiple-test procedure for controlling the false discovery rate, the expected proportion of false discoveries amongst the rejected hypotheses.

Value

return a list of adjusted alpha values.

Author(s)

Yuan-De Tan <tanyuande@gmail.com>

References


See Also

p.adjust
Examples

```r
mtprocedure(alpha=0.5,N=200,C=1.22)
# [1] 0.007501404 0.011906423 0.015914688 0.019682621 0.023284917 0.026763656
# [7] 0.030145311 0.033447843 0.036684127 0.039863779 0.042994217 0.046081313
# ....
# [175] 0.444073506 0.446322519 0.448570478 0.450817390 0.453063265 0.455308110
# [181] 0.457551933 0.459794741 0.462036542 0.464277343 0.466517153 0.468755977
# [187] 0.470993825 0.473230781 0.475466614 0.477701571 0.479935578 0.482168642
# [193] 0.484400770 0.486631969 0.488862244 0.491091683 0.493320052 0.495547597
# [199] 0.497774244 0.500000000
```

Description

Given a set of N p-values, it returns a set of N p-values adjusted by choosing C-value

Usage

```r
mtpvadjust(pv, C)
```

Arguments

- `pv`: numeric vector of p-values.
- `C`: numeric constant, the value can be taken from any number > 0 or equal to 0. C is used to choose multiple-test procedure.

Details

This is a multiple-test procedure family including Benjamini-Hochberg procedure, Bonferroni procedure and single-test procedure. By choosing C-value, it can generate a multiple-test procedure for controlling the false discovery rate, the expected proportion of false discoveries amongst the rejected hypotheses. Benjamini-Hochberg procedure is given with C=1.22, Bonferroni procedure is given with C = N and single-test procedure can be given with C=0.

Value

return a list of adjusted p-values.

Note

p-value must be ordered from the largest value to the smallest value before executing `mtpvadjust`.

Author(s)

Yuan-De Tan <tanyuande@gmail.com>
References


See Also

`p.adjust`

Examples

```r
set.seed(123)
x <- rnorm(50, mean = c(rep(0, 25), rep(3, 25)))
p <- 2*pnorm(sort(-abs(x)))
round(mtpvadjust(pv=p, C=1.22),4)
# [1] 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000
# [11] 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 0.6875 0.6174 0.4588
# [21] 0.4115 0.3644 0.2216 0.1554 0.1443 0.1249 0.0964 0.0763 0.0319
# [31] 0.0166 0.0135 0.0123 0.0096 0.0091 0.0068 0.0045 0.0041 0.0020 0.0007
# [41] 0.0004 0.0003 0.0002 0.0001 0.0001 0.0001 0.0001 0.0000 0.0000 0.0000
```

**myheatmap**

**Description**

This function is used to display heatmap of differential expressions of genes or isoforms or differential splicings of genes detected by the multiple beta t-test method in the real data.

**Usage**

```r
myheatmap(dat, r1, r2, W, colrs, tree, method, rwangle, clangle, maptitle)
```

**Arguments**

- `dat` data outputted by `mbeta_ttest`, includes information columns, data columns, t-value, rho and symbol columns;
- `r1` numeric argument: number of replicate libraries in condition 1;
- `r2` numeric argument: number of replicate libraries in condition 2;
- `W` numeric argument: threshold for choosing genes or isoforms for heatmap. W value can be set to be 0 to any large number. If you set W =0, then the function will select all differentially expressed genes with symb="+". To choose a appropriate W, user needs to refere to rho values in the result file. Default W=1.
- `colrs` heatmap colors. User has 5 options: "redgreen", "greenred", "redblue", "bluered" and "heat.colors". Default colrs="redgreen".
- `tree` object of heatmap. User has four options: "both" for row and column trees,"row" for only row tree,"column" for only column tree, and "none" for no tree specified. Default tree="both".
myheatmap

method

method to be chosen to calculate distance between columns or rows. It has four options: "euclidean", "pearson","spearman" and "kendall". The latter three are d=1-cc where cc is correlation coefficients. Default="euclidean".

rwangle

angle of xlab under heatmap. Default value is 30.

ciangle

angle of ylab. Default value is 30

maptitle

string for heatmap title.

