

# Package ‘Ringo’

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**Type** Package

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**Suggests** rtracklayer (>= 1.3.1), mclust, topGO (>= 1.15.0)

**Description** The package Ringo facilitates the primary analysis of ChIP-chip data. The main functionalities of the package are data read-in, quality assessment, data visualisation and identification of genomic regions showing enrichment in ChIP-chip. The package has functions to deal with two-color oligonucleotide microarrays from NimbleGen used in ChIP-chip projects, but also contains more general functions for ChIP-chip data analysis, given that the data is supplied as RGList (raw) or ExpressionSet (pre-processed).

The package employs functions from various other packages of the Bioconductor project and provides additional ChIP-chip-specific and NimbleGen-specific functionalities.

**Reference** Joern Toedling, Oleg Sklyar, Tammo Krueger, Jenny J. Fischer, Silke Sperling, and Wolfgang Huber (2007). Ringo - an R/Bioconductor package for analyzing ChIP-chip readouts. BMC Bioinformatics, 8:221.

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asExprSet	<i>converts a Ringo MAList into an ExpressionSet</i>
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---

### Description

Function to convert an object of class `MAList` into an object of class `ExpressionSet`. Note that the otherwise optional `targets` component is required in this case to generate the `phenoData` of the new `ExpressionSet`.

### Usage

```
asExprSet(from, idColumn="PROBE_ID")
```

### Arguments

<code>from</code>	object of class <code>MAList</code> to convert into an <code>ExpressionSet</code>
<code>idColumn</code>	string; indicating which column of the <code>genes</code> data.frame of the <code>MAList</code> holds the identifier for reporters on the microarray. This column, after calling <code>make.names</code> on it, will make up the unique <code>featureNames</code> of the resulting <code>ExpressionSet</code> .

### Value

an object of class `ExpressionSet`

### Note

There is a more general function for converting `MALists` to `ExpressionSets` in the package `convert`. This function here is solely intended for converting Ringo-generated `MALists` into `ExpressionSets`.

### Author(s)

Joern Toedling

### See Also

[ExpressionSet](#), [preprocess](#)

### Examples

```
exDir <- system.file("exData", package="Ringo")
exRG <- readNimblegen("example_targets.txt", "spottypes.txt", path=exDir)
exMA <- preprocess(exRG, "none", returnMAList=TRUE)
exX <- asExprSet(exMA)
```

---

autocor

*Function to compute auto-correlation of probe intensities*


---

### Description

Function to compute auto-correlation of probe intensities at specified offsets from the original positions.

### Usage

```
autocor(x, probeAnno, chrom, samples = NULL, lag.max = 2000,
        lag.step = 100, cor.method = "pearson",
        channel = c("red", "green", "logratio"),
        idColumn = "ID", verbose = TRUE)
```

### Arguments

x	an object either of class <code>ExpressionSet</code> containing the normalized probe intensities or of class <code>RGList</code> containing the raw intensities.
probeAnno	Object of class <code>probeAnno</code> holding chromosomal match positions and indices of reporters in data matrix.
chrom	character; chromosome to compute the autocorrelation for
samples	which samples of the data to use; if more than 1 for each probe the mean intensity over these samples is taken.
lag.max	integer; maximal offset from the original position, the auto-correlation is to be computed for.
lag.step	integer; step size of lags between 0 and maximal lag.
cor.method	character; which type of correlation to compute, translates to argument method of function <code>cor</code>
channel	character; in case x is an <code>RGList</code> , which channel to plot, either red, green or the logratio $\log_2(\text{red}) - \log_2(\text{green})$
idColumn	string; indicating which column of the genes data.frame of the <code>RGList</code> holds the identifier for reporters on the microarray. Character entries of the index elements of the <code>probeAnno</code> will be matched against these identifiers. If the index elements of the <code>probeAnno</code> are numeric or x is of class <code>ExpressionSet</code> , this argument will be ignored.
verbose	logical; extended output to <code>STDOUT</code>

### Details

the lags, i.e. the offsets from the original position, the auto-correlation is to be computed for, are constructed from the given arguments as `seq(0, lag.max, by=lag.step)`.

**Value**

Object of class `autocor.result`, a numeric vector of auto-correlation values at the offsets specified in argument `lags`. The lag values are stored as the names of the vector. Argument `chrom` is stored as attribute `chromosome` of the result.

**Author(s)**

Joern Toedling

**See Also**

[cor,plot.autocor.result](#)

**Examples**

```
exDir <- system.file("exData",package="Ringo")
load(file.path(exDir,"exampleProbeAnno.rda"))
load(file.path(exDir,"exampleX.rda"))
exAc <- autocor(exampleX, probeAnno=exProbeAnno,
                chrom="9", lag.max=1000)
plot(exAc)
```

---

cher-class

*Class "cher" - ChIP-enriched region*

---

**Description**

An object of class `cher` (ChIP-enriched region) holds characteristics of an enriched genomic region from ChIP chip data.

**Objects from the Class**

Objects can be created by calls of the form `new("cher", name, chromosome, start, end, cellType, antibody, maxLevel, ...)`

**Slots**

**name:** character vector of length 1 unequivocally describing the cher, e.g. "Suz12.Nudt2.upstream.cher"

**chromosome:** character vector of length one, naming the chromosome of the region, e.g. "9"

**start:** integer, region start position on the chromosome, e.g. 34318900

**end:** integer, region end position on the chromosome, e.g. 34320100

**cellType:** character vector describing the cell type the ChIP chip experiment has been done on, e.g. "HeLa" or "human"

**antibody:** character vector describing the antibody or characteristic for which fragments were supposedly enriched in immuno-precipitation step, e.g. "Suz12" for the protein Suz12

**maxLevel:** numeric, maximal (smoothed) probe level in the cher, e.g. 2.00

**score:** numeric of a cher score, currently we use the sum of smoothed probe levels (log fold changes), e.g. 69.16

**probes:** vector of probe identifiers of all probes with match positions in the cher

**extras:** list of further elements used to annotate the cher; examples of such that are used in Ringo are:

**typeUpstream** optional character vector of features that this cher is located upstream of, e.g. the transcriptional start site of "ENST00000379158". See [relateChers](#) for details.

**typeInside** optional character vector of features that this cher is located inside of

**distMid2TSS** optional named numeric vector of distances of the cher's middle position to features, e.g. TSSs of features upstream and inside; names are the features to which the distances are given; only meaningful in combination with typeUpstream and typeInside; e.g. 55 with name "ENST00000379158"

**upSymbol** optional character vector of gene symbols of features the cher is located upstream of; supplements typeUpstream; e.g. "Nudt2"

**inSymbol** optional character vector of gene symbols of features the cher is located upstream of; supplements typeInside.

... further list elements can be added using the update method.

## Methods

**initialize** create a new cher; see section examples below

**plot** calls [chipAlongChrom](#) to plot the cher; see [plot.cher](#) for more details

**update** signature(cher,...); updates elements of the cher object; The further arguments in '...' are interpreted. Arguments corresponding to defined slot names of the cher result in the value by that slot being replaced by the specified value for the argument; argument names that do not correspond to slot names of the object result in list elements of the extras list of the cher being replaced by the given values for these arguments or the values are appended to the current extras list and the argument names make up the list names of the appended arguments. See section examples below for an example how to use this method.

**cellType** obtain or replace the description of the cell type, the ChIP-enriched regions was found in with this antibody

**probes** obtain the vector of probes involved in a ChIP-enriched region

## cherList

A list in which each element is of class cher, is called a cherList. This class, however, is rarely used (yet).

## Note

The cher class used to be an S3 list before.

The term 'cher' is shorthand for 'ChIP-enriched region'. We think this term is more appropriate than the term 'peak' commonly used in ChIP-chip context. Within such regions the actual signal could show two or more actual signal peaks or none at all (long plateau).

