

Package ‘samr’

July 2, 2014

Title SAM: Significance Analysis of Microarrays

Version 2.0

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Description Significance Analysis of Microarrays

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Depends impute, matrixStats

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URL <http://www-stat.stanford.edu/~tibs/SAM>

Repository CRAN

Date/Publication 2011-06-29 09:42:31

NeedsCompilation yes

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Description

Correlates a large number of features (eg genes) with an outcome variable, such as a group indicator, quantitative variable or survival time. This is a simple user interface for the `samr` function applied to array data. For sequencing data applications, see the function `SAMseq`.

Usage

```
SAM(x,y=NULL,censoring.status=NULL,
    resp.type=c("Quantitative","Two class unpaired","Survival","Multiclass",
    "One class", "Two class paired","Two class unpaired timecourse",
    "One class timecourse","Two class paired timecourse", "Pattern discovery"),
    geneid = NULL,
    genenames = NULL,
    s0=NULL,
    s0.perc=NULL,
    nperms=100,
    center.arrays=FALSE,
    testStatistic=c("standard","wilcoxon"),
    time.summary.type=c("slope","signed.area"),
    regression.method=c("standard","ranks"),
    return.x=TRUE,
    knn.neighbors=10,
    random.seed=NULL,
    logged2 = FALSE,
    fdr.output = 0.20,
    eigengene.number = 1)
```

Arguments

<code>x</code>	Feature matrix: p (number of features) by n (number of samples), one observation per column (missing values allowed)
<code>y</code>	n-vector of outcome measurements
<code>censoring.status</code>	n-vector of censoring <code>censoring.status</code> (1= died or event occurred, 0=survived, or event was censored), needed for a censored survival outcome
<code>resp.type</code>	Problem type: "Quantitative" for a continuous parameter; "Two class unpaired"; "Survival" for censored survival outcome; "Multiclass": more than 2 groups; "One class" for a single group; "Two class paired" for two classes with paired observations; "Two class unpaired timecourse", "One class time course", "Two class.paired timecourse", "Pattern discovery"
<code>geneid</code>	Optional character vector of geneids for output.
<code>genenames</code>	Optional character vector of genenames for output.

<code>s0</code>	Exchangeability factor for denominator of test statistic; Default is automatic choice. Only used for array data.
<code>s0.perc</code>	Percentile of standard deviation values to use for <code>s0</code> ; default is automatic choice; -1 means <code>s0=0</code> (different from <code>s0.perc=0</code> , meaning <code>s0=zeroeth</code> percentile of standard deviation values= min of sd values. Only used for array data.
<code>nperms</code>	Number of permutations used to estimate false discovery rates
<code>center.arrays</code>	Should the data for each sample (array) be median centered at the outset? Default =FALSE. Only used for array data.,
<code>testStatistic</code>	Test statistic to use in two class unpaired case. Either "standard" (t-statistic) or "wilcoxon" (Two-sample wilcoxon or Mann-Whitney test). Only used for array data.
<code>time.summary.type</code>	Summary measure for each time course: "slope", or "signed.area"). Only used for array data.
<code>regression.method</code>	Regression method for quantitative case: "standard", (linear least squares) or "ranks" (linear least squares on ranked data). Only used for array data.
<code>return.x</code>	Should the matrix of feature values be returned? Only useful for time course data, where <code>x</code> contains summaries of the features over time. Otherwise <code>x</code> is the same as the input data <code>data\$x</code>
<code>knn.neighbors</code>	Number of nearest neighbors to use for imputation of missing features values. Only used for array data.
<code>random.seed</code>	Optional initial seed for random number generator (integer)
<code>logged2</code>	Has the data been transformed by log (base 2)? This information is used only for computing fold changes
<code>fdr.output</code>	(Approximate) False Discovery Rate cutoff for output in significant genes table
<code>eigengene.number</code>	Eigengene to be used (just for <code>resp.type="Pattern discovery"</code>)

Details

This is a simple, user-friendly interface to the `samr` package used on array data. It calls `samr`, `samr.compute.delta.table` and `samr.compute.siggenes.table`. `samr` detects differential expression for microarray data, and sequencing data, and other data with a large number of features. `samr` is the R package that is called by the "official" SAM Excel Addin. The format of the response vector `y` and the calling sequence is illustrated in the examples below. A more complete description is given in the SAM manual at <http://www-stat.stanford.edu/~tibs/SAM>

Value

A list with components

<code>samr.obj</code>	Output of <code>samr</code> . See documentation for <code>samr</code> for details.
<code>siggenes.table</code>	Table of significant genes, output of <code>samr.compute.siggenes.table</code> . This has components: <code>genes.up</code> — matrix of significant genes having positive correlation with the outcome and <code>genes.lo</code> —matrix of significant genes having negative correlation with the outcome. For survival data, <code>genes.up</code> are those genes

	having positive correlation with risk- that is, increased expression corresponds to higher risk (shorter survival) genes. lo are those whose increased expression corresponds to lower risk (longer survival).
delta.table	Output of samr.compute.delta.table.
del	Value of delta (distance from 45 degree line in SAM plot) for used for creating delta.table and siggenes.table. Changing the input value fdr.output will change the resulting del.
call	The calling sequence

Author(s)

Jun Li and Balasubramanian Narasimhan and Robert Tibshirani

References

Tusher, V., Tibshirani, R. and Chu, G. (2001): Significance analysis of microarrays applied to the ionizing radiation response PNAS 2001 98: 5116-5121, (Apr 24). <http://www-stat.stanford.edu/~tibs/SAM>

Li, Jun and Tibshirani, R. (2011). Finding consistent patterns: a nonparametric approach for identifying differential expression in RNA-Seq data. To appear, Statistical Methods in Medical Research.

