Package ‘macat’

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Description This library contains functions to investigate links between differential gene expression and the chromosomal localization of the genes. MACAT is motivated by the common observation of phenomena involving large chromosomal regions in tumor cells. MACAT is the implementation of a statistical approach for identifying significantly differentially expressed chromosome regions. The functions have been tested on a publicly available data set about acute lymphoblastic leukemia (Yeoh et al. Cancer Cell 2002), which is provided in the library 'stjudem'.
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R topics documented:

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**buildMACAT**

Create MACAT list from objects in workspace

**Description**

This is a wrapper around the preprocessedLoader function. Use it, when you want to build a MACAT-list structure from objects already in your workspace.

**Usage**

```r
buildMACAT(matrix, chip, labels = NULL, chromLocObj = NULL)
```

**Arguments**

- `matrix`: expression matrix with rows=genes and columns=samples; Rownames have to match chip; Columnnames are not mandatory.
- `chip`: Identifier for used microarray
- `labels`: Classlabels for samples, has to have length=number of columns in matrix
- `chromLocObj`: Object of class `chromLocation` specifying the genomic position, each probe on the array is mapped to. If not provided, it is build in the function using `annotate`'s function `buildChromLocation`.

**Details**

This is only a convenience wrapper around the function `preprocessedLoader` for the case, that you want to build a MACAT-list from objects in your workspace.

**Value**

A MACAT-list structure. For an example and a description of the format see data `stjude` in package 'stjudem'.

**Author(s)**

MACAT development team

**See Also**

`preprocessedLoader`, `stjude` in package 'stjudem'
Examples

```r
tax <- matrix(rnorm(200),nrow=20,ncol=10)
rownames(text) <- c('34916_s_at','34917_at','34462_at','163_at','35219_at',
 '31641_s_at','33300_at','33301_g_at','38950_r_at','41249_at',
 '294_s_at','32004_s_at','33299_at','41243_at','33341_at','362_at',
 '1918_at','41499_at','41500_at','41282_s_at')
colnames(text) <- paste("Sample",1:10,sep="")
y <- rep(c("A","B"),c(5,5))
toy <- buildMACAT(text,"hgu95av2.db",y)
summary(toy)
```

compute.sliding

Compute and plot smoothing of expression values or scores along the chromosome

Description

`compute.sliding` computes a smoothing of the expression data or scores along the chromosome using the specified kernel function. This function is also used within the `evalScoring` function. `plotSliding` creates a plot of the smoothed expression values / scores.

Usage

```r
compute.sliding(data, chromosome, sample, kernel, kernelparams=NULL, step.width = 1e+06)
plotSliding(data, chromosome, sample, kernel, kernelparams=NULL, step.width=1000000, ...)
```

Arguments

- `data`: A MACATData list holding the Expression values and gene locations
- `chromosome`: the chromosome to be smoothed
- `sample`: the sample (patient) whose expression values are smoothed
- `kernel`: a kernel function (one of rbf, kNN, basePairDistance or your own)
- `kernelparams`: a list of named parameters for the kernel (by default estimated from the data)
- `step.width`: the smoothing is computed stepwise every step.width basepairs (default is 100000)
- `...`: further graphical parameters passed on to `plot.default`

Value

- `compute.sliding`: a matrix of dimension (steps x 2) with in the first column the locations in basepairs where an interpolation is computed, and in the second column the smoothed values.
- `plotSliding` does not return anything and is merely called for its side-effect producing the plot.

Author(s)

MACAT development team

See Also

- `kernelize`, `evalScoring`
Examples

data(stjd)
# just compute smoothed values:
smooth = compute.sliding(stjd, chromosome=3, sample=6, rbf,
kernelparams=list(gamma=1/10^13))
# compute and plot smoothed values:
plotSliding(stjd, chromosome=3, sample=6, rbf,
kernelparams=list(gamma=1/10^13), pch=20,
main="Chromosome 3")

discreteKernelize  Discretize and smooth expression values

Description

returns discretized kernelized expression values and saves them to a file if argument ‘saveToFile’ is TRUE. For details on discretization see discretize.