**Author(s)**

Joern Toedling, Tammo Krueger

**See Also**

[plot.cher](#), [findChersOnSmoothed](#), [relateChers](#)

**Examples**

```
## how to create a cher object from scratch
cherNudt2 <- new("cher", name="nudt2.cher", chromosome=9,
  start=34318954, end=34319944, antibody="Suz12",
  maxLevel=2.00, score=69.2, upSymbol="NUDT2")
#extras=list(upSymbol="NUDT2")

cherNudt2
str(cherNudt2)

## use the update method (note: this update is biologically meaningless)
cher2 <- update(cherNudt2, cellType="HeLa", downSymbol="P53",
  probes=c("probe1", "probe2"))
cher2; str(cher2)

## plot a cher object
exDir <- system.file("exData", package="Ringo")
load(file.path(exDir, "exampleProbeAnno.rda"))
load(file.path(exDir, "exampleX.rda"))
smoothX <- computeRunningMedians(exampleX, probeAnno=exProbeAnno,
  modColumn = "Cy5", allChr = "9", winHalfSize = 400)
plot(cherNudt2, smoothX, probeAnno=exProbeAnno, gff=exGFF, extent=5000)
```

---

cherByThreshold

*Function to identify chers based on thresholds*

---

**Description**

Given a vector of probe positions on the chromosome, a vector of smoothed intensities on these positions, and a threshold for intensities to indicated enrichment, this function identifies *Chers* (ChIP-enriched regions) on this chromosome.

This function is called by the function `findChersOnSmoothed`.

**Usage**

```
cherByThreshold(positions, scores, threshold, distCutOff,
  minProbesInRow = 3)
```

**Arguments**

positions	numeric vector of genomic positions of probes
scores	scores (intensities) of probes on those positions
threshold	threshold for scores to be called a cher
distCutOff	maximal positional distance between two probes to be part of the same cher
minProbesInRow	integer; minimum number of enriched probes required for a cher; see details for further explanation.

**Details**

Specifying a minimum number of probes for a cher (argument `minProbesInRow`) guarantees that a cher is supported by a reasonable number of measurements in probe-sparse regions. For example, if there's only one enriched probe within a certain genomic 1kb region and no other probes can be mapped to that region, this single probe does arguably not provide enough evidence for calling this genomic region enriched.

**Value**

A LIST with `n` components, where the first `n` components are the cher clusters, each one holding the scores and, as their names, the genomic positions of probes in that cluster.

**Author(s)**

Joern Toedling

**See Also**

[findChersOnSmoothed](#)

**Examples**

```
## example with random generated data:
rpos <- cumsum(round(runif(200)*5))
rsco <- rnorm(200)+0.2
plot(rpos, rsco, type="l", col="seagreen3", lwd=2)
rug(rpos, side=1, lwd=2); abline(h=0, lty=2)
rchers <- cherByThreshold(rpos, rsco, threshold=0, distCutOff=2)
sapply(rchers[-length(rchers)], function(thisClust){
  points(x=as.numeric(names(thisClust)), y=thisClust, type="h", lwd=2,
  col="gold")})
```



---

chipAlongChrom	<i>Visualize ChIP intensities along the chromosome</i>
----------------	--

---

## Description

This function can visualize the array intensities from a ChIP chip experiment for a chromosomal region or the whole chromosome. It's based on the `plotAlongChrom` function from the package `tilingArray`, but provides a different visualization.

## Usage

```
## S4 method for signature 'ExpressionSet,probeAnno'
plot(x, y, ...)

chipAlongChrom(eSet, probeAnno, chrom, xlim, ylim,
  samples = NULL, paletteName = "Set2", colPal = NULL,
  ylab = "Fold change [log]", ipch = 16, ilwd = 3, ilty = 1,
  icex = 3, gff = NULL,
  featureExclude=c("chromosome", "nucleotide_match","insertion"),
  zeroLine = TRUE, sampleLegend = TRUE, sampleLegendPos = "topleft",
  featureLegend = FALSE, maxInterDistance = 200, coord = NULL,
  highlight, main, ...)
```

## Arguments

eSet	An expression set containing the (normalized) ChIP intensities, e.g. the result objects from functions <code>preprocess</code> and <code>computeRunningMedians</code> .
x	Corresponds to argument <code>eSet</code> when calling the S4 method
probeAnno	An object of class <code>probeAnno</code> holding genomic position, index and gene association of probes on array.
y	Corresponds to argument <code>probeAnno</code> when calling the S4 method
chrom	character; the chromosome to visualize
xlim	start and end genomic coordinates on the chromosome to visualize
ylim	minimum and maximum probe intensities for the plot, if missing (default) set to <code>range(exprs(eSet))</code>
samples	numeric; which samples from the <code>eSet</code> are to be shown. Default is to show all samples in the <code>eSet</code> ,
paletteName	character; Name of the <code>RColorBrewer</code> palette to use for sample colors. If the number of samples is greater than the palette size, random colors are taken.
colPal	vector of colors to use for the sample intensities. This is alternative to the argument <code>paletteName</code> for specifying which colors to use.
ylab	character; label for the y-axis, passed on to the plotting function as <code>ylab</code>
ipch	plot character to use

icex	character expansion to use for plotting symbol
ilwd	line width of plotted data lines
ilty	line type of plotted data lines; passed on to <code>par(lty)</code> .
gff	data frame containing annotation for genomic feature to be used to further annotate the plot.
featureExclude	character vector specifying the feature types in the data.frame gff that should not be shown in the plot
zeroLine	logical; should a dashed horizontal line at $y=0$ be put into the plot?
sampleLegend	logical; should a sample legend be put into the plot?
sampleLegendPos	character; where to put the sample legend; one of 'topleft' (default), 'bottom-left', 'topright', or 'bottomright'
featureLegend	logical; should a feature legend be put beneath the plot?
maxInterDistance	numeric; only used when <code>itype</code> is either "r" or "u"; specifies the maximal distance up to which adjacent probe positions should be connected by a line.
coord	optional integer of length 2; can be used instead of <code>xlim</code> to specify the start and end coordinates of the genomic region to plot
highlight	optional list specifying a genomic region to be highlighted in the shown plot
main	optional main title for the plot; if not specified: the default is 'Chromosome coordinate [bp]'
...	further parameters passed on to <code>grid.polyline</code> and <code>grid.points</code>

**Value**

`invisible` list of probe positions (element `x`) and probe levels (element `y`) in the selected genomic region.

**Note**

The `S4` method is provided as a mere convenience wrapper.

When plotting a new 'grid' plot in an active `x11` window that already contains a plot, remember to call `grid.newpage()` before.

**Author(s)**

Joern Toedling

**See Also**

[ExpressionSet-class](#), [probeAnno-class](#), [grid.points](#), [plotAlongChrom](#) in package `tilingArray`

## Examples

```
### load data
ringoExampleDir <- system.file("exData",package="Ringo")
load(file.path(ringoExampleDir,"exampleProbeAnno.rda"))
load(file.path(ringoExampleDir,"exampleX.rda"))

### show a gene that is well represented on this microarray
plot(exampleX, exProbeAnno, chrom="9",
      xlim=c(34318000,34321000), ylim=c(-2,4), gff=exGFF)

### this should give you the same result as:
chipAlongChrom(exampleX, chrom="9", xlim=c(34318000,34321000),
               ylim=c(-2,4), probeAnno=exProbeAnno, gff=exGFF)
```

---

compute.gc

*Compute the GC content of DNA and probe sequences*

---

## Description

Simple auxiliary function to compute the GC content of a given set of DNA sequences, such as microarray probe sequences.

## Usage

```
compute.gc(probe.sequences, digits = 2)
```

## Arguments

probe.sequences	character vector of DNA or probe sequences of which the GC content is to be computed
digits	integer specifying the desired precision

## Value

a numeric vector with sequence-wise GC contents; the names of this vector are the names of the supplied probe.sequences.

## Author(s)

Joern Toedling

## See Also

Function basecontent in package matchprobes for a more general function to compute base occurrence in sequences

**Examples**

```
ex.seqs <- c("gattaca", "GGGNTT", "ggAtT", "tata", "gcccg")
names(ex.seqs) <- paste("sequence", 1:5, sep="")
compute.gc(ex.seqs)
```

---

computeRunningMedians *Function to compute running medians on a tiling expression set*

---

**Description**

Function to compute running medians (or other quantiles) on a tiling expression set.