Examples

```
##### two class unpaired comparison
# y must take values 1,2

set.seed(100)
x<-matrix(rnorm(1000*20),ncol=20)
dd<-sample(1:1000,size=100)

u<-matrix(2*rnorm(100),ncol=10,nrow=100)
x[dd,11:20]<-x[dd,11:20]+u
y<-c(rep(1,10),rep(2,10))

samfit<-SAM(x,y,resp.type="Two class unpaired")

# examine significant gene list

print(samfit)

# plot results
plot(samfit)

##### two class paired

# y must take values -1, 1, -2,2 etc, with (-k,k) being a pair

set.seed(100)
x<-matrix(rnorm(1000*20),ncol=20)
dd<-sample(1:1000,size=100)
```

```
u<-matrix(2*rnorm(100),ncol=10,nrow=100)
x[dd,11:20]<-x[dd,11:20]+u

y=c(-(1:10),1:10)

samfit<-SAM(x,y, resp.type="Two class paired",fdr.output=.25)

#####quantitative response

set.seed(30)
p=1000
x<-matrix(rnorm(p*20),ncol=20)
y<-rnorm(20)
x[1:20,y>0]=x[1:20,y>0]+4
a<-SAM(x,y,resp.type="Quantitative",nperms=50,fdr.output=.5)

#####survival data
# y is numeric; censoring.status=1 for failures, and 0 for censored

set.seed(84048)
x=matrix(rnorm(1000*50),ncol=50)
x[1:50,26:50]= x[1:50,26:50]+2
x[51:100,26:50]= x[51:100,26:50]-2

y=abs(rnorm(50))
y[26:50]=y[26:50]+2
censoring.status <- sample(c(0,1),size=50,replace=TRUE)

a<-SAM(x,y,censoring.status=censoring.status,resp.type="Survival",
nperms=20)

#####multi-class example
# y takes values 1,2,3,...k where k= number of classes

set.seed(84048)
x=matrix(rnorm(1000*10),ncol=10)

y=c(rep(1,3),rep(2,3),rep(3,4))
x[1:50,y==3]=x[1:50,y==3]+5
```

```

a <- SAM(x,y,resp.type="Multiclass",nperms=50)

##### pattern discovery
# here there is no outcome y; the desired eigengene is indicated by
# the argument eigengene.numbe in the data object

set.seed(32)
x=matrix(rnorm(1000*9),ncol=9)
mu=c(3,2,1,0,0,0,1,2,3)
b=3*runif(100)+.5
x[1:100,]=x[1:100,]+ b

d=list(x=x,eigengene.number=1,
geneid=as.character(1:nrow(x)),genenames=paste("gene", as.character(1:nrow(x))))

a <- SAM(x, resp.type="Pattern discovery", nperms=50)

##### timecourse data

# elements of y are of the form kTimet where k is the class label and t
# is the time; in addition, the suffixes Start or End indicate the first
# and last observation in a given time course
# the class label can be that for a two class unpaired, one class or
# two class paired problem

set.seed(8332)
y=paste(c(rep(1,15),rep(2,15)),"Time",rep(c(1,2,3,4,5,1.1,2.5, 3.7, 4.1,5.5),3),
sep="")
start=c(1,6,11,16,21,26)
for(i in start){
y[i]=paste(y[i],"Start",sep="")
}
for(i in start+4){
y[i]=paste(y[i],"End",sep="")
}
x=matrix(rnorm(1000*30),ncol=30)
x[1:50,16:20]=x[1:50,16:20]+matrix(3*c(0,1,2,3,4),ncol=5,nrow=50,byrow=TRUE)
x[1:50,21:25]=x[1:50,21:25]+matrix(3*c(0,1,2,3,4),ncol=5,nrow=50,byrow=TRUE)
x[1:50,26:30]=x[1:50,26:30]+matrix(3*c(0,1,2,3,4),ncol=5,nrow=50,byrow=TRUE)

x[51:100,16:20]=x[51:100,16:20]-matrix(3*c(0,1,2,3,4),ncol=5,nrow=50,byrow=TRUE)
x[51:100,21:25]=x[51:100,21:25]-matrix(3*c(0,1,2,3,4),ncol=5,nrow=50,byrow=TRUE)
x[51:100,26:30]=x[51:100,26:30]-matrix(3*c(0,1,2,3,4),ncol=5,nrow=50,byrow=TRUE)

```

```
a<- SAM(x,y, resp.type="Two class unpaired timecourse",
  nperms=100, time.summary.type="slope")
```

samr

Significance analysis of microarrays

Description

Correlates a large number of features (eg genes) with an outcome variable, such as a group indicator, quantitative variable or survival time. NOTE: for most users, the interface function SAM— which calls samr— will be more convenient for array data, and the interface function SAMseq— which also calls samr— will be more convenient for sequencing data.

Usage

```
samr(data, resp.type=c("Quantitative", "Two class unpaired",
  "Survival", "Multiclass", "One class", "Two class paired",
  "Two class unpaired timecourse", "One class timecourse",
  "Two class paired timecourse", "Pattern discovery"),
  assay.type=c("array", "seq"), s0=NULL, s0.perc=NULL, nperms=100,
  center.arrays=FALSE, testStatistic=c("standard", "wilcoxon"),
  time.summary.type=c("slope", "signed.area"),
  regression.method=c("standard", "ranks"), return.x=FALSE,
  knn.neighbors=10, random.seed=NULL, nresamp=20, nresamp.perm=NULL,
  xl.mode=c("regular", "firsttime", "next20", "lasttime"),
  xl.time=NULL, xl.prevfit=NULL)
```

Arguments

data	Data object with components x- p by n matrix of features, one observation per column (missing values allowed); y- n-vector of outcome measurements; censoring.status- n-vector of censoring censoring.status (1= died or event occurred, 0=survived, or event was censored), needed for a censored survival outcome
resp.type	Problem type: "Quantitative" for a continuous parameter (Available for both array and sequencing data); "Two class unpaired" (for both array and sequencing data); "Survival" for censored survival outcome (for both array and sequencing data); "Multiclass": more than 2 groups (for both array and sequencing data); "One class" for a single group (only for array data); "Two class paired" for two classes with paired observations (for both array and sequencing data); "Two class unpaired timecourse" (only for array data), "One class time course" (only for array data), "Two class.paired timecourse" (only for array data), or "Pattern discovery" (only for array data)
assay.type	Assay type: "array" for microarray data, "seq" for counts from sequencing