Details

This function uses W (omega) and symb to choose genes or isoforms in the data ordered by t-values and then to normalize the selected data by using z-scale. This function has multiple options to select map color, distance, cluster and x- and y-lab angles. The heatmap was designed for publication and presentation, that is, zoom of the figure can be reduced without impacting solution.

Value

no return value but create a heatmap.

Note

myheatmap requires gplots

Author(s)

Yuan-De Tan <tanyuande@gmail.com>

See Also

heatmap.2

Examples

require(gplots)
data(dat)

#dat<-mbetattest(X=jkttcell,na=3, nb=3, W=1, alpha=0.05, 
#file="C:/mBeta_ttest/R_package/jurkat_NS_48h_tag_mbetattest.csv")

# data(mtcars)
#x <-as.matrix(mtcars)
#myheatmap(dat=x,r1=3,r2=3, maptitle="mtcars_heatmap")

myheatmap(dat=dat,r1=3,r2=3,maptitle="Jurkat T-cell heatmap2")

myheatmap(dat=dat,r1=3,r2=3,tree="none",maptitle="Jurkat T-cell heatmap")
**oddratio**

---

**Calculation of Zeta**

**Description**

Zeta is used to measure homogeneity intensity of two subdatasets. If $\zeta > 1$, these two subdatasets have good homogeneity; otherwise, $\zeta < 1$ indicates that two subdatasets have poor homogeneity (big noise).

**Usage**

```
oddratio(XX, na, nb)
```

**Arguments**

- **XX**: count data of RNA reads generated by next generation sequencing.
- **na**: number of replicate libraries in condition A.
- **nb**: number of replicate libraries in condition B.

**Details**

Zeta is defined as

\[
\zeta = \log(1+(\text{mean}(XA, XB)\ast\text{var}(XA, XB))/(\text{mean}(XA)\ast\text{var}(XA)+\text{mean}(XB)\ast\text{var}(XB)))
\]

$\zeta$ is different from $\psi$. If two subdatasets have big a gap and good homogeneity, then $\zeta$ value has much larger than 1.

**Value**

```
oddrat list of zeta values
```

**Author(s)**

Yuan-De Tan <tanyuande@gmail.com>

**References**


**See Also**

`pratio`, `mbetatest`.
pratio

Examples

XX <- matrix(NA, 2, 8)
XX[,2] <- c(511, 230, 754, 335, 771, 842, 1014, 798)
#XX
# [1,] 112 122 108 127 302 314 322 328
# [2,] 511 230 754 335 771 842 1014 798
oddratio(XX=XX, na=4, nb=4)
#[1] 3.9432676 0.8762017

# see example in mbetattest

---

pratio Calculation of Psi

Description

Psi is also called polar ratio.

\[
\psi = \max\left(\min(X_A), \min(X_B)\right) / \max\left(\max(X_B), \max(X_A)\right)
\]

Usage

pratio(xx, na, nb)

Arguments

xx count data of RNA reads generated by next generation sequencing.
na number of replicate libraries in condition A.
nb number of replicate libraries in condition B.

Details

Psi is defined as

\[
\psi = \max\left(\min(X_A), \min(X_B)\right) / \max\left(\max(X_B), \max(X_A)\right)
\]

It is used to measure overlap of two subdatasets. Psi>1, these two subdatasets have a gap, not overlap. Psi <1 indicates that two subdatasets overlap.

Value

pratio pratio list

Author(s)

Yuan-De Tan <tanyuande@gmail.com>
**References**


**See Also**

`mbetattest, oddratio`

**Examples**

```r
XX <- matrix(NA, 2, 8)
XX[1,] <- c(112, 122, 108, 127, 302, 314, 322, 328)
XX[2,] <- c(511, 230, 754, 335, 771, 842, 1014, 798)
# XX
# [1,] 112 122 108 127 302 314 322 328
# [2,] 511 230 754 335 771 842 1014 798
pratio(xx=XX, na=4, nb=4)
```

---

**simulat**  
*Simulation Data*

**Description**

This function uses negative binomial (NB) pseudorandom generator to create any count datasets of RNA isoform reads based on real data.