**Usage**

```
computeRunningMedians(xSet, probeAnno, modColumn = "Cy5",
  allChr, winHalfSize = 400, min.probes = 5, quant = 0.5,
  combineReplicates = FALSE, nameSuffix = ".sm", checkUnique=TRUE,
  uniqueCodes=c(0), verbose = TRUE)
```

**Arguments**

xSet	Object of class ExpressionSet holding the normalized probe intensity data
probeAnno	Environment holding the genomic positions of probes in the ExpressionSet
modColumn	Column of the ExpressionSet's phenoData holding the samples' difference of interest
allChr	Character vector of all chromosomes in genome; if not specified (default) all chromosomes annotated in the supplied probeAnno are used.
winHalfSize	Half the size of the window centered at a probe position, in which all other probes contribute to the calculation of the median.
min.probes	integer; if less probes are in the sliding window, NA instead of the median is returned. This meant to avoid to computing non-meaningful medians. If unwanted, set this to 1 or less
quant	numeric; which quantile to use for the smoothing. The default 0.5 means compute the median over the values in the sliding window.
combineReplicates	logical; should the median not be computed over individual samples in the ExpressionSet, but should samples be combined according to the column modColumn of the phenoData. The median is then computed across all probe levels and samples of the same type in the window. The resulting ExpressionSet has so many columns as are there different entries in the column modColumn
nameSuffix	character; suffix attached to the sample labels of the supplied ExpressionSet xSet for the sample names of the resulting ExpressionSet.
checkUnique	logical; indicates whether the uniqueness indicator of probe matches from the probeAnno environment should be used.

uniqueCodes      numeric; which numeric codes in the chromosome-wise match-uniqueness elements of the probeAnno environment indicate uniqueness?

verbose            logical; detailed progress output to STDOUT?

**Value**

An object of class ExpressionSet, holding smoothed intensity values for the probes of the supplied ExpressionSet. The number of results samples is either the number of levels in the supplied modColumn of the supplied ExpressionSet's phenoData if combineReplicates is set to TRUE or equal to the number of samples in the supplied ExpressionSet xSet otherwise.

**Author(s)**

Joern Toedling

**See Also**

[ExpressionSet](#), [sliding.quantile](#), [probeAnno-class](#)

**Examples**

```
exDir <- system.file("exData",package="Ringo")
load(file.path(exDir,"exampleProbeAnno.rda"))
load(file.path(exDir,"exampleX.rda"))
smoothX <- computeRunningMedians(exampleX, probeAnno=exProbeAnno,
                                  winHalfSize = 400)
combX <- combine(exampleX, smoothX)
if (interactive()){
  grid.newpage()
  plot(combX, exProbeAnno, chrom="9", xlim=c(34318000,34321000),
       ylim=c(-2,4), gff=exGFF)
}
```

---

computeSlidingT

*Function to compute sliding T statistics on a tiling expression set*

---

**Description**

Function to compute sliding (regularized) one- or two-sample T statistics on a tiling expression set.

**Usage**

```
computeSlidingT(xSet, probeAnno, allChr = c(1:19, "X", "Y"), test = "one.sample", grouping = NULL, winHalfSize = 400)
```

**Arguments**

xSet	Object of class ExpressionSet holding the normalized probe intensity data
probeAnno	Environment holding the genomic positions of probes in the ExpressionSet
allChr	Character vector of all chromosomes in genome
test	character; one of one.sample or two.sample
grouping	factor vector of length equal to number of samples, not required if test=one.sample
winHalfSize	Half the size of the window centered at a probe position, in which all other probes contribute to the calculation of the mean and standard deviation.
min.probes	integer; if less probes are in the sliding window, NA instead of the mean and sd is returned. This is meant to avoid to computing non-meaningful means and standard deviations. If unwanted, set this to 1 or less
checkUnique	logical; indicates whether the uniqueness indicator of probe matches from the probeAnno environment should be used.
uniqueCodes	numeric; which numeric codes in the chromosome-wise match-uniqueness elements of the probeAnno environment indicate uniqueness?
verbose	logical; detailed progress output to STDOUT?

**Value**

An object of class ExpressionSet, holding the T statistics values for the probes of the supplied ExpressionSet. The number of results samples is the number of levels in the supplied factor grouping.

**Author(s)**

Joern Toedling

**See Also**

[sliding.meansd](#)

**Examples**

```
exDir <- system.file("exData",package="Ringo")
load(file.path(exDir,"exampleProbeAnno.rda"))
load(file.path(exDir,"exampleX.rda"))
tX <- computeSlidingT(exampleX, probeAnno=exProbeAnno,
                      allChr=c("9"), winHalfSize=400)
sampleNames(tX) <- "t-Stat_Suz12vsTotal"
if (interactive()){
  grid.newpage()
  plot(cbind2(exampleX, tX), exProbeAnno, chrom="9",
        xlim=c(34318000,34321000), ylim=c(-2,8.5), gff=exGFF,
        paletteName="Paired")
}
```

---

`corPlot`*Function to plot correlation of different samples*

---

**Description**

This function can be used to visualise the (rank) correlation in expression data between different samples or sample groups.

**Usage**

```
corPlot(eset, samples = NULL, grouping = NULL, ref = NULL,  
        useSmoothScatter = TRUE, ...)
```

**Arguments**

<code>eset</code>	object of class <code>ExpressionSet</code> holding the array data, or a numeric matrix instead
<code>samples</code>	which samples' expression shall be correlated to each other; either a numeric vector of sample numbers in the <code>ExpressionSet</code> or a character vector that must be contained in the <code>sampleNames</code> of the <code>ExpressionSet</code> , default <code>NULL</code> means take all samples in the <code>ExpressionSet</code>
<code>grouping</code>	an optional factor vector defining if the correlation should be assessed between groups of samples, rather than individual samples. If two or more samples are assigned into the same group, the mean over these samples' expression values is taken before computing correlation. Default <code>NULL</code> means assess correlation between individual samples only.
<code>ref</code>	reference than only applies if argument <code>grouping</code> is given; see <a href="#">relevel</a>
<code>useSmoothScatter</code>	logical; should the function <a href="#">smoothScatter</a> be used?
<code>...</code>	additional arguments, not used yet

**Value**

The function only returns `NULL` (invisible). The function is called for its side-effect producing the pairs plot.

**Author(s)**

Joern Toedling

**See Also**

[ExpressionSet](#), [relevel](#), [pairs](#), [smoothScatter](#)

**Examples**

```
data(sample.ExpressionSet)
if (interactive())
corPlot(sample.ExpressionSet,
grouping=paste(sample.ExpressionSet$sex,
sample.ExpressionSet$type, sep="."))
```

---

exportCherList	<i>Function to export cherList into a file</i>
----------------	--

---

**Description**

Function to export cherList into a file of gff or BED format. This files can be imported as tracks into genome browsers.

**Usage**

```
exportCherList(object, filename = "chers.gff", format = "gff3",
genome="hg18", ...)
```

**Arguments**

object	an object of class cherList
filename	character; path to file to be written
format	Format of exported file; currently only "gff3" and "bed" are supported
genome	character; which genome the ChIP-enriched regions were found in denoting species and assembly, e.g. 'hg18' or 'mm9'
...	further arguments to be passed on to the trackSet method

**Details**

First converts the cherList into an object of class trackSet from package **rtracklayer** and then calls the export method as defined for a trackSet.

**Value**

returns invisible NULL; called for the side effect of writing the file filename.