Usage

discreteKernelize(data, chrom, margin = 10, step.width = 1e+05, kernel = rbf,
kernelparams = list(gamma = 1/10^13), saveToFile = FALSE)

Arguments

data  MACATData Object  
chrom  chromosome to kernelize  
margin  symmetric quantile in percent  
step.width  size of the interpolation steps  
kernel  kernel function one of rbf, kNN, basePairDistance or your own  
kernelparams  list of named kernel parameters  
saveToFile  logical val indicating whether to write a flatfile or not; default is FALSE

Details

Filename of the flatfile is: discrete_kernelized_seq_margin_<margin>_<chrom>_<chrom>.py  
where <margin> is the discretization parameter and <chrom> the name of the chromosome.

Value

discretized and kernelized expression matrix

Author(s)

The MACAT Development team

See Also

pydata, kernelizeAll
discretize

Examples

```r
#loaddatapkg("stjude")
data(stjude)
data(stjd)
discretizedKernelized = discreteKernelize(stjd, 13)
```

Discretize expression values

Description

'discretize' returns the discretized expression data for all chromosomes in `chrom` and all samples that have a label listed in `label`. Discretization is performed by comparing the value gene-wise (location-wise) with the symmetric upper and lower quantile given by `margin` (in percent `margin/2` lower and upper quantile).

Usage

```r
discretize(data, chrom, label, margin = 10)
discretizeChromosome(data, chrom, margin=10)
discretizeOne(data, chrom, sample, margin=10)
```

Arguments

- `data`: MACATData object
- `chrom`: list of chromosomes
- `label`: list of labels
- `margin`: symmetric quantile in percent
- `sample`: the sample for which you want discretized expression data

Value

returns a discretized expression matrix for all genes on the chromosomes in `chrom` and all samples that have a label in `label`.

Author(s)

MACAT development team

See Also

discretizeAll
discretize.tscores  

Discretize regularized t-scores

Description

discretize.tscores returns a discretized version of the scores in the MACATevalScoring object. Discretization is performed by comparing the value gene-wise (location-wise) with the symmetric upper and lower quantile given by margin (in percent margin/2 lower and upper quantile). discretizeAllClasses produces a flatfile readable by PYTHON.

Usage

discretize.tscores(scores)
discretizeAllClasses.tscores(data, chrom, nperms=10, kernel=rbf, kernelparams=NULL, step.width=100000)

Arguments

scores  
a MACATevalScoring object obtained from evalScoring

data  
a MACATData Object containing all expression values, geneLocations and labels (obtained from preprocessedLoader)

chrom  
chromosome that is discretized

nperms  
number of permutations for the computation of empirical p values (evalScoring)

kernel  
kernel function used for smoothing one of rbf, kNN, basePairDistance or your own

kernelparams  
list of parameters for the kernels

step.width  
size of a interpolation step in basepairs

Details

The filename for the python flat files are discrete_chrom_<chrom>_class_<label>.py where <chrom> and <label> are the names of the chromosome and class label.

Value

  discretize.tscores
  a vector of discretized t-scores

  discretizeAllClasses.tscores
  creates python flatfiles (see details)

Author(s)

The MACAT development team

See Also

evalScoring, kernels, pythondata
### evalScoring

**Score differential expression, assess significance, and smooth scores along the chromosome**

**Description**

This function computes for all genes on one chromosome the regularized t-statistic to score differential gene expression for two given groups of samples. Additionally, these scores are computed for a number of permutations to assess significance. Afterwards, these scores are smoothed with a given kernel along the chromosome to give scores for chromosomal regions.