<code>s0</code>	Exchangeability factor for denominator of test statistic; Default is automatic choice. Only used for array data.
<code>s0.perc</code>	Percentile of standard deviation values to use for <code>s0</code> ; default is automatic choice; -1 means <code>s0=0</code> (different from <code>s0.perc=0</code> , meaning <code>s0=zeroeth</code> percentile of standard deviation values= min of <code>sd</code> values. Only used for array data.
<code>nperms</code>	Number of permutations used to estimate false discovery rates
<code>center.arrays</code>	Should the data for each sample (array) be median centered at the outset? Default =FALSE. Only used for array data.,
<code>testStatistic</code>	Test statistic to use in two class unpaired case. Either "standard" (t-statistic) or "wilcoxon" (Two-sample wilcoxon or Mann-Whitney test). Only used for array data.
<code>time.summary.type</code>	Summary measure for each time course: "slope", or "signed.area"). Only used for array data.
<code>regression.method</code>	Regression method for quantitative case: "standard", (linear least squares) or "ranks" (linear least squares on ranked data). Only used for array data.
<code>return.x</code>	Should the matrix of feature values be returned? Only useful for time course data, where <code>x</code> contains summaries of the features over time. Otherwise <code>x</code> is the same as the input data <code>data[extract_itex]x</code>
<code>knn.neighbors</code>	Number of nearest neighbors to use for imputation of missing features values. Only used for array data.
<code>random.seed</code>	Optional initial seed for random number generator (integer)
<code>nresamp</code>	For <code>assay.type="seq"</code> , number of resamples used to construct test statistic. Default 20. Only used for sequencing data.
<code>nresamp.perm</code>	For <code>assay.type="seq"</code> , number of resamples used to construct test statistic for permutations. Default is equal to <code>nresamp</code> and it must be at most <code>nresamp</code> . Only used for sequencing data.
<code>x1.mode</code>	Used by Excel interface
<code>x1.time</code>	Used by Excel interface
<code>x1.prevfit</code>	Used by Excel interface

Details

Carries out a SAM analysis. Applicable to microarray data, sequencing data, and other data with a large number of features. This is the R package that is called by the "official" SAM Excel package v2.0. The format of the response vector `y` and the calling sequence is illustrated in the examples below. A more complete description is given in the SAM manual at <http://www-stat.stanford.edu/~tibs/SAM>

Value

A list with components

`n` Number of observations

x	Data matrix p by n (p=# genes or features). Equal to the matrix data\%x in the original call to samr except for (1) time course analysis, where it contains the summarized data or (2) quantitative outcome with rank regression, where it contains the data transformed to ranks. Hence it is null except for in time course analysis.
y	Vector of n outcome values. equal the values data\%y in the original call to samr, except for (1) time course analysis, where it contains the summarized y or (2) quantitative outcome with rank regression, where it contains the y values transformed to ranks
argy	The values data\%y in the original call to samr
censoring.status	Censoring status indicators if applicable
testStatistic	Test Statistic used
,	
nperms	Number of permutations requested
nperms.act	Number of permutations actually used. Will be < nperms when % of possible permutations <= nperms (in which case all permutations are done)
tt	tt=numer/sd, the vector of p test statistics for original data
numer	Numerators for tt
sd	Denominators for tt. Equal to standard deviation for feature plus s0
s0	Computed exchangeability factor
s0.perc	Computed percentile of standard deviation values. s0= s0.perc percentile of the gene standard deviations
eva	p-vector of expected values for tt under permutation sampling
perms	nperms.act by n matrix of permutations used. Each row is a permutation of 1,2,...n
permsy	nperms.act by n matrix of permutations used. Each row is a permutation of y1,y2,...yn. Only one of perms or permsy is non-Null, depending on resp.type
all.perms.flag	Were all possible permutations used?
ttstar	p by nperms.act matrix t of test statistics from permuted data. Each column is sorted in descending order
ttstar0	p by nperms.act matrix of test statistics from permuted data. Columns are in order of data
eigengene.number	The number of the eigengene (eg 1,2,...) that was requested for Pattern discovery
eigengene	Computed eigengene
pi0	Estimated proportion of non-null features (genes)
foldchange	p-vector of foldchanges for original data
foldchange.star	p by nperms.act matrix estimated foldchanges from permuted data
sdstar.keep	n by nperms.act matrix of standard deviations from each permutation

censoring.status.star.keep
 n by nperms.act matrix of censoring.status indicators from each permutation

resp.type The response type used. Same as resp.type.arg, except for time course data, where time data is summarized and then treated as non-time course. Eg if resp.type.arg="oneclass.timecourse" then resp.type="oneclass"

resp.type.arg The response type requested in the call to samr

stand.contrasts
 For multiclass data, p by nclass matrix of standardized differences between the class mean and the overall mean

stand.contrasts.star
 For multiclass data, p by nclass by nperms.act array of standardized contrasts for permuted datasets

stand.contrasts.95
 For multiclass data, 2.5 of standardized contrasts. Useful for determining which class contrast for significant genes, are large

depth For array.type="seq", estimated sequencing depth for each sample.

call calling sequence

Author(s)

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References

Tusher, V., Tibshirani, R. and Chu, G. (2001): Significance analysis of microarrays applied to the ionizing radiation response PNAS 2001 98: 5116-5121, (Apr 24). <http://www-stat.stanford.edu/~tibs/SAM>

Li, Jun and Tibshirani, R. (2011). Finding consistent patterns: a nonparametric approach for identifying differential expression in RNA-Seq data. To appear, Statistical Methods in Medical Research.

Examples

```
##### two class unpaired comparison
# y must take values 1,2

set.seed(100)
x<-matrix(rnorm(1000*20),ncol=20)
dd<-sample(1:1000,size=100)

u<-matrix(2*rnorm(100),ncol=10,nrow=100)
x[dd,11:20]<-x[dd,11:20]+u
y<-c(rep(1,10),rep(2,10))

data=list(x=x,y=y, geneid=as.character(1:nrow(x)),
          genenames=paste("g",as.character(1:nrow(x)),sep=""), logged2=TRUE)

samr.obj<-samr(data, resp.type="Two class unpaired", nperms=100)
```

```

delta=.4
samr.plot(samr.obj,delta)

delta.table <- samr.compute.delta.table(samr.obj)

siggenes.table<-samr.compute.siggenes.table(samr.obj,delta, data, delta.table)

# sequence data

set.seed(3)
x<-abs(100*matrix(rnorm(1000*20),ncol=20))
x=trunc(x)
y<- c(rep(1,10),rep(2,10))
x[1:50,y==2]=x[1:50,y==2]+50
data=list(x=x,y=y, geneid=as.character(1:nrow(x)),
genenames=paste("g",as.character(1:nrow(x)),sep=""))

samr.obj<-samr(data, resp.type="Two class unpaired",assay.type="seq", nperms=100)

delta=5
samr.plot(samr.obj,delta)

delta.table <- samr.compute.delta.table(samr.obj)

siggenes.table<-samr.compute.siggenes.table(samr.obj,delta, data, delta.table)


##### two class paired

# y must take values -1, 1, -2,2 etc, with (-k,k) being a pair

set.seed(100)
x<-matrix(rnorm(1000*20),ncol=20)
dd<-sample(1:1000,size=100)

u<-matrix(2*rnorm(100),ncol=10,nrow=100)
x[dd,11:20]<-x[dd,11:20]+u
y=c(-(1:10),1:10)

d=list(x=x,y=y, geneid=as.character(1:nrow(x)),
genenames=paste("g",as.character(1:nrow(x)),sep=""), logged2=TRUE)

samr.obj<-samr(d, resp.type="Two class paired", nperms=100)


#####quantitative response