**Author(s)**

Joern Toedling

**See Also**

Class trackset in package **rtracklayer**



## Examples

```
## Not run:
exDir <- system.file("exData",package="Ringo")
load(file.path(exDir,"exampleProbeAnno.rda"))
load(file.path(exDir,"exampleX.rda"))
smoothX <- computeRunningMedians(exampleX, probeAnno=exProbeAnno,
  modColumn = "Cy5", allChr = "9", winHalfSize = 400)
chersX <- findChersOnSmoothed(smoothX, probeAnno=exProbeAnno,
  thresholds=0.45, allChr="9", distCutOff=600, cellType="human")
exportCherList(chersX, file="chers.gff")

## End(Not run)
```

---

extractProbeAnno	<i>Build probeAnno from match positions in an RGList</i>
------------------	--

---

## Description

This function can be used to build a probeAnno object from the reporter match positions given in the 'genes' slot of an RGList if present, as is the case with some ChIP-chip microarray platforms, e.g. with certain Agilent ones after reading in the data with read.maimages(...,"agilent").

## Usage

```
extractProbeAnno(object, format = "agilent", ...)
```

## Arguments

object	an object that holds the data and the probe match positions, currently can only be of class RGList
format	in which format are the reporter match positions stored in the object; see details; currently only "agilent" is implemented
...	further arguments that are passed on to the function postToProbeAnno

## Details

Which information is used for creating the probeAnno is specified by the argument format.

**agilent** expects that the object is of class RGList. The 'genes' element of the object is taken. This element is expected to have at least a column 'ProbeName', which stores the unique reporter/probe identifiers, and a column 'SystematicName', which holds the probe match position in the format "chr<chromosome>:coordinate1-coordinate2", e.g. "chr1:087354051-087354110".

## Value

An object of class probeAnno holding the mapping between reporters and genomic positions.

**Author(s)**

Joern Toedling

**See Also**[posToProbeAnno](#), [probeAnno-class](#)

features2Probes

*Function for mapping genomic features to probes***Description**

This function creates a mapping between annotated genomic features and probes on the array whose matching genomic positions are stored in a probeAnno environment.

**Usage**

```
features2Probes(gff, probeAnno, upstream = 5000, checkUnique = TRUE, uniqueCodes = c(0), mem.limit=1e8,
```

**Arguments**

gff	data.frame holding genomic feature annotation
probeAnno	Object of class environment holding the genomic positions of probes in the ExpressionSet
upstream	up to how many bases upstream of annotated genomic features should probes be counted as related to that feature (see details)
checkUnique	logical; indicates whether the uniqueness indicator of probe matches from the probeAnno environment should be used.
uniqueCodes	numeric; which numeric codes in the chromosome-wise match-uniqueness elements of the probeAnno environment indicate uniqueness?
mem.limit	integer value; what is the maximal allowed size of matrices during the computation; see regionOverlap
verbose	logical; detailed progress output to STDOUT?

**Value**

The results is a list of length equal to the number of rows in the provided gff, the data.frame of genomic features. The names of the list are the names specified in the gff. Each element of the list is specified by the probes mapping into the genomic region from upstream bases upstream of the feature's start site to the feature's end site. The entries itself are either NULL, if no probe was mapped into this region, or a named numeric vector, with its values being the distances of the probes' middle positions to the feature's start site (which depends on the strand the feature is on) and its names being the identifiers of these probes.

**Note**

This resulting mapping is not used excessively by other Ringo functions, so creating this mapping is optional at this time, but it may simplify subsequent gene/transcript-based analyses.

Here, the term *feature* describes a genomic entity such as a gene, transcript, non-coding RNA or a similar feature annotated to a genome. It does NOT refer to oligo-nucleotide or cDNA probes on the microarray.

**Author(s)**

Joern Toedling

**See Also**

[regionOverlap](#)

**Examples**

```
ringoExampleDir <- system.file("exData",package="Ringo")
load(file.path(ringoExampleDir,"exampleProbeAnno.rda"))
trans2Probe <- features2Probes(exGFF, exProbeAnno)
trans2Probe[exGFF$name[match("NUDT2", exGFF$symbol)]]
exGFF[match(names(trans2Probe)[listLen(trans2Probe)>0],exGFF$name),]
trans2Probe[listLen(trans2Probe)==1]
```

---

findChersOnSmoothed     *Find ChIP-enriched regions on smoothed ExpressionSet*

---

**Description**

Given an ExpressionSet of smoothed probe intensities, an environment with the mapping of probes to chromosomes, and a vector of thresholds for calling genomic sites enriched, this function finds the 'chers' (ChIP-enriched regions) consisting of enriched genomic positions, with probes mapped to them. 'Adjacent' enriched positions are condensed into a single Cher.

**Usage**

```
findChersOnSmoothed(smoothedX, probeAnno, thresholds, allChr = NULL,
  distCutOff = 600, minProbesInRow = 3, cellType = NULL,
  antibodyColumn=NULL, checkUnique = TRUE, uniqueCodes = c(0),
  verbose = TRUE)
```

**Arguments**

smoothedX	Object of class ExpressionSet holding the smoothed probe intensities, e.g. the result of function computeRunningMedians.
probeAnno	environment containing the probe to genome mapping

thresholds	numeric vector of threshold above which smoothed probe intensities are considered to correspond to enriched probes. The vector has to be of length equal the number of samples in <code>smoothedX</code> , with a single threshold for each sample.
allChr	character vector of all chromosomes on which enriched regions are sought. Every chromosome here has to have probes mapped to it in the <code>probeAnno</code> environment. By default (NULL) the <code>chromosomeNames</code> of the <code>probeAnno</code> object are used.
distCutOff	integer; maximum amount of base pairs at which enriched probes are condensed into one Cher.
minProbesInRow	integer; minimum number of enriched probes required for a Cher; see details for further explanation.
cellType	character; name of cell type the data comes from, is either a. of length one indicating the column of <code>pData(smoothedX)</code> that holds the cell type OR b. of length one indicating the common cell type for all samples in the <code>ExpressionSet</code> OR c. of length equal to <code>ncol(smoothedX)</code> specifying the cell type of each sample individually.
antibodyColumn	the name or number of the column of the <code>pData(smoothedX)</code> that holds the description of the antibody used for each sample. This information is used to annotate found ChIP-enriched regions accordingly. If NULL (default), the <code>sampleNames</code> of <code>smoothedX</code> are used.
checkUnique	logical; indicates whether the uniqueness indicator of probe matches from the <code>probeAnno</code> environment should be used.
uniqueCodes	numeric; which numeric codes in the chromosome-wise match-uniqueness elements of the <code>probeAnno</code> environment indicate uniqueness?
verbose	logical; extended output to STDOUT?

**Details**

Specifying a minimum number of probes for a Cher (argument `minProbesInRow`) guarantees that a Cher is supported by a reasonable number of measurements in probe-sparse regions. For example, if there's only one enriched probe within a certain genomic 1kb region and no other probes can be mapped to that region, this single probe does arguably not provide enough evidence for calling this genomic region enriched.

**Value**

A list of class `cherList`, holding objects of class `cher` that were found on the supplied data.

**Author(s)**

Joern Toedling

**See Also**

[cherByThreshold](#), [computeRunningMedians](#), [relateChers](#)

## Examples

```
exDir <- system.file("exData",package="Ringo")
load(file.path(exDir,"exampleProbeAnno.rda"))
load(file.path(exDir,"exampleX.rda"))
smoothX <- computeRunningMedians(exampleX, probeAnno=exProbeAnno,
  modColumn = "Cy5", allChr = "9", winHalfSize = 400)
chersX <- findChersOnSmoothed(smoothX, probeAnno=exProbeAnno,
  thresholds=0.45, allChr="9", distCutOff=600, cellType="human")
if (interactive())
  plot(chersX[[1]], smoothX, probeAnno=exProbeAnno, ggf=exGFF)
chersX <- relateChers(chersX, exGFF)
as.data.frame.cherList(chersX)
```

---

ftr2xys

*Convert a NimbleScan ftr-file into a xys-file*

---

## Description

Auxiliary function to convert a NimbleScan feature-report file into a xys-file that can be used with the function `read.xysfiles` of package `oligo`.

## Usage

```
ftr2xys(ftr.file, path=getwd())
```

## Arguments

<code>ftr.file</code>	character; file path of feature report file to convert into an xys file
<code>path</code>	file path to directory where the xys-file should be written to; defaults to the current working directory

## Details

The output file is names as the input ftr file; with the file extension `.ftr` replaced by `.xys`.