**Usage**

```r
evalScoring(data, class, chromosome, nperms=1000, permute="labels", pcompute="empirical", subset=NULL, newlabels=NULL, kernel=rbf, kernelparams=NULL, cross.validate=TRUE, paramMultipliers=2^-4:4, ncross=10, step.width=100000, memory.limit=TRUE, verbose=TRUE)
```

**Arguments**

- **data** Gene expression data in the MACAT list format. See `data(stjude)` for an example.
- **class** Which of the given class labels is to be analyzed
- **chromosome** Chromosome to be analyzed
- **nperms** Number of permutations
- **permute** Method to do permutations. Default 'labels' does permutations of the class labels, which is the common and faster way to assess significance of differential expression. The alternative 'locations' does permutations of gene locations, is much slower and right now should be considered preliminary at best.
- **pcompute** Method to determine the p-value for differential expression of each gene. Is only evaluated if the argument `permute='labels'` and in that case passed on to the function `scoring`
- **subset** If a subset of samples is to be used, give vector of column-indices of these samples in the original matrix here.
- **newlabels** If other labels than the ones in the MACAT-list-structure are to be used, give them as character vector/factor here. Make sure argument 'class' is one of them.
- **kernel** Choose kernel to smooth scores along the chromosome. Available are 'kNN' for K-Nearest-Neighbors, 'rbf' for radial-basis-function (Gaussian), 'basePairDistance' for a kernel, which averages over all genes within a given range of base pairs around a position.
kernelparams: Additional parameters for the kernel as list, e.g., kernelparams=list(k=5) for taking the 5 nearest neighbours in the kNN-kernel. If NULL some defaults are set within the function.
cross.validate: Logical. Should the parameter settings for the kernel function be optimized by a cross-validation?
paramMultipliers: Numeric vector. If you do cross-validation of the kernel parameters, specify the multipliers of the given (standard) parameters to search over for the optimal one.
ncross: Integer. If you do cross-validation, specify how many folds.
step.width: Defines the resolution of smoothed scores on the chromosome, is in fact the distance in base pairs between 2 positions, for which smoothed scores are to be calculated.
memory.limit: If you have a computer with lots of RAM, setting this to FALSE will increase speed of computations.
verbose: logical; should function’s progress be reported to STDOUT?; default: TRUE.

details: Please see the package vignette for more details on this function.

value: List of class 'MACATevalScoring' with 11 components:
original.geneid: Gene IDs of the genes on the chosen chromosome, sorted according to their position on the chromosome.
original.loc: Location of genes on chromosome in base pairs from 5’end.
original.score: Regularized t-score of genes on chromosome.
original.pvalue: Empirical p-value of genes on chromosome. How often was a higher score observed than this one with random permutations? In other words, how significant seems this score to be?
steps: Positions on the chromosome in bp from 5’, for which smoothed scores have been computed.
sliding.value: Smoothed regularized t-scores at step-positions.
lower.permuted.border: Smoothed scores from permutations, lower significance border, currently 2.5%-quantile of permutation scores.
upper.permuted.border: Smoothed scores from permutations, upper significance border, currently 97.5%-quantile of permutation scores.
chromosome: Chromosome, which has been analyzed.
class: Class, which has been analyzed.
chip: Identifier for used microarray.

Author(s):
MACAT development team.
evaluateParameters

Evaluate Performance of Kernel Parameters by Cross-validation

Description

For a given data set, chromosome, class, and kernel function, this function helps in determining optimal settings for the kernel parameter(s). The performance of individual parameter setting is assessed by cross-validation.

Usage

evaluateParameters(data, class, chromosome, kernel, kernelparams = NULL, paramMultipliers = 2^(-4:4), subset = NULL, newlabels = NULL, ncross = 10, verbose = TRUE)

Arguments

data Gene expression data in the MACAT list format. See data(stjude) for an example.

class Sample class to be analyzed

chromosome Chromosome to be analyzed

kernel Choose kernel to smooth scores along the chromosome. Available are 'kNN' for k-Nearest-Neighbors, 'rbf' for radial-basis-function (Gaussian), 'basePairDistance' for a kernel, which averages over all genes within a given range of base pairs around a position.
evaluateParameters

kernelparams  Additional parameters for the kernel as list, e.g., kernelparams=list(k=5) for taking the 5 nearest neighbours in the kNN-kernel. If NULL some defaults are set within the function.

paramMultipliers  Numeric vector. If you do cross-validation of the kernel parameters, specify these as multipliers of the given (standard) kernel parameter, depending on your kernel choice (see page 5 of the vignette). The multiplication results are the kernel argument settings, among which you want to search for the optimal one using cross-validation.

subset  If a subset of samples is to be used, give vector of column- indices of these samples in the original matrix here.

newlabels  If other labels than the ones in the MACAT-list-structure are to be used, give them as character vector/factor here. Make sure argument 'class' is one of them.

ncross  Integer. Specify how many folds in cross-validation.

verbose  Logical. Should progress be reported to STDOUT?