```

```

# y must take numeric values

set.seed(84048)
x=matrix(rnorm(1000*9),ncol=9)

mu=c(3,2,1,0,0,0,1,2,3)
b=runif(100)+.5
x[1:100,]=x[1:100,]+ b

y=mu

d=list(x=x,y=y,
geneid=as.character(1:nrow(x)),genenames=paste("gene", as.character(1:nrow(x))))

samr.obj =samr(d, resp.type="Quantitative", nperms=50)

##### oneclass
# y is a vector of ones

set.seed(100)
x<-matrix(rnorm(1000*20),ncol=20)
dd<-sample(1:1000,size=100)

u<-matrix(2*rnorm(100),ncol=10,nrow=100)
x[dd,11:20]<-x[dd,11:20]+u

y<-c(rep(1,20))

data=list(x=x,y=y, geneid=as.character(1:nrow(x)),
genenames=paste("g",as.character(1:nrow(x)),sep=""), logged2=TRUE)

samr.obj<-samr(data, resp.type="One class", nperms=100)

#####survival data
# y is numeric; censoring.status=1 for failures, and 0 for censored

set.seed(84048)
x=matrix(rnorm(1000*50),ncol=50)
x[1:50,26:50]= x[1:50,26:50]+2
x[51:100,26:50]= x[51:100,26:50]-2

y=abs(rnorm(50))
y[26:50]=y[26:50]+2
censoring.status=sample(c(0,1),size=50,replace=TRUE)
d=list(x=x,y=y,censoring.status=censoring.status,
geneid=as.character(1:1000),genenames=paste("gene", as.character(1:1000)))

samr.obj=samr(d, resp.type="Survival", nperms=20)

```

```
#####multi-class example
# y takes values 1,2,3,...k where k= number of classes

set.seed(84048)
x=matrix(rnorm(1000*10),ncol=10)
x[1:50,6:10]= x[1:50,6:10]+2
x[51:100,6:10]= x[51:100,6:10]-2

y=c(rep(1,3),rep(2,3),rep(3,4))
d=list(x=x,y=y,genid=as.character(1:1000),
      genenames=paste("gene", as.character(1:1000)))

samr.obj <- samr(d, resp.type="Multiclass")

##### timecourse data

# elements of y are of the form kTimet where k is the class label and t
# is the time; in addition, the suffixes Start or End indicate the first
# and last observation in a given time course
# the class label can be that for a two class unpaired, one class or
# two class paired problem

set.seed(8332)
y=paste(c(rep(1,15),rep(2,15)),"Time",rep(c(1,2,3,4,5,1.1,2.5, 3.7, 4.1,5.5),3),
      sep="")
start=c(1,6,11,16,21,26)
for(i in start){
  y[i]=paste(y[i],"Start",sep="")
}
for(i in start+4){
  y[i]=paste(y[i],"End",sep="")
}
x=matrix(rnorm(1000*30),ncol=30)
x[1:50,16:20]=x[1:50,16:20]+matrix(3*c(0,1,2,3,4),ncol=5,nrow=50,byrow=TRUE)
x[1:50,21:25]=x[1:50,21:25]+matrix(3*c(0,1,2,3,4),ncol=5,nrow=50,byrow=TRUE)
x[1:50,26:30]=x[1:50,26:30]+matrix(3*c(0,1,2,3,4),ncol=5,nrow=50,byrow=TRUE)

x[51:100,16:20]=x[51:100,16:20]-matrix(3*c(0,1,2,3,4),ncol=5,nrow=50,byrow=TRUE)
x[51:100,21:25]=x[51:100,21:25]-matrix(3*c(0,1,2,3,4),ncol=5,nrow=50,byrow=TRUE)
x[51:100,26:30]=x[51:100,26:30]-matrix(3*c(0,1,2,3,4),ncol=5,nrow=50,byrow=TRUE)

data=list(x=x,y=y, genid=as.character(1:nrow(x)),
      genenames=paste("g",as.character(1:nrow(x)),sep=""), logged2=TRUE)

samr.obj<- samr(data, resp.type="Two class unpaired timecourse",
      nperms=100, time.summary.type="slope")

##### pattern discovery
```

```
# here there is no outcome y; the desired eigengene is indicated by
# the argument eigengene.numbe in the data object

set.seed(32)
x=matrix(rnorm(1000*9),ncol=9)
mu=c(3,2,1,0,0,0,1,2,3)
b=3*runif(100)+.5
x[1:100,]=x[1:100,]+ b

d=list(x=x,eigengene.number=1,
geneid=as.character(1:nrow(x)),genenames=paste("gene", as.character(1:nrow(x))))

samr.obj=samr(d, resp.type="Pattern discovery", nperms=50)
```

samr.assess.samplesize

Assess the sample size for a SAM analysis

Description

Estimate the false discovery rate, false negative rate, power and type I error for a SAM analysis. Currently implemented only for two class (unpaired or paired), one-sample and survival problems).