## Value

Function returns only NULL invisibly and is only called for its side effect to write the xys-file into the current working directory.

## Note

This function should only be used with one-color Nimblegen microarrays and when the correct xys-file of the raw data is not available. The output file can be used with the function `read.xysfiles` of package `oligo`.

## Author(s)

Joern Toedling

## Examples

```
## Not run:
  sapply(list.files(pattern=".ftr$"),ftr2xys)
  library(oligo)
  fs = read.xysfiles(list.xysfiles())

## End(Not run)
```

---

getFeats

*Utility function to extract all features from a cherList*

---

## Description

This is a small utility function for extracting all related features from a *cherList*, a list of *ChIP-enriched regions*.

## Usage

```
getFeats(cl)
```

## Arguments

`cl` object of class `cherList`, a list of `cher` objects

## Value

a character vector containing the names of all features that were associated to any ChIP-enriched region in the list before, using the function [relateChers](#)

## Author(s)

Joern Toedling

## See Also

[relateChers](#), [cher-class](#)

---

 image.RGList

*Function to visualize spatial distribution of raw intensities*


---

**Description**

Function to visualize spatial distribution of raw intensities on NimbleGen Oligoarrays. Requires RGList with component genes complete with genes\$X and genes\$X coordinates of probes on array. arrayImage is a synonym of image.RGList.

**Usage**

```
## S3 method for class 'RGList'
image(x,arrayno,channel=c("red","green","logratio"),
      mycols=NULL, mybreaks=NULL, dim1="X", dim2="Y",
      ppch=20, pcex=0.3, verbose=TRUE, ...)
```

**Arguments**

x	object of class RGList containing red and green channel raw intensities; possibly result of readNimblegen.
arrayno	integer; which array to plot; one of 1:ncol(x\$R)
channel	character; which channel to plot, either red, green or the logratio $\log_2(\text{red}) - \log_2(\text{green})$
mycols	vector of colors to use for image; if NULL defaults to colorRampPalette(c("White", "Yellow", "Red"))
mybreaks	optional numeric vector of breaks to use as argument breaks in image.default; default NULL means take length(mycols)+1 quantiles of the data as breaks.
dim1	string; which column of the 'genes' element of the supplied RGList indicates the first dimension of the reporter position on the microarray surface; for example this column is called 'X' with some NimbleGen arrays and 'Row' with some Agilent arrays.
dim2	string; which column of the 'genes' element of the supplied RGList indicates the second dimension of the reporter position on the microarray surface; for example this column is called 'Y' with some NimbleGen arrays and 'Col' with some Agilent arrays.
ppch	which symbol to use for intensities; passed on as pch to points.default
pcex	enlargement factor for intensity symbols; passed on as cex to points.default
verbose	logical; extended output to STDOUT?
...	further arguments passed on to plot.default and points.default

**Value**

invisibly returns NULL; function is called for its side effect, this is producing the plot

**Author(s)**

Joern Toedling

**See Also**

[readNimblegen](#), [plot.default](#), [points](#)

**Examples**

```
exDir <- system.file("exData", package="Ringo")
exRG <- readNimblegen("example_targets.txt", "spottypes.txt", path=exDir)
image(exRG, 1, channel="red", mycols=c("black", "darkred", "red"))
## this example looks strange because the example data files only
## includes the probe intensities of probes mapped to the forward
## strand of chromosome 9.
## you can see these probes are distributed all over the array
```

---

nonzero-methods

*Methods for Function nonzero*

---

**Description**

Auxiliary functions to retrieve the indices of non-zero elements in sparse matrices.

**Value**

A two-column matrix. Each row gives the row and column index of a non-zero element in the supplied matrix `x`.

**Methods**

`x = "dgCMatrix"` returns the indices of non-zero elements in matrices of class `dgCMatrix`

`x = "matrix.csr"` returns the indices of non-zero elements in matrices of class `matrix.csr`

`x = "matrix"` returns the indices of non-zero elements in matrices of base class `matrix`; equivalent to `which(x != 0, arr.ind=TRUE)`

**Note**

Originally we used the `matrix.csr` class from `SparseM`, but we have switched to the class `dgCMatrix` from package `Matrix`, as that package is part of the R distribution bundle now.

The idea is to have a function similar to `which(x != 0, arr.ind=TRUE)` if `x` is a matrix.

**See Also**

[dgCMatrix-class](#)



**Examples**

```
(A <- matrix(c(0,0,0,0,0,1,0,0,0,0,0,0,0,-34),
             nrow=5, byrow=TRUE))
str(A.dgc <- as(A, "dgCMatrix"))
nonzero(A.dgc)
A2.dgc <- cbind(A.dgc, A.dgc)
as.matrix(A2.dgc)
nonzero(A2.dgc)
```

---

plot.autocor.result     *Plots auto-correlation of probe intensities*

---

**Description**

Function to plot the auto-correlation of probe intensities computed by function autocor.

**Usage**

```
## S3 method for class 'autocor.result'
plot(x,
      plot.title = "ChIP: Autocorrelation of Intensities", ...)
```

**Arguments**

x	an object of class autocor.result, the result of function autocor
plot.title	main title of the plot
...	further arguments passed on to plot.default, see details

**Details**

The following arguments to plot.default are already defined in the function and thus cannot be specified by the user as further arguments in ...: type,lwd,xlab,ylab,col. Argument main is specified in plot.title.

**Value**

invisible NULL

**Author(s)**

Joern Toedling

**See Also**

[autocor](#)

**Examples**

```
## see the help page of 'autocor' for an example
```

---

`plot.cher`*Plot identified Chers*

---

**Description**

Function for plotting identified *Chers* (ChIP-enriched regions).

**Usage**

```
## S4 method for signature 'cher,ExpressionSet'  
plot(x, y, probeAnno, samples=NULL, extent = 1000, gff = NULL, ...)
```

**Arguments**

<code>x</code>	object of class <code>cher</code>
<code>y</code>	data object of class <code>ExpressionSet</code> that was used for function <code>findChersOnSmoothed</code>
<code>probeAnno</code>	object of class <code>probeAnno</code> holding the reporter/probe to genome mappings
<code>samples</code>	which samples to plot, either a numeric vector of entries in <code>1</code> to <code>ncol(dat)</code> , or character vector with entries in <code>sampleNames(dat)</code> or <code>NULL</code> meaning plot the levels from all samples in the <code>ExpressionSet</code>
<code>extent</code>	integer; how many base pairs to the left and right should the plotted genomic region be extended
<code>gff</code>	data frame with gene/transcript annotation
<code>...</code>	further arguments passed on to function <code>chipAlongChrom</code>

**Value**

called for generating the plot; `invisible(NULL)`

**Author(s)**

Joern Toedling

**See Also**

[chipAlongChrom](#), [cher-class](#)

---

plotBM *Visualization of a binary matrix*

---

**Description**

This function produces simple, heatmap-like visualizations of binary matrices.

**Usage**

```
plotBM(x, boxCol = "darkblue", reorder = FALSE, frame = TRUE, ...)
```

**Arguments**

x	Binary matrix to visualize
boxCol	Color to use for boxes of '1's
reorder	logical; states whether the rows shall be reordered according to the size of the category
frame	logical; states whether a frame should be drawn around the visualization. In contrast to the frame drawn in <code>plot.default</code> , there is no gap between the visualization and this frame.
...	further arguments passed on to <code>plot.default</code>

**Details**

For reordering, each row is interpreted as a binary matrix, for example a row  $z=(1,0,0,1)$  would be interpreted as the binary number  $1001 = 9$  in the decimal system. Rows are then reordered by the frequency of each binary number with the rows that correspond to the most frequent binary number shown at the top in the visualization.

**Value**

The function invisibly returns the (reordered) matrix `x`, but its mainly called for its side effect of producing the visualization.