Value

A list of class 'MACATevP' with 4 components:

[parameterName]  List of assessed settings for the parameter [parameterName].

avgResid  Average Residual Sum of Squares for the parameter settings in the same order as the first component.

multiplier  Multiplier of the original parameters in the same order as the first component.

best  List of parameter settings considered optimal by cross-validation. Can be directly inserted under the argument 'kernelparams' of the 'evalScoring' function.

Author(s)

MACAT development team

See Also

evalScoring

Examples

data(stjd)
evalkNN6 <- evaluateParameters(stjd, class="T", chromosome=6, kernel=kNN,
  paramMultipliers=c(0.01,seq(0.2,2.0,0.2),2.5))
if (interactive()&&capabilities("X11"))
  plot(evalkNN6)
getResults  

Access results of `evalScoring`

**Description**

This function processes the result of the `evalScoring` function and returns a list of probe sets within chromosome regions deemed significant by MACAT. Additional annotation for these probe sets is provided along with their identifiers.

**Usage**

```r
getResults(MACATevalScoringOBJ)
```

**Arguments**

- `MACATevalScoringOBJ` 
  - Object of class `MACATevalScoring`, usually the result from `evalScoring`

**Details**

The p-values have been computed individually for probe sets (genes), not for whole chromosome regions. Thus, regions deemed significant by sliding window approach do not have to consist only of probe sets with low p-values. These probe-set p-values are not used to determine whether a region is considered significant or not. Instead the comparison between actual and interpolated scores to actual and interpolated boundaries determines whether a region is considered significant.

This function is called within the plot function for the results of `evalScoring`, when HTML output is desired.

**Value**

A list with the following components, describing probe sets within chromosome regions deemed significant:

- `probeID`  
  - IDs of probe sets within these chromosome regions

- `cytoband`  
  - Chromosomal bands these probe sets have been annotated to

- `geneSYM`  
  - Gene symbols these probe sets have been annotated to

- `pvalue`  
  - P-values for probe sets; see details

- `locusid`  
  - EntrezGene-(formerly LocusLink) IDs of these probe sets

- `genedescription`  
  - Description of genes the probe sets have been annotated to

- `probeScore`  
  - Differential expression scores for the probe sets

- `chromosome`  
  - Chromosome, the analysis has been done for

- `class`  
  - Sample class, the analysis has been done for

**Author(s)**

MACAT development team
kernelize

Smooth expression values or scores

Description

'kernelize' uses a kernel to smooth the data given in geneLocations by computing a weighted sum of the values vector. The weights for each position are given in the kernelweights matrix. A kernelweights matrix can be obtained by using the kernelmatrix function.

Usage

getsteps(geneLocations, step.width)
kernelmatrix(steps, geneLocations, kernel, kernelparams)
kernelize(values, kernelweights)

Arguments

geneLocations a list of gene locations (length n)
step.width the width of steps in basepairs
steps a list of locations where the kernelization shall be computed
kernel kernel function one of rbf, kNN or basePairDistance (or your own)
kernelparams a list of named parameters for the kernel (default is fitted to the data)
values vector of length n or matrix (m x n) of values that are to be smoothed
kernelweights a matrix of (n x steps) where n is the length of the values vector and steps is the number of points where you wish to interpolate

Value

getsteps a list of locations starting at min(geneLocations) going to max(geneLocations) with steps of size step.width
kernelmatrix a matrix of (n x steps) containing the kernel weights for each location in steps
kernelize a vector of length steps or a matrix (m x steps) containing the smoothed values

Author(s)