Usage

```
samr.assess.samplesize(samr.obj, data, dif, samplesize.factors=c(1,2,3,5),
min.genes = 10, max.genes = nrow(data$x)/2)
```

Arguments

samr.obj	Object returned from call to samr
data	Data list, same as that passed to samr.train
dif	Change in gene expression between groups 1 and 2, for genes that are differentially expressed. For log base 2 data, a value of 1 means a 2-fold change. For One-sample problems, dif is the number of units away from zero for differentially expressed genes. For survival data, dif is the numerator of the Cox score statistic (this info is provided in the output of samr).
samplesize.factors	Integer vector of length 4, indicating the sample sizes to be examined. The values are factors that multiply the original sample size. So the value 1 means a sample size of ncol(data\$x), 2 means a sample size of ncol(data\$x), etc.
min.genes	Minimum number of genes that are assumed to truly changed in the population
max.genes	Maximum number of genes that are assumed to truly changed in the population

Details

Estimates false discovery rate, false negative rate, power and type I error for a SAM analysis. The argument `samplesize.factor` allows the use to assess the effect of varying the sample size (total number of samples). A detailed description of this calculation is given in the SAM manual at <http://www-stat.stanford.edu/~tibs/SAM>

Value

A list with components

<code>Results</code>	A matrix with columns: number of genes- both the number differentially expressed genes in the population and number called significant; cutpoint- the threshold used for the absolute SAM score d ; FDR, 1-power- the median false discovery rate, also equal to the power for each gene; FDR-90perc- the upper 90th percentile of the FDR; FNR, Type 1 error- the false negative rate, also equal to the type I error for each gene; FNR-90perc- the upper 90th percentile of the FNR
<code>dif.call</code>	Change in gene expression between groups 1 and 2, that was provided in the call to <code>samr.assess.samplesize</code>
<code>difm</code>	The average difference in SAM score d for the genes differentially expressed vs unexpressed
<code>samplesize.factor</code>	The <code>samplesize.factor</code> that was passed to <code>samr.assess.samplesize</code>
<code>n</code>	Number of samples in input data (i.e. <code>ncol</code> of <code>x</code> component in data)

Author(s)

Jun Li and Balasubramanian Narasimhan and Robert Tibshirani

References

Tusher, V., Tibshirani, R. and Chu, G. (2001): Significance analysis of microarrays applied to the ionizing radiation response" PNAS 2001 98: 5116-5121, (Apr 24). <http://www-stat.stanford.edu/~tibs/sam>

Taylor, J., Tibshirani, R. and Efron. B. (2005). The "Miss rate" for the analysis of gene expression data. Biostatistics 2005 6(1):111-117.

A more complete description is given in the SAM manual at <http://www-stat.stanford.edu/~tibs/SAM>

Examples

```
#generate some example data
set.seed(100)
x<-matrix(rnorm(1000*20),ncol=20)
dd<-sample(1:1000,size=100)

u<-matrix(2*rnorm(100),ncol=10,nrow=100)
x[dd,11:20]<-x[dd,11:20]+u

y<-c(rep(1,10),rep(2,10))
```

```

data=list(x=x,y=y, geneid=as.character(1:nrow(x)),
          genenames=paste("g",as.character(1:nrow(x)),sep=""), logged2=TRUE)

log2=function(x){log(x)/log(2)}

# run SAM first
samr.obj<-samr(data, resp.type="Two class unpaired", nperms=100)

# assess current sample size (20), assuming 1.5fold difference on log base 2 scale

samr.assess.samplesize.obj<- samr.assess.samplesize(samr.obj, data, log2(1.5))

# assess the effect of doubling the sample size

samr.assess.samplesize.obj2<- samr.assess.samplesize(samr.obj, data, log2(1.5))

```

```
samr.assess.samplesize.plot
```

Make a plot of the results from samr.assess.samplesize

Description

Plots of the results from samr.assess.samplesize

Usage

```
samr.assess.samplesize.plot(samr.assess.samplesize.obj, logx=TRUE,
call.win.metafile=FALSE)
```

Arguments

samr.assess.samplesize.obj	Object returned from call to samr.assess.samplesize
logx	Should logs be used on the horizontal (\# of genes) axis? Default TRUE
call.win.metafile	Used by Excel interface

Details

Plots results: FDR (or 1-power) and FNR (or 1-type 1 error) from samr.assess.samplesize

Author(s)

Jun Li and Balasubrimanian Narasimhan and Robert Tibshirani

References

Tusher, V., Tibshirani, R. and Chu, G. (2001): Significance analysis of microarrays applied to the ionizing radiation response" PNAS 2001 98: 5116-5121, (Apr 24). <http://www-stat.stanford.edu/~tibs/sam>

Examples

```
#generate some example data
set.seed(100)
x<-matrix(rnorm(1000*20),ncol=20)
dd<-sample(1:1000,size=100)

u<-matrix(2*rnorm(100),ncol=10,nrow=100)
x[dd,11:20]<-x[dd,11:20]+u

y<-c(rep(1,10),rep(2,10))

data=list(x=x,y=y, geneid=as.character(1:nrow(x)),
          genenames=paste("g",as.character(1:nrow(x)),sep=""), logged2=TRUE)

log2=function(x){log(x)/log(2)}

# run SAM first
samr.obj<-samr(data, resp.type="Two class unpaired", nperms=100)

# assess current sample size (20), assuming 1.5fold difference on the log base 2 scale

samr.assess.samplesize.obj<- samr.assess.samplesize(samr.obj, data, log2(1.5))

samr.assess.samplesize.plot(samr.assess.samplesize.obj)
```

samr.compute.delta.table

Compute delta table for SAM analysis

Description

Computes tables of thresholds, cutpoints and corresponding False Discovery rates for SAM (Significance analysis of microarrays) analysis

Usage

```
samr.compute.delta.table(samr.obj, min.foldchange=0, dels=NULL, nvals=50)
```

Arguments

samr.obj	Object returned from call to samr
min.foldchange	The minimum fold change desired; should be >1; default is zero, meaning no fold change criterion is applied

dels	vector of delta values used. Delta is the vertical distance from the 45 degree line to the upper and lower parallel lines that define the SAM threshold rule. By default, for array data, 50 values are chosen in the relevant operating change for delta. For sequencing data, the maximum number of effective delta values are chosen automatically according to the data.
nvals	Number of delta values used. For array data, the default value is 50. For sequencing data, the value will be chosen automatically.

Details

Returns a table of the FDR and upper and lower cutpoints for various values of delta, for a SAM analysis.