**Note**

An alternative way to display such matrices are given by `heatmap` or, the simpler version thereof, `image`. However, image files produced with this functions tend to be very large. This function uses `plot.default` and `polygon` which results in much smaller file sizes and is sufficient for binary matrices.

**Author(s)**

Joern Toedling

**See Also**[polygon,colors](#)**Examples**

```
A <- matrix(round(runif(80)), ncol=4, byrow=TRUE)
dimnames(A)=list(letters[seq(nrow(A))],
                 as.character(as.roman(seq(ncol(A))))))
show(A)
plotBM(A, reorder=FALSE)
plotBM(A, reorder=TRUE)
```

---

`posToProbeAnno`*Function for creating a probeAnno environment*

---

**Description**

This function allows the user to create a probeAnno environment that holds the mapping between probes on the array and their genomic match position(s). As input, the function takes either a.) one of NimbleGen's POS file or a similar file that holds the mapping of probes to the genome. OR b.) a data.frame holding this information

**Usage**

```
posToProbeAnno(pos, chrNameColumn = "CHROMOSOME",
               probeColumn = "PROBE_ID", chrPositionColumn = "POSITION",
               lengthColumn = "LENGTH", sep="\t", genome="unknown",
               microarrayPlatform="unknown", verbose = TRUE, ...)
```

**Arguments**

<code>pos</code>	either a file-name that specifies the path to the POS or other mapping file OR a data.frame holding the mapping
<code>chrNameColumn</code>	name of the column in the file or data.frame that holds the chromosome name of the match
<code>probeColumn</code>	name of the column that holds the matching probe's unique identifier
<code>chrPositionColumn</code>	name of the column that holds the match genomic position/coordinate on the chromosome
<code>lengthColumn</code>	name of the column that holds the length of the match position, in case of perfect match should correspond to the sequence length of the probe
<code>sep</code>	string; denotes the separator between elements in the supplied mappings file pos; passed on to function scan; ignored if pos is not a filename.
<code>genome</code>	string; denotes genome (and assembly) the reporters have been mapped to for this probeAnno object, e.g. "M. musculus (mm9)"

microarrayPlatform	string; denotes the commercial or custom microarray platform/design that holds the reporters whose mapping is stored in this probeAnno object, e.g. "Nimble-Gen MOD SUZ12"
verbose	logical; should progress be written to STDOUT?
...	further arguments passed on to function scan, which is used for reading in the file pos.

## Details

The default column names correspond to the column names in a NimbleGen POS file.

For custom mappings, using the tools Exonerate, BLAT or MUMmer, the scripts directory of this package holds Perl scripts to generate such a POS file from the respective output files.

## Value

The results is an object of class probeAnno.

## Author(s)

Joern Toedling

## See Also

[probeAnno-class](#), [scan](#)

## Examples

```
exPos <- read.delim(file.path(system.file("exData", package="Ringo"),
  "MOD_2003-12-05_SUZ12_1in2.pos"),
  header=TRUE, as.is=TRUE)
str(exPos)
exProbeAnno <- posToProbeAnno(exPos,
  genome="M. musculus (assembly mm8)",
  microarrayPlatform="NimbleGen 2005-06-17_Ren_MM5Tiling_Set1")
## is equivalent to
exProbeAnno2 <- posToProbeAnno(file.path(
  system.file("exData", package="Ringo"), "MOD_2003-12-05_SUZ12_1in2.pos"),
  genome="M. musculus (assembly mm8)",
  microarrayPlatform="NimbleGen 2005-06-17_Ren_MM5Tiling_Set1")
ls(exProbeAnno)
chromosomeNames(exProbeAnno2)
```

preprocess

*Preprocess Raw ChIP-chip Intensities***Description**

Calls one of various (limma) functions to transform raw probe intensities into (background-corrected) normalized log ratios (M-values).

**Usage**

```
preprocess(myRG, method="vsn", ChIPChannel="R", inputChannel="G",
           returnMAList=FALSE, idColumn="PROBE_ID", verbose=TRUE, ...)
```

**Arguments**

myRG	object of class RGList
method	string; denoting which normalization method to choose, see below for details
ChIPChannel	string; which element of the RGList holds the ChIP result, see details
inputChannel	string; which element of the RGList holds the untreated <i>input</i> sample; see details
returnMAList	logical; should an MAList object be returned? Default is to return an ExpressionSet object.
idColumn	string; indicating which column of the genes data.frame of the RGList holds the identifier for reporters on the microarray. This column, after calling <code>make.names</code> on it, will make up the unique featureNames of the resulting ExpressionSet. If argument returnMAList is TRUE, this argument is ignored.
verbose	logical; progress output to STDOUT?
...	further arguments to be passed on normalizeWithinArrays and normalizeBetweenArrays

**Details**

The procedure and called limma functions depend on the choice of method.

**loess** Calls normalizeWithinArrays with method="loess".

**vsn** Calls normalizeBetweenArrays with method="vsn".

**Gquantile** Calls normalizeBetweenArrays with method="Gquantile".

**Rquantile** Calls normalizeBetweenArrays with method="Rquantile".

**median** Calls normalizeWithinArrays with method="median".

**nimblegen** Scaling procedure used by Nimblegen. Yields scaled log-ratios by a two step procedure:  $srat = \log_2(R) - \log_2(G)$   $srat = srat - \text{tukey.biweight}(srat)$

**Gvsn** Learns vsn model on green channel intensities only and applies that transformation to both channels before computing fold changes.

**Rvsn** Learns vsn model on red channel intensities only and applies that transformation to both channels before computing fold changes.

**none** No normalization of probe intensities, takes raw  $\log_2(R) - \log_2(G)$  as component M and  $(\log_2(R) + \log_2(G))/2$  as component A; uses `normalizeWithinArrays` with `method="none"`.

Mostly with two-color ChIP-chip, the ChIP sample is marked with the red Cy5 dye and for the untreated *input* sample the green Cy3 dye is used. In that case the `RGListmyRG`'s element R holds the ChIP data, and element G holds the input data. If this is not the case with your data, use the arguments `ChIPChannel` and `inputChannel` to specify the respective elements of `myRG`.

### Value

Returns normalized, transformed values as an object of class `ExpressionList` or `MAList`.

### Note

Since Ringo version 1.5.6, this function does not call `limma`'s function `backgroundCorrect` directly any longer. If wanted by the user, background correction should be indicated as additional arguments passed on to `normalizeWithinArrays` or `normalizeBetweenArrays`, or alternatively call `backgroundCorrect` on the `RGList` before preprocessing.

### Author(s)

Joern Toedling

### See Also

[normalizeWithinArrays](#), [normalizeBetweenArrays](#), [malist](#), [ExpressionSet](#), [vsnMatrix](#)

### Examples

```
exDir <- system.file("exData",package="Ringo")
exRG <- readNimblegen("example_targets.txt","spottypes.txt",
                    path=exDir)
exampleX <- preprocess(exRG)
sampleNames(exampleX) <- make.names(paste(exRG$targets$Cy5,"vs",
                                          exRG$targets$Cy3,sep="_"))

print(exampleX)
### compare VSN to NimbleGen's tukey-biweight scaling
exampleX.NG <- preprocess(exRG, method="nimblegen")
sampleNames(exampleX.NG) <- sampleNames(exampleX)
if (interactive())
  corPlot(cbind(exprs(exampleX),exprs(exampleX.NG)),
          grouping=c("VSN normalized","Tukey-biweight scaled"))
```

---

probeAnno-class      *Class "probeAnno"*

---

### Description

A class that holds the mapping between reporters/probes on a microarray and their genomic match position(s) in a chosen genome.

### Objects from the Class

Objects can be created by calls of the form `new("probeAnno", map, arrayName, genome)`.

### Slots

**map:** Object of class "environment" This map consists of four vectors for each chromosome/strand, namely, say for chromosome 1:

**1.start** genomic start coordinates of probe matches on chromosome 1

**1.end** genomic end coordinates of probe matches on chromosome 1

**1.index** identifier of probes matching at these coordinates

**1.unique** vector of the same length as the three before; encoding how many matches the corresponding probe has in the given file or data.frame. An entry of '0' indicates that the probe matching at this position has only this one match.