MACAT Development team
See Also

compute.sliding, evalScoring

Examples

```r
data(stjd)
genes = seq(100)
geneLocations = abs(stjd$geneLocation[genes])
geneExpression = stjd$expr[genes,]
step.width = 100000
steps = getsteps(geneLocations, step.width)
weights = kernelmatrix(steps, geneLocations, rbf, list(gamma=1/10^13))
kernelized = kernelize(geneExpression, weights)
plot(steps, kernelized[,1])
```

Description

Function plots scores, 0.025 and 0.975 quantiles of the permuted scores (grey lines), and sliding average score (red line) along the chromosome. Yellow dots highlight regions, in which the smoothed absolute scores exceed the permutation-derived quantile boundaries.

Usage

```r
## S3 method for class 'MACATevalScoring'
plot(x, output = "x11",
     HTMLfilename = NULL, mytitle = NULL, new.device = TRUE, ...)
```

Arguments

- `x` MACATevalScoring object.
- `output` plot "x11" or create a "html"-file with further information. HTML-page will open automatically.
- `HTMLfilename` HTML-filename, default: Results<CHROMOSOME>_<CLASS>.html
- `mytitle` Title of HTML-page, default: "Results of class <CLASS> on chromosome <CHROMOSOME>"
- `new.device` if FALSE: Possibility to plot several plots in one device
- `...` further arguments passed on to generic function plot

Details

One can create a HTML-page on-the-fly if argument output='html'. The HTML-page provides informations about highlighted regions in the plot. Furthermore there are click-able Entrezgene-IDs for further analysis.

Author(s)

MACAT development team
See Also

evalScoring, getResult

Examples

# see function 'evalScoring' for an example

```
preprocessedLoader    Read in data and produce MACAT list

Description

This function reads expression data either from a saved R-file (.RData,.rda), or from a tab-separated text-file (.xls). For building a MACAT-list structure from objects in your workspace, you can either use this function or the convenience wrapper 'buildMACAT'.

Usage

preprocessedLoader(rdatafile, chip, labels = NULL, chromLocObj = NULL, rdafile = TRUE, tabfile = FALSE, labelfile = FALSE)

Arguments

rdatafile       Complete name of the expression data file, or the expression matrix
chip            Identifier of the used microarray. To date only commercial Affymetrix microarrays are supported by MACAT
labels          Classlabels of the samples, vector of same length as number of columns in expression matrix; alternatively complete name of textfile with one label per line
chromLocObj     Object of class chromLocation specifying the genomic position, each probe on the array is mapped to. If not provided, it is build in the function using annotate's function buildChromLocation.
rdafile         Logical; is first argument a saved R-file?
tabfile         Logical; is first argument a tab-separated text file?
labelfile       Logical; is third argument a file with one label per line?

Value

List of class 'MACATData' with 6 components:
geneName        Identifiers of genes/probe sets in expression data
geneLocation    Location of genes on their chromosome as distance from 5'end in base pairs
                 Negative numbers denote genes on the antisense strand.
chromosome      Chromosome of the respective gene. Components 'geneName’, ‘geneLocation’, and ‘chromosome’ are in the same order.
expr            expression matrix with rows = genes and columns = samples/patients
labels          (disease) subtype of each sample, has length = number of columns of expression matrix
chip            Identifier for Microarray used for the experiments
**scoring**

**Note**

At present, macat can only work with Affymetrix microarrays, for which an annotation package is installed on your system. Such annotation packages can either be obtained from the Bioconductor annotation packages repository or be constructed using the Bioconductor package AnnBuilder. For an example, see the common annotation package hgu95av2.

**Author(s)**

MACAT development team

**See Also**

buildMACAT, read.table, stj.d, stjude in package 'stjudem'

**Examples**

```r
## Not run:
# assume you have your HG-U95Av2 expression values in a
tab-separated text file, called 'foo.txt'
mydata <- preprocessedLoader("foo.txt","hgu95av2",rdafile=FALSE,tabfile=TRUE)
## End(Not run)
```

**scoring**

_Compute (regularized) t-scores for gene expression data_

**Description**

This function computes for all genes in an expression matrix the (regularized) t-scores (statistics) with the given class labels and a number of permutations of these labels. Each gene is also assigned a p-value either empirically from the permutation scores or from a t-distribution.

**Usage**