Author(s)

Balasubramanian Narasimhan and Robert Tibshirani

References

Tusher, V., Tibshirani, R. and Chu, G. (2001): Significance analysis of microarrays applied to the ionizing radiation response" PNAS 2001 98: 5116-5121, (Apr 24). <http://www-stat.stanford.edu/~tibs/sam>

Examples

```
#generate some example data
set.seed(100)
x<-matrix(rnorm(1000*20),ncol=20)
dd<-sample(1:1000,size=100)

u<-matrix(2*rnorm(100),ncol=10,nrow=100)
x[dd,11:20]<-x[dd,11:20]+u

y<-c(rep(1,10),rep(2,10))

data=list(x=x,y=y, geneid=as.character(1:nrow(x)),
          genenames=paste("g",as.character(1:nrow(x)),sep=""), logged2=TRUE)

samr.obj<-samr(data, resp.type="Two class unpaired", nperms=50)

delta.table<- samr.compute.delta.table(samr.obj)
```

samr.compute.siggenes.table

Compute significant genes table

Description

Computes significant genes table, starting with samr object "samr.obj" and delta.table "delta.table"

Usage

```
samr.compute.siggenes.table(samr.obj, del, data, delta.table,
min.foldchange=0, all.genes=FALSE, compute.localfdr=FALSE)
```

Arguments

samr.obj	Object returned from call to samr
del	Value of delta to define cutoff rule
data	Data object, same as that used in call to samr
delta.table	Object returned from call to samr.compute.delta.table
min.foldchange	The minimum fold change desired; should be >1; default is zero, meaning no fold change criterion is applied
all.genes	Should all genes be listed? Default FALSE
compute.localfdr	Should the local fdrs be computed (this can take some time)? Default FALSE

Value

```
return(list(genes.up=res.up, genes.lo=res.lo, color.ind.for.multi=color.ind.for.multi, ngenes.up=ngenes.up,
ngenes.lo=ngenes.lo))
```

genes.up	Matrix of significant genes having positive correlation with the outcome. For survival data, genes.up are those genes having positive correlation with risk- that is, increased expression corresponds to higher risk (shorter survival).
genes.lo	Matrix of significant genes having negative correlation with the outcome. For survival data, genes.lo are those whose increased expression corresponds to lower risk (longer survival).
color.ind.for.multi	For multiclass response: a matrix with entries +1 if the class mean is larger than the overall mean at the 95 levels, -1 if less, and zero otherwise. This is useful in determining which class or classes causes a feature to be significant
ngenes.up	Number of significant genes with positive correlation
ngenes.lo	Number of significant genes with negative correlation

Author(s)

Balasubramanian Narasimhan and Robert Tibshirani

References

Tusher, V., Tibshirani, R. and Chu, G. (2001): Significance analysis of microarrays applied to the ionizing radiation response" PNAS 2001 98: 5116-5121, (Apr 24). <http://www-stat.stanford.edu/~tibs/sam>

Examples

```
#generate some example data
set.seed(100)
x<-matrix(rnorm(1000*20),ncol=20)
dd<-sample(1:1000,size=100)

u<-matrix(2*rnorm(100),ncol=10,nrow=100)
x[dd,11:20]<-x[dd,11:20]+u

y<-c(rep(1,10),rep(2,10))

data=list(x=x,y=y, geneid=as.character(1:nrow(x)),
          genenames=paste("g",as.character(1:nrow(x)),sep=""), logged2=TRUE)

samr.obj<-samr(data, resp.type="Two class unpaired", nperms=100)

delta.table<-samr.compute.delta.table(samr.obj)
del<- 0.3
siggenes.table<- samr.compute.siggenes.table(samr.obj, del, data, delta.table)
```

samr.estimate.depth	<i>estimate the sequencing depth</i>
---------------------	--------------------------------------

Description

Estimate the sequencing depth of each experiment for sequencing data.

Usage

```
samr.estimate.depth(x)
```

Arguments

x the original count matrix. p by n matrix of features, one observation per column.

Details

normalize the data matrix so that each number looks roughly like Gaussian distributed and each experiment has the same sequencing depth. To do this, we first use Anscombe transformation to stabilize the variance and makes each number look like Gaussian, and then divide each experiment by the square root of the sequencing depth.

Value

depth sequencing depth of each experiment. a vector of length n.

Author(s)

Jun Li and Balasubrimanian Narasimhan and Robert Tibshirani

References

Tusher, V., Tibshirani, R. and Chu, G. (2001): Significance analysis of microarrays applied to the ionizing radiation response PNAS 2001 98: 5116-5121, (Apr 24). <http://www-stat.stanford.edu/~tibs/SAM>

Examples

```
set.seed(100)
mu <- matrix(100, 1000, 20)
mu[1:100, 11:20] <- 200
mu <- scale(mu, center=FALSE, scale=runif(20, 0.5, 1.5))
x <- matrix(rpois(length(mu), mu), 1000, 20)
y <- c(rep(1, 10), rep(2, 10))
data=list(x=x,y=y, geneid=as.character(1:nrow(x)),
          genenames=paste("g",as.character(1:nrow(x)),sep=""))
depth <- samr.estimate.depth(data$x)
```

samr.missrate

Estimate the miss rate table for a SAM analysis

Description

Estimates the miss rate table, showing the local false negative rate, for a SAM analysis

Usage

```
samr.missrate(samr.obj, del, delta.table, quant=NULL)
```

Arguments

samr.obj	Object returned from call to samr
del	Value of delta to define cutoff rule
delta.table	Object returned from call to samr.compute.delta.table
quant	Optional vector of quantiles to used in the miss rate calculation

Author(s)

Jun Li and Balasubrimanian Narasimhan and Robert Tibshirani

References

Tusher, V., Tibshirani, R. and Chu, G. (2001): Significance analysis of microarrays applied to the ionizing radiation response" PNAS 2001 98: 5116-5121, (Apr 24). <http://www-stat.stanford.edu/~tibs/sam>

Taylor, J., Tibshirani, R. and Efron. B. (2005). The "Miss rate" for the analysis of gene expression data. Biostatistics 2005 6(1):111-117.

Examples

```
#generate some example data
set.seed(100)
x<-matrix(rnorm(1000*20),ncol=20)
dd<-sample(1:1000,size=100)

u<-matrix(2*rnorm(100),ncol=10,nrow=100)
x[dd,11:20]<-x[dd,11:20]+u

y<-c(rep(1,10),rep(2,10))

data=list(x=x,y=y, geneid=as.character(1:nrow(x)),
          genenames=paste("g",as.character(1:nrow(x)),sep=""), logged2=TRUE)

samr.obj<-samr(data, resp.type="Two class unpaired", nperms=100)

delta.table<-samr.compute.delta.table(samr.obj)
del<- 0.3
siggenes.table<- samr.compute.siggenes.table(samr.obj, del, data, delta.table)

samr.missrate(samr.obj, del, delta.table)
```

samr.norm.data	<i>output normalized sequencing data</i>
----------------	--

Description

Output a normalized sequencing data matrix from the original count matrix.