**arrayName:** Object of class "character", the name or identifier of the microarray design, e.g. 2005-06-17\\_Ren\\_MM5Tiling\\_Set1

**genome:** Object of class "character", which genome the reporters have been mapped to

### Methods

**arrayName** obtain the microarray platform name

**arrayName<-** replace the microarray platform name

**[** get elements from the map environment

**[<-** assign elements to the map environment

**chromosomeNames** obtain a character vector holding the names of the chromosomes for which the probeAnno objects holds a mapping.

**get** get elements from the map environment

**initialize** create new probeAnno object

**ls** list elements of the map environment

**genome** obtain the description of the genome the reporters were mapped to

**genome<-** replace the description of the genome the reporters were mapped to

**as** signature(from="environment"); function to coerce old-style 'probeAnno' environments to new-style 'probeAnno' objects. Simply creates a new object with the old environment in its map slot



**Note**

'probeAnno' objects used to be environments and still are used as such in package tilingArray

**Author(s)**

Joern Toedling; Wolfgang Huber

**See Also**

posToProbeAnno

**Examples**

```
pa <- new("probeAnno")
pa["X.start"] <- seq(5000,10000,by=1000)
if (interactive()) show(pa)
pa2 <- posToProbeAnno(file.path(system.file("exData",package="Ringo"),
                                         "MOD_2003-12-05_SUZ12_1in2.pos"))
arrayName(pa2) <- "NimbleGen MOD_2003-12-05_SUZ12_1in2"
genome(pa2) <- "H. sapiens (hg18)"
show(pa2)
head(pa2["9.start"])
```

---

quantilesOverPositions

*show ChIP-chip data aligned over genome features, e.g. TSSs*

---

**Description**

Function to show the ChIP-chip data aligned over certain genome features, for example transcription start sites (TSSs).

**Usage**

```
quantilesOverPositions(xSet, selGenes, g2p,
                      positions = seq(-5000, 10000, by = 250),
                      quantiles = c(0.1, 0.5, 0.9))
```

**Arguments**

xSet	an ExpressionSet holding the ChIP-chip data
selGenes	character; vector of genome features, e.g. transcripts, to use for the plot
g2p	A list object containing the mapping between genome positions and probes on the microarray. Created with the function features2Probes.
positions	Numeric vector of positions related to the coordinates of the genome features, such as in which distances of the TSS the values should be computed over the aligned data
quantiles	numeric; which quantiles to compute over the aligned data

**Value**

An object of class `qop`, which can be visualized by its `plot` method.

**Author(s)**

Joern Toedling

**See Also**

[features2Probes](#), [qop-class](#)

**Examples**

```
ringoExampleDir <- system.file("exData",package="Ringo")
load(file.path(ringoExampleDir,"exampleProbeAnno.rda"))
trans2Probe <- features2Probes(exGFF, exProbeAnno)
load(file.path(ringoExampleDir,"exampleX.rda"))
exampleSX <- computeRunningMedians(exampleX, probeAnno=exProbeAnno,
  modColumn = "Cy5", allChr = "9", winHalfSize = 400)
exampleC <- findChersOnSmoothed(exampleSX, probeAnno=exProbeAnno,
  thresholds=0.2, allChr="9", distCutOff=600, cellType="human")
exampleC <- relateChers(exampleC, exGFF)
exampleQop <- quantilesOverPositions(exampleSX,
  selGenes=getFeats(exampleC), quantiles=c(0.5, 0.9),
  g2p=trans2Probe, positions=seq(-4000, 1000, by=250))
show(exampleQop)
plot(exampleQop, ylim=c(-0.5, 2.1))
```

---

readNimblegen

*Function to read in Nimblegen Intensity Text Files*

---

**Description**

Function to read in Nimblegen Intensity Text Files into an `RGList`. Calls some other functions for actual reading of data. This function is to be used with two-color NimbleGen array data. Use the function `read.xyfiles` of the `oligo` package for single-color data.

**Usage**

```
readNimblegen(hybesFile, spotTypesFile, path = getwd(),
  headerPattern="# software=NimbleScan",verbose = TRUE, ...)
```

**Arguments**

<code>hybesFile</code>	Name of the file describing the arrays. In <code>limma</code> this file would be called <code>targets</code> file.
<code>spotTypesFile</code>	spot types also used by <code>limma</code>

path	Path to directory that holds the files hybesFile, spotTypesFile and also the intensity files. Set this to NULL if you prefer the arguments hybesFile, spotTypesFile and the file-name entries of the hybes file to be treated as absolute or relative file paths themselves.
headerPattern	string; pattern used to identify explanatory header lines in the supplied pair-format files
verbose	logical; progress output to STDOUT?
...	further arguments passed on the readNgIntensitiesTxt

**Value**

Returns raw intensity values in form of an RGList.

**Author(s)**

Joern Toedling

**See Also**

[rglist](#), [readTargets](#)

**Examples**

```
exDir <- system.file("exData", package="Ringo")
exRG <- readNimblegen("example_targets.txt", "spottypes.txt", path=exDir)
print(exRG)
```

---

regionOverlap

*Function to compute overlap of genomic regions*

---

**Description**

Given two data frames of genomic regions, this function computes the base-pair overlap, if any, between every pair of regions from the two lists.

**Usage**

```
regionOverlap(xdf, ydf, chrColumn = "chr", startColumn = "start",
              endColumn = "end", mem.limit=1e8)
```

**Arguments**

xdf	data.frame that holds the first set of genomic regions
ydf	data.frame that holds the first set of genomic regions
chrColumn	character; what is the name of the column that holds the chromosome name of the regions in xdf and ydf

startColumn	character; what is the name of the column that holds the start position of the regions in xdf and ydf
endColumn	character; what is the name of the column that holds the start position of the regions in xdf and ydf
mem.limit	integer value; what is the maximal allowed size of matrices during the computation

**Value**

Originally, a matrix with `nrow(xdf)` rows and `nrow(ydf)` columns, in which entry `X[i, j]` specifies the length of the overlap between region `i` of the first list (`xdf`) and region `j` of the second list (`ydf`). Since this matrix is very sparse, we use the `dgMatrix` representation from the `Matrix` package for it.

**Note**

The function only return the absolute length of overlapping regions in base-pairs. It does not return the position of the overlap or the fraction of region 1 and/or region 2 that overlaps the other regions.

The argument `mem.limit` is not really a limit to used RAM, but rather the maximal size of matrices that should be allowed during the computation. If larger matrices would arise, the second regions list is split into parts and the overlap with the first list is computed for each part. During computation, matrices of size `nrow(xdf)` times `nrow(ydf)` are created.

**Author(s)**

Joern Toedling

**See Also**

[dgMatrix-class](#)

**Examples**

```
## toy example:
regionsH3ac <- data.frame(chr=c("chr1", "chr7", "chr8", "chr1", "chrX", "chr8"), start=c(100, 100, 100, 510, 100, 60), e
regionsH4ac <- data.frame(chr=c("chr1", "chr2", "chr7", "chr8", "chr9"),
start=c(500, 100, 50, 80, 100), end=c(700, 200, 250, 120, 200))

## compare the regions first by eye
## which ones do overlap and by what amount?
regionsH3ac
regionsH4ac

## compare it to the result:
as.matrix(regionOverlap(regionsH3ac, regionsH4ac))
nonzero(regionOverlap(regionsH3ac, regionsH4ac))
```

---

relateChers	<i>Relate found Chers to genomic features</i>
-------------	---

---

### Description

This function relates found 'cher's (ChIP-enriched regions) to annotated genomic features, such as transcripts.

### Usage

```
relateChers(pl, gff, upstream = 5000, verbose = TRUE)
```

### Arguments

pl	Object of class <code>cherList</code>
gff	<code>data.frame</code> holding genomic feature annotation
upstream	up to how many bases upstream of annotated genomic features should chers be counted as related to that feature (see details)
verbose	logical; extended output to STDOUT?