```r
scoring(data, labels, method = "SAM", pcompute = "tdist",
nperms = 1000, memory.limit = TRUE, verbose = TRUE)
```

**Arguments**

- **data**
  Expression matrix with rows = genes and columns = samples
- **labels**
  Vector or factor of class labels; Scoring works only with two classes!
- **method**
  Either "SAM" to compute regularized t-scores, or "t.test" to compute Student’s t-statistic
- **pcompute**
  Method to compute p-values for each genes, either "empirical" to do permutations and compute p-values from them, or "tdist" to compute p-values based on respective t-distribution
- **nperms**
  Number of permutations of the labels to be investigated, if argument 'pcompute="empirical"'
- **memory.limit**
  Logical, if you have a really good computer (>2GB RAM), setting this FALSE will increase speed of computations
- **verbose**
  Logical, if progress should be reported to STDOUT
Details

If `pcompute="empirical"`, the statistic is computed based on the given class labels, afterwards for `nperms` permutations of the labels. The p-value for each gene is then the proportion of permutation statistics that are higher or equal than the statistic from the real labels. For each gene the 2.5%- and the 97.5%-quantile of the permutation statistics are also returned as lower and upper 'significance threshold'.

If `pcompute="tdist", the statistic is computed only based on the given class labels, and the p-value is computed from the t-distribution with (Number of samples - 2) degrees of freedom.

Value

A list, with four components:

- **observed**: (Regularized) t-scores for all genes based on the given labels
- **pvalues**: P-values for all genes, either from permutations or t-distribution
- **expected.lower**: 2.5%-quantile of permutation test-statistics, supposed to be a lower 'significance border' for the gene; or NULL if p-values were computed from t-distribution
- **expected.upper**: 97.5%-quantile of permutation test-statistics, supposed to be an upper 'significance border' for the gene; or NULL if p-values were computed from t-distribution

Note

In package macat, this function is only called internally by the function `evalScoring`.

Author(s)

MACAT development team

References

Regarding the regularized t-score please see the macat vignette.

See Also

evalScoring

Examples

data(stjd)
# compute gene-wise regularized t-statistics for
# T- vs. B-lymphocyte ALL:
# ist <- as.numeric(stjd$labels=="T")
TvsB <- scoring(stjd$expr,ist,method="SAM",pcompute="none")
summary(TvsB$observed)
stjd

Subset Microarray Data from St.Jude Children Research Hospital (USA)

Description
Example for list-structure used by many functions in MACAT. It’s based on the gene expression data published by Yeoh et al. (2002) The data has been preprocessed using ‘vsn’ on probe level and the probe values have been summed up to probe set values using the 'median polish' procedure. This is a subset of the data, containing only the data for the 5000 probe sets with the highest variance across the samples and for 10 exemplary samples, 5 from T-lymphocytic Acute Lymphocytic Leukemia (ALL) and 5 from B-lymphocytic ALL.

Usage
data(stjd)

Format
List of class 'MACATData' with 6 components:

geneName: Identifiers of genes/probe sets in expression data
geneLocation: Location of genes on their chromosome as distance from 5’end in base pairs Negative numbers denote genes on the antisense strand.
chromosome: Chromosome of the respective gene. Components 'geneName', 'geneLocation', and 'chromosome' are in the same order.
expr: expression matrix with rows = genes and columns = samples/patients
labels: (disease) subtype of each sample, has length = number of columns of expression matrix
chip: Identifier for Microarray used for the experiments (here for the Affymetrix HG-U95av2 Oligonucleotide GeneChip)

Note
For the full data package see the Bioconductor data package stjudem. If it is not already installed on your system, try source("http:\www.bioconductor.org\biocLite.R"); biocLite("stjudem")

References

See Also
buildMACAT, stjude in package 'stjudem' for the complete expression data

Examples
data(stjd)
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