Usage

```
samr.norm.data(x, depth=NULL)
```

Arguments

x	the original count matrix. p by n matrix of features, one observation per column.
depth	sequencing depth of each experiment. a vector of length n. This function will estimate the sequencing depth if it is not specified.

Details

normalize the data matrix so that each number looks roughly like Gaussian distributed and each experiment has the same sequencing depth. To do this, we first use Anscombe transformation to stabilize the variance and makes each number look like Gaussian, and then divide each experiment by the square root of the sequencing depth.

Value

x the normalized data matrix.

Author(s)

Jun Li and Balasubramanian Narasimhan and Robert Tibshirani

References

Tusher, V., Tibshirani, R. and Chu, G. (2001): Significance analysis of microarrays applied to the ionizing radiation response PNAS 2001 98: 5116-5121, (Apr 24). <http://www-stat.stanford.edu/~tibs/SAM>

Examples

```
set.seed(100)
mu <- matrix(100, 1000, 20)
mu[1:100, 11:20] <- 200
mu <- scale(mu, center=FALSE, scale=runif(20, 0.5, 1.5))
x <- matrix(rpois(length(mu), mu), 1000, 20)
y <- c(rep(1, 10), rep(2, 10))
data=list(x=x,y=y, geneid=as.character(1:nrow(x)),
  genenames=paste("g",as.character(1:nrow(x)),sep=""))
x.norm <- samr.norm.data(data$x)
```

samr.plot

Make Q-Q plot for SAM analysis

Description

Makes the Q-Q plot for a SAM analysis

Usage

```
samr.plot(samr.obj, del, min.foldchange=0)
```

Arguments

samr.obj	Object returned from call to samr
del	Value of delta to use. Delta is the vertical distance from the 45 degree line to the upper and lower parallel lines that define the SAM threshold rule.
min.foldchange	The minimum fold change desired; should be >1; default is zero, meaning no fold change criterion is applied

Details

Creates the Q-Q plot fro a SAm analysis, marking features (genes) that are significant, ie. lie outside a slab around teh 45 degreee line of width delta. A gene must also pass the min.foldchange criterion to be called significant, if this criterion is specified in the call.

Author(s)

Jun Li and Balasubramanian Narasimhan and Robert Tibshirani

References

Tusher, V., Tibshirani, R. and Chu, G. (2001): Significance analysis of microarrays applied to the ionizing radiation response" PNAS 2001 98: 5116-5121, (Apr 24). <http://www-stat.stanford.edu/~tibs/sam>

Examples

```
#generate some example data
set.seed(100)
x<-matrix(rnorm(1000*20),ncol=20)
dd<-sample(1:1000,size=100)

u<-matrix(2*rnorm(100),ncol=10,nrow=100)
x[dd,11:20]<-x[dd,11:20]+u

y<-c(rep(1,10),rep(2,10))

data=list(x=x,y=y, geneid=as.character(1:nrow(x)),
  genenames=paste("g",as.character(1:nrow(x)),sep=""), logged2=TRUE)

samr.obj<-samr(data, resp.type="Two class unpaired", nperms=50)

samr.plot(samr.obj, del=.3)
```

samr.pvalues.from.perms

Report estimated p-values for each gene, from a SAM analysis

Description

Report estimated p-values for each gene, from set of permutations in a SAM analysis

Usage

```
samr.pvalues.from.perms(tt, ttstar)
```

Arguments

tt	Vector of gene scores, returned by samr in component tt
ttstar	Matrix of gene scores (p by nperm) from nperm permutations. Returned by samr in component ttstar

Author(s)

Jun Li and Balasubrimanian Narasimhan and Robert Tibshirani

References

Taylor, J. and Tibshirani, R. (2005): A tail strength measure for assessing the overall significance in a dataset. Submitted.

Examples

```
#generate some example data
set.seed(100)
x<-matrix(rnorm(1000*20),ncol=20)
dd<-sample(1:1000,size=100)

u<-matrix(2*rnorm(100),ncol=10,nrow=100)
x[dd,11:20]<-x[dd,11:20]+u

y<-c(rep(1,10),rep(2,10))

data=list(x=x,y=y, geneid=as.character(1:nrow(x)),
  genenames=paste("g",as.character(1:nrow(x)),sep=""), logged2=TRUE)
samr.obj<-samr(data, resp.type="Two class unpaired", nperms=100)

pv=samr.pvalues.from.perms(samr.obj$tt, samr.obj$ttstar)
```

samr.tail.strength	<i>Estimate tail strength for a dataset, from a SAM analysis</i>
--------------------	--

Description

Estimate tail strength for a dataset, from a SAM analysis

Usage

```
samr.tail.strength(samr.obj)
```

Arguments

samr.obj	Object returned by samr
----------	-------------------------

Value

A list with components

ts	Estimated tail strength. A number less than or equal to 1. Zero means all genes are null; 1 means all genes are differentially expressed.
----	---

,

se.ts	Estimated standard error of tail strength.
-------	--

Author(s)

Jun Li and Balasubramanian Narasimhan and Robert Tibshirani

References

Taylor, J. and Tibshirani, R. (2005): A tail strength measure for assessing the overall significance in a dataset. Submitted.

Examples

```
#generate some example data
set.seed(100)
x<-matrix(rnorm(1000*20),ncol=20)
dd<-sample(1:1000,size=100)

u<-matrix(2*rnorm(100),ncol=10,nrow=100)
x[dd,11:20]<-x[dd,11:20]+u

y<-c(rep(1,10),rep(2,10))

data=list(x=x,y=y, geneid=as.character(1:nrow(x)),
  genenames=paste("g",as.character(1:nrow(x)),sep=""), logged2=TRUE)

samr.obj<-samr(data, resp.type="Two class unpaired", nperms=100)

samr.tail.strength(samr.obj)
```

SAMseq

Significance analysis of sequencing data - simple user interface

Description

Correlates a large number of features (eg. genes) with an outcome variable, such as a group indicator, quantitative variable or survival time. This is a simple user interface for the samr function applied to sequencing data. For array data applications, see the function SAM.