**Usage**

```r
simulat(yy, nci, r1, r2, p, q, A)
```

**Arguments**

- `yy`: Real count data.
- `nci`: Numeric argument: column number of information related to genes or isoforms.
- `r1`: Numeric argument: number of replicate libraries in condition 1.
- `r2`: Numeric argument: number of replicate libraries in condition 2.
- `p`: Numeric argument: proportion of genes or isoforms differentially expressed. The value is in range of 0~1. Default value is 0.
- `q`: Numeric argument: proportion of genes or isoforms artificially noised. The value is in range of 0~1. Default value is 0.
- `A`: Numeric argument: conditional effect value. The value is larger than or equal to 0. Default value is 0.
Details

Null count data are created by using R negative binomial pseudorandom generator `rnbinom` with mu and size. Parameters mu and size are given by mean and variance drawn from real read counts of a gene or an isoforms in a condition. Condition (or treatment) effect on differential transcription of isoforms is linearly and randomly assigned to genes or isoforms. The conditional effect = AU where U is uniform variable and A is input constant. P percent of genes or isoforms are set to be differentially expressed or differentially spliced. Q percent of genes or isoforms have technical noise. If P=0, then simulation is null simulation, the data are null data or baseline data.

Value

Return count data.

Author(s)

Yuan-De Tan <tanyuande@gmail.com>

References


See Also

`NegBinomial`

Examples

```r
data(jktcell)
jknul1<-simulat(yy=jktcell[1:500,],nci=7,r1=3,r2=3,p=0,q=0.2,A=0)
```

### skjt

**Simulated Null Transcriptomic data**

Description

The dataset skjt generated by using R negative binomial pseudorandom generator `rnbinom` is used as an example for calculating omega.

Usage

```r
data("skjt")
```

Format

A data frame with 13409 observations on the following 14 variables.

- `geneid` a string vector
- `tagid` a numeric vector
- `geneid.1` a numeric vector
name a string vector
chr a string vector
strand a character vector
pos a numeric vector
anno a string vector
Jurk.NS.A a numeric vector
Jurk.NS.B a numeric vector
Jurk.NS.C a numeric vector
Jurk.48h.A a numeric vector
Jurk.48h.B a numeric vector
Jurk.48h.C a numeric vector

Details

The dataset skjt was generated by using R negative binomial pseudorandom generator rnbinom with mu and size. Parameters mu and size are given by mean and variance drawn from real Jurkat T cell transcriptomic count data. Condition (or treatment) effect on differential transcription of isoforms was set to zero. The data have 13409 genes and 7 information columns: geneid tagid name chr,strand,pos,anno, and 6 data columns: Jurk.NS.A,Jurk.NS.B,Jurk.NS.C,Jurk.48h.A,Jurk.48h.B,Jurk.48h.C.

Value

ID, information, count data of RNA reads

Source

Simulation.

References


Examples

data(skjt)
## maybe str(skjt) ; plot(skjt) ...

smbetattest

Performance of multiple Beta t-test on simulated data

Description

This function is to perform mBeta t-test with rho=1 and omega=1 on simulated data. The result lists differentially expressed genes or isoforms marked by symbol="+" and their rho values. The rho values are used to calculate omega value for performance of mBeta t-tests on the real data.
Usage

\texttt{smbetattest(X, na, nb, alpha)}

Arguments

\texttt{X} \hspace{1em} \text{simulated count data with N genes or isoforms.}
\texttt{na} \hspace{1em} \text{number of replicate libraries in condition A.}
\texttt{nb} \hspace{1em} \text{number of replicate libraries in condition B.}
\texttt{alpha} \hspace{1em} \text{statistical probabilistic threshold, default value is 0.05.}

Details

Before performing mbeta t-test on real data, user needs omega value for the threshold of rho. To determine omega value, user is required to simulate null data having the same gene or isoform number and the same numbers of replicate libraries in two conditions and then performs mbeta t-test on the simulated null data by setting rho =1 and omega =1. To calculate accurately omega value, user needs such performance on 4-6 simulated null datasets. Manual provides method for omega calculation.

Value

Return results from multiple beta t-tests on simulated data.

Author(s)

Yuan-De Tan <tanyuande@gmail.com>

References


See Also

See Also as \texttt{mbetattest}

Examples

data(skjt)
mysim<-smbetattest(X=skjt[1:500,], na=3, nb=3, alpha=0.05)
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