### Details

chers will be counted as related to genomic features, if

- their middle position is located between start and end position of the feature
- their middle position is located not more than argument `upstream` bases upstream of the feature start

One can visualize such cher-feature relations as a graph using the Bioconductor package `Rgraphviz`. See the script `'graphChers2Transcripts.R'` in Ringo's scripts directory for an example.

### Value

An object of class `cherList` with for each cher the elements `typeUpstream` and `typeInside` filled in with the names of the features that have been related to.

### Author(s)

Joern Toedling

### Examples

```
# see findChersOnSmoothed for an example
```

---

sliding.meansd	<i>Compute mean and standard deviation of scores in a sliding window</i>
----------------	--

---

**Description**

This functions is used to slide a window of specified size over scores at given positions. Computed is the mean and standard deviation over the scores in the window.

**Usage**

```
sliding.meansd(positions, scores, half.width)
```

**Arguments**

positions	numeric; sorted vector of (genomic) positions of scores
scores	numeric; scores to be smoothed associated to the positions
half.width	numeric, half the window size of the sliding window

**Value**

Matrix with three columns:

mean	means over scores in running window centered at the positions that were specified in argument positions.
sd	standard deviations over scores in running window centered at the positions that were specified in argument positions.
count	number of points that were considered for computing the mean and standard deviation at each position

**Author(s)**

Joern Toedling and Oleg Sklyar

**See Also**

[sliding.quantile](#)

**Examples**

```
set.seed(123)
sampleSize <- 10
ap <- cumsum(1+round(runif(sampleSize)*10))
as <- c(rnorm(floor(sampleSize/3)),
        rnorm(ceiling(sampleSize/3),mean=1.5),
        rnorm(floor(sampleSize/3)))
sliding.meansd(ap, as, 20)
ap
mean(as[1:3])
sd(as[1:3])
```

---

sliding.quantile	<i>Compute quantile of scores in a sliding window</i>
------------------	---

---

### Description

This function is used to slide a window of specified size over scores at given positions. Computed is the quantile over the scores in the window.

### Usage

```
sliding.quantile(positions, scores, half.width, prob = 0.5,  
                 return.counts = TRUE)
```

### Arguments

positions	numeric; sorted vector of (genomic) positions of scores
scores	numeric; scores to be smoothed associated to the positions
half.width	numeric, half the window size of the sliding window
prob	numeric specifying which quantile is to be computed over the scores in the window; default 0.5 means compute the median over the scores.
return.counts	logical; should the number of points, e.g. probes, that were used for computing the median in each sliding window also returned?

### Value

Matrix with two columns:

quantile	medians over running window centered at the positions that were specified in argument positions.
count	number of points that were considered for computing the median at each position

These positions are given as row.names of the resulting vector. If argument return.counts is FALSE, only a vector of the medians is returned, with the positions as names.

### Author(s)

Oleg Sklyar and Joern Toedling

### See Also

[quantile](#)

**Examples**

```

sampleSize <- 1000
ap <- cumsum(1+round(runif(sampleSize)*10))
as <- c(rnorm(floor(sampleSize/3)),
        rnorm(ceiling(sampleSize/3),mean=1.5),
        rnorm(floor(sampleSize/3)))
arm <- sliding.quantile(ap, as, 20)
arq <- sliding.quantile(ap, as, 20, prob=0.25)
plot(ap, as, pch=20, xlab="position",ylab="level")
points(ap, arm[,1], type="l", col="red", lwd=2)
points(ap, arq[,1], type="l", col="green", lwd=2)
legend(x="topleft", legend=c("median","1st quartile"),
       col=c("red","green"), lty=1, lwd=2)

```

---

twoGaussiansNull

*Estimate a threshold from Gaussian mixture distribution*


---

**Description**

Function to estimate a threshold from Gaussian mixture distribution. The data is assumed to follow a mixture of two Gaussian distributions. The one Gaussian with the lower mean value is assumed to be the null distribution and probe levels are assigned p-values based on this null distribution. The threshold is then the minimal data value with an adjusted p-value smaller than a specified threshold.

**Usage**

```
twoGaussiansNull(x, p.adj.method = "BY", max.adj.p = 0.1, var.equal = FALSE, ...)
```

**Arguments**

<code>x</code>	numeric vector of data values
<code>p.adj.method</code>	method for adjusting the p-values for multiple testing; must be one of <code>p.adjust.methods</code>
<code>max.adj.p</code>	which adjusted p-value to use as upper limit for estimating the threshold
<code>var.equal</code>	logical; is the variance of the two Gaussians assumed to be equal or different
<code>...</code>	further arguments passed on to function <code>Mclust</code>

**Details**

This function uses the package `mclust` to fit a mixture of two Gaussians to the data. The threshold is then estimated from the fitted Gaussian with the lower mean value.

**Value**

Single numeric value. The threshold that is the minimal data value with an adjusted p-value smaller than a specified threshold.



**Note**

Please note that the use of the package 'mclust' is only free for strict academic use (see the license of 'mclust' here: <http://www.stat.washington.edu/mclust/license.txt> ). The alternative function `upperBoundNull` does not have this restriction.

Thanks to Richard Bourgon for pointing out the necessity of providing this method as an alternative way of estimating the threshold.

**Author(s)**

Joern Toedling, Aleksandra Pekowska

**See Also**

`mclust`, `p.adjust`, `upperBoundNull`

**Examples**

```
exDir <- system.file("exData", package="Ringo")
load(file.path(exDir, "exampleProbeAnno.rda"))
load(file.path(exDir, "exampleX.rda"))
smoothX <- computeRunningMedians(exampleX, probeAnno=exProbeAnno,
  modColumn = "Cy5", allChr = "9", winHalfSize = 400)

## compare the two different ways of estimating the threshold
y0a <- apply(exprs(smoothX), 2, upperBoundNull)
y0b <- apply(exprs(smoothX), 2, twoGaussiansNull)

hist(exprs(smoothX)[,1], n=10, main=NA,
  xlab="Smoothed expression level [log2]")
abline(v=c(y0a, y0b), col=c("blue", "orange"), lwd=2)
legend(x="topright", col=c("blue", "orange"), lwd=2,
  legend=c(expression(paste(y[0], " Non-parametric")),
    expression(paste(y[0], " Gaussian"))))
```

---

`upperBoundNull`

*function to estimate upper limit of null distribution*

---

**Description**

The data is assumed to arise from a mixture of two distributions, a symmetric null distribution with its mode close to zero, and an alternative distribution that is stochastically larger than the null. This function tries to pinpoint the minimum of data values that are more likely to arise from the alternative distribution, i.e. an upper bound for values following the null distribution.

**Usage**

```
upperBoundNull(x, modeMethod = "shorth", limits = c(-1, 1), prob = 0.99, ...)
```

### Arguments

x	numeric vector of data values
modeMethod	character string; which method to use for estimating the mode of the null distribution; see details
limits	numeric of length 2; data values within this range are used for estimating the mode of the null distribution
prob	quantile of the null distribution that will be used as an upper bound
...	additional arguments that are passed on to the function for mode estimation

### Details

For estimating the mode of the null distribution, current options are

**"shorth"** the function [shorth](#)

**"half.range.mode"** the function [half.range.mode](#)

**"null"** does not estimate the mode from the data, but sets it to 0

### Value

a single numeric value which is the estimated upper bound for the null distribution.

### Note

This way of estimating the null distribution is mentioned in the PhD thesis of Richard Bourgon.

### Author(s)

Joern Toedling, based on suggestions by Richard Bourgon

### See Also

[shorth](#), [half.range.mode](#)

### Examples

```
exDir <- system.file("exData", package="Ringo")
load(file.path(exDir, "exampleProbeAnno.rda"))
load(file.path(exDir, "exampleX.rda"))
smoothX <- computeRunningMedians(exampleX, probeAnno=exProbeAnno,
  modColumn = "Cy5", allChr = "9", winHalfSize = 400)
apply(exprs(smoothX), 2, upperBoundNull)
```

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