Usage

```
SAMseq(x, y, censoring.status = NULL,
  resp.type = c("Quantitative", "Two class unpaired",
    "Survival", "Multiclass", "Two class paired"),
  geneid = NULL, genenames = NULL, nperms = 100,
  random.seed = NULL, nresamp = 20, fdr.output = 0.20)
```

Arguments

<code>x</code>	Feature matrix: p (number of features) by n (number of samples), one observation per column (missing values allowed)
<code>y</code>	n -vector of outcome measurements
<code>censoring.status</code>	n -vector of censoring <code>censoring.status</code> (1=died or event occurred, 0=survived, or event was censored), needed for a censored survival outcome
<code>resp.type</code>	Problem type: "Quantitative" for a continuous parameter; "Two class unpaired" for two classes with unpaired observations; "Survival" for censored survival outcome; "Multiclass": more than 2 groups; "Two class paired" for two classes with paired observations.
<code>geneid</code>	Optional character vector of geneids for output.
<code>genenames</code>	Optional character vector of genenames for output.
<code>nperms</code>	Number of permutations used to estimate false discovery rates
<code>random.seed</code>	Optional initial seed for random number generator (integer)
<code>nresamp</code>	Number of resamples used to construct test statistic. Default 20.
<code>fdr.output</code>	(Approximate) False Discovery Rate cutoff for output in significant genes table

Details

This is a simple, user-friendly interface to the `samr` package used on sequencing data. It automatically disables arguments/features that do not apply to sequencing data. It calls `samr`, `samr.compute.delta.table` and `samr.compute.siggenes.table`. `samr` detects differential expression for microarray data, and sequencing data, and other data with a large number of features. `samr` is the R package that is called by the "official" SAM Excel Addin. The format of the response vector `y` and the calling sequence is illustrated in the examples below. A more complete description is given in the SAM manual at <http://www-stat.stanford.edu/~tibs/SAM>

Value

A list with components

<code>samr.obj</code>	Output of <code>samr</code> . See documentation for <code>samr</code> for details
<code>siggenes.table</code>	Table of significant genes, output of <code>samr.compute.siggenes.table</code> . This has components: <code>genes.up</code> —matrix of significant genes having positive correlation with the outcome and <code>genes.lo</code> —matrix of significant genes having negative correlation with the outcome. For survival data, <code>genes.up</code> are those genes having positive correlation with risk- that is, increased expression corresponds to higher risk (shorter survival) <code>genes.lo</code> are those whose increased expression corresponds to lower risk (longer survival).
<code>delta.table</code>	Output of <code>samr.compute.delta.table</code> .
<code>del</code>	Value of delta (distance from 45 degree line in SAM plot) for used for creating <code>delta.table</code> and <code>siggenes.table</code> . Changing the input value <code>fdr.output</code> will change the resulting <code>del</code> .
<code>call</code>	The calling sequence

Author(s)

Jun Li and Balasubramanian Narasimhan and Robert Tibshirani

References

Tusher, V., Tibshirani, R. and Chu, G. (2001): Significance analysis of microarrays applied to the ionizing radiation response PNAS 2001 98: 5116-5121, (Apr 24). <http://www-stat.stanford.edu/~tibs/SAM>

Li, Jun and Tibshirani, R. (2011). Finding consistent patterns: a nonparametric approach for identifying differential expression in RNA-Seq data. To appear, Statistical Methods in Medical Research.

Examples

```
##### two class unpaired comparison
set.seed(100)
mu <- matrix(100, 1000, 20)
mu[1:100, 11:20] <- 200
mu <- scale(mu, center=FALSE, scale=runif(20, 0.5, 1.5))
x <- matrix(rpois(length(mu), mu), 1000, 20)
y <- c(rep(1, 10), rep(2, 10))

samfit <- SAMseq(x, y, resp.type = "Two class unpaired")

# examine significant gene list
print(samfit)

# plot results
plot(samfit)

##### two class paired comparison
set.seed(100)
mu <- matrix(100, 1000, 20)
mu[1:100, 11:20] <- 200
mu <- scale(mu, center=FALSE, scale=runif(20, 0.5, 1.5))
x <- matrix(rpois(length(mu), mu), 1000, 20)
y <- c(-(1:10), 1:10)

samfit <- SAMseq(x, y, resp.type = "Two class paired")

# examine significant gene list
print(samfit)

# plot results
plot(samfit)

##### Multiclass comparison
set.seed(100)
mu <- matrix(100, 1000, 20)
mu[1:20, 1:5] <- 120
mu[21:50, 6:10] <- 80
mu[51:70, 11:15] <- 150
```

```
mu[71:100, 16:20] <- 60
mu <- scale(mu, center=FALSE, scale=runif(20, 0.5, 1.5))
x <- matrix(rpois(length(mu), mu), 1000, 20)
y <- c(rep(1:4, rep(5, 4)))

samfit <- SAMseq(x, y, resp.type = "Multiclass")

# examine significant gene list
print(samfit)

# plot results
plot(samfit)

##### Quantitative comparison
set.seed(100)
mu <- matrix(100, 1000, 20)
y <- runif(20, 1, 3)
mu[1 : 100, ] <- matrix(rep(100 * y, 100), ncol=20, byrow=TRUE)
mu <- scale(mu, center=FALSE, scale=runif(20, 0.5, 1.5))
x <- matrix(rpois(length(mu), mu), 1000, 20)
samfit <- SAMseq(x, y, resp.type = "Quantitative")

# examine significant gene list
print(samfit)

# plot results
plot(samfit)

##### Survival comparison
set.seed(100)
mu <- matrix(100, 1000, 20)
y <- runif(20, 1, 3)
mu[1 : 100, ] <- matrix(rep(100 * y, 100), ncol=20, byrow=TRUE)
mu <- scale(mu, center=FALSE, scale=runif(20, 0.5, 1.5))
x <- matrix(rpois(length(mu), mu), 1000, 20)
y <- y + runif(20, -0.5, 0.5)
censoring.status <- as.numeric(y < 2.3)
y[y >= 2.3] <- 2.3
samfit <- SAMseq(x, y, censoring.status = censoring.status,
resp.type = "Survival")

# examine significant gene list
print(samfit)

# plot results
plot(samfit)
```

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