# Package 'macat' 

April 5, 2014
Title MicroArray Chromosome Analysis Tool
Version 1.36.0
Date 2011-06-03
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Depends Biobase, annotate
Suggests hgu95av2.db, stjudem
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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License Artistic-2.0
biocViews Microarray, DifferentialExpression, Visualization
Reference Toedling J, Schmeier S, Heinig M, Georgi B, Roepcke S (2005). MACAT - MicroArray Chromosome Analysis Tool. Bioinformatics. 21(9):2112--2113.

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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

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 st jude in package 'stjudem'.

## Author(s)

MACAT development team

## See Also

preprocessedLoader,stjude in package 'stjudem'

## Examples

```
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.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

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) |
| $\ldots$ | 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

```
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 $=1 \mathrm{e}+05$, kernel $=$ rbf, kernelparams = list(gamma = 1/10^13), saveToFile = FALSE)

## Arguments

data MACATData Object
chrom chromosome to kernelize
margin symmetric quatile 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 logicval 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

## Examples

```
#loaddatapkg("stjudem")
    #data(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

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 tscores
discretizeAllClasses.tscores
creates python flatfiles (see details)

## Author(s)

The MACAT development team

## See Also

evalScoring, kernels, pythondata

## Examples

```
#loaddatapkg("stjudem")
#data(stjude)
data(stjd)
# simple scoring with short running time
scores = evalScoring(stjd, "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", 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 altenative '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 <br> only evaluated if the argument permute=labels and in that case passed on to <br> the function scoring |
| :--- | :--- |
| subset | If a subset of samples is to be used, give vector of column- indices of these <br> samples in the original matrix here. |
| newlabels | If other labels than the ones in the MACAT-list-structure are to be used, give <br> them as character vector/factor here. Make sure argument 'class' is one of them. |
| choose kernel to smooth scores along the chromose. Available are 'kNN' for |  |

## 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

## See Also

```
scoring,plot.MACATevalScoring, getResults
```


## Examples

```
    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
    #loaddatapkg("stjudem")
    #data(stjude)
    # T-lymphocyte versus B-lymphocyte on chromosome 1,
    # smoothed with k-Nearest-Neighbours kernel(k=15),
    # few permutations for higher speed
    chrom1Tknn <- evalScoring(stjd,"T",chromosome="1",permute="labels",
    nperms=100,kernel=kNN,kernelparams=list(k=15),step.width=100000)
    # plotting on x11:
    if (interactive())
        plot(chrom1Tknn)
    # plotting on HTML:
    if (interactive())
        plot(chrom1Tknn,"html")
```


## 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 <br> evaluateParameters(data, class, chromosome, kernel, kernelparams = NULL, paramMultipliers $=2^{\wedge}(-4: 4)$, 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.
kernelparams Additional parameters for the kernel as list, e.g., kernelparams=list $(\mathrm{k}=5)$ 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

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 <br> 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

## 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 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) <br> values |
| vector of length n or matrix ( $\mathrm{m} x \mathrm{n}$ ) of values that are to be smoothed  <br> kernelweights a matrix of ( $\mathrm{n} \times \mathrm{x}$ steps) where n is the length of the values vector and steps is the <br> number of points where you wish to interpolate. |  |

## Value

getsteps a list of locations starting at min(genLocations) 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 ( $\mathrm{m} \times$ steps) containing the smoothed values

## Author(s)

MACAT Development team

## 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

$x \quad$ MACATevalScoring object.
output plot "x11" or create a "html" -file with further information. HTML-page will open automatically.
HTMLfilename HTML-filename, default:Results<CHOMOSOME>_<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 EntrezgeneIDs for further analysis.

## Author(s)

MACAT development team

## See Also

evalScoring, getResults

## 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

\(\left.$$
\begin{array}{ll}\text { rdatafile } & \begin{array}{l}\text { Complete name of the expression data file, or the expression matrix } \\
\text { chip }\end{array}
$$ <br>
Identifier of the used microarray. To date only commercial Affymetrix microar- <br>

rays are supported by MACAT\end{array}\right]\)| Classlabels of the samples, vector of same length as number of columns in ex- |
| :--- |
| pression 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. |

## 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 <br> 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

```
## 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

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

## Arguments

data $\quad$ 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 $\quad$ 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 <br> 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 'signifi- <br> cance 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 $\begin{aligned} & \text { Subset Microarray Data from St.Jude Children Research Hospital } \\ & (U S A)\end{aligned}$
(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

Yeoh et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell. March 2002. 1: 133-143.

## See Also

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

## Examples

```
data(stjd)
summary(stjd)
```


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