**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**

```
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
X <- matrix(rnorm(200),nrow=20,ncol=10)
rownames(X) <- 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(X) <- paste("Sample",1:10,sep="")
y <- rep(c("A","B"),c(5,5))
toy <- buildMACAT(X,"hgu95av2",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**

- for `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

```r
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

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

discretize

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
**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**

```r
discretize.tscores(scores)
discretizeAllClasses.tscores(data, chrom, nperms=10, kernel=rbf, kernelparams=NA)
```

**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`
Examples

```r
# load data pkg("stjude")
data(stjude)
# simple scoring with short running time
scores = evalScoring(stjude, "T", 1, nperms=100, cross.validate=FALSE)
discrete = discretize.tscores(scores)
```

`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

```
evalScoring(data, class, chromosome, nperms=1000, permute="labels", 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 only preliminary.
- `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

```r
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.

Examples

```r
data(stjd)  # load example data

# if you have the data package 'stjudem' installed,
# you should work on the full data therein, of which
# the provided example data, is just a piece
# load datapkg("stjudem")

# T-lymphocyte versus B-lymphocyte on chromosome 1,
# few permutations for higher speed
chrom1Tknn <- evalScoring(stjd,"T",chromosome="1",permute="labels",
                          nperms=100,kernel=kNN,kerneparams=list(k=15),step.width=100000)

# plotting on x11:
if (interactive())
  plot(chrom1Tknn)

# plotting on HTML:
if (interactive())
  plot(chrom1Tknn,"html")
```
**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 components.
- **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`: the 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

See Also
evalScoring

Examples
data(stjd)
myevalres <- evalScoring(stjd, class="T", chromosome=6, nperms=10, 
cross.validate=FALSE)
results <- getResults(myevalres)
summary(results)
results$probeID[1:20]

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 kernel-
weights matrix can be obtained by using the kernelmatrix function.

Usage
getsteps(geneLocations, step.width)
kernmatrix(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

kernmatrix 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
plot.MACATevalScoring

See Also
compute.sliding, evalScoring

Examples

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,])

plot.MACATevalScoring

Plot function for MACATevalScoring objects.

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

## 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
preprocessedLoader

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
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, stjd, 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)
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)
summary(stjd)
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