

Package ‘oligo’

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Title Preprocessing tools for oligonucleotide arrays

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VignetteBuilder knitr

Description A package to analyze oligonucleotide arrays (expression/SNP/tiling/exon) at probe-level. It currently supports Affymetrix (CEL files) and NimbleGen arrays (XYS files).

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Collate AllGenerics.R methods-GenericArrays.R methods-GeneFeatureSet.R methods-ExonFeatureSet.R methods-ExpressionFeatureSet.R methods-ExpressionSet.R methods-LDS.R methods-FeatureSet.R methods-SnpFeatureSet.R methods-SnpCnvFeatureSet.R methods-TilingFeatureSet.R methods-HtaFeatureSet.R methods-DBPDInfo.R methods-background.R methods-normalization.R methods-summarization.R read.celfiles.R read.xysfiles.R utils-general.R utils-selectors.R todo-snp.R functions-crlmm.R functions-snpRMA.R justSNPRMA.R justCRLMM.R methods-snp6.R methods-genotype.R methods-PLMset.R zzz.R

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oligo-package	<i>The oligo package: a tool for low-level analysis of oligonucleotide arrays</i>
---------------	---

Description

The **oligo** package provides tools to preprocess different oligonucleotide arrays types: expression, tiling, SNP and exon chips. The supported manufacturers are Affymetrix and NimbleGen.

It offers support to large datasets (when the **bigmemory** is loaded) and can execute preprocessing tasks in parallel (if, in addition to **bigmemory**, the **snow** package is also loaded).

Details

The package will read the raw intensity files (CEL for Affymetrix; XYS for NimbleGen) and allow the user to perform analyses starting at the feature-level.

Reading in the intensity files require the existence of data packages that contain the chip specific information (X/Y coordinates; feature types; sequence). These data packages packages are built using the **pdInfoBuilder** package.

For Affymetrix SNP arrays, users are asked to download the already built annotation packages from BioConductor. This is because these packages contain metadata that are not automatically created. The following annotation packages are available:

50K Xba - pd.mapping50kxba.240 50K Hind - pd.mapping50khind.240 250K Sty - pd.mapping250k.sty
 250K Nsp - pd.mapping250k.nsp GenomeWideSnp 5 (SNP 5.0) - pd.genomewidesnp.5 GenomeWideSnp
 6 (SNP 6.0) - pd.genomewidesnp.6

For users interested in genotype calls for SNP 5.0 and 6.0 arrays, we strongly recommend the use use the **crlmm** package, which implements a more efficient version of CRLMM.

Author(s)

Benilton Carvalho - <carvalho@bclab.org>

References

Carvalho, B.; Bengtsson, H.; Speed, T. P. & Irizarry, R. A. Exploration, Normalization, and Genotype Calls of High Density Oligonucleotide SNP Array Data. Biostatistics, 2006.

basecontent	<i>Sequence Base Contents</i>
-------------	-------------------------------

Description

Function to compute the amounts of each nucleotide in a sequence.

Usage

```
basecontent(seq)
```

Arguments

seq character vector of length n containing a valid sequence (A/T/C/G)

Value

matrix with n rows and 4 columns with the counts for each base.

Examples

```
sequences <- c("ATATATCCCCG", "TTTCCGAGC")
basecontent(sequences)
```

basicPLM	<i>Simplified interface to PLM.</i>
----------	-------------------------------------

Description

Simplified interface to PLM.

Usage

```
basicPLM(pmMat, pnVec, normalize = TRUE, background = TRUE, transfo =
  log2, method = c('plm', 'plmr', 'plmrr', 'plmrc'), verbose = TRUE)
```

Arguments

pmMat	Matrix of intensities to be processed.
pnVec	Probeset names
normalize	Logical flag: normalize?
background	Logical flag: background adjustment?
transfo	function: function to be used for data transformation prior to summarization.
method	Name of the method to be used for normalization. 'plm' is the usual PLM model; 'plmr' is the (row and column) robust version of PLM; 'plmrr' is the row-robust version of PLM; 'plmrc' is the column-robust version of PLM.
verbose	Logical flag: verbose.

Value

A list with the following components:

Estimates	A (length(pnVec) x ncol(pmMat)) matrix with probeset summaries.
StdErrors	A (length(pnVec) x ncol(pmMat)) matrix with standard errors of 'Estimates'.
Residuals	A (nrow(pmMat) x ncol(pmMat)) matrix of residuals.

Note

Currently, only RMA-bg-correction and quantile normalization are allowed.

Author(s)

Benilton Carvalho

See Also

[rcModelPLM](#), [rcModelPLMr](#), [rcModelPLMrr](#), [rcModelPLMrc](#), [basicRMA](#)

Examples

```
set.seed(1)
pms <- 2^matrix(rnorm(1000), nc=20)
colnames(pms) <- paste("sample", 1:20, sep="")
pns <- rep(letters[1:10], each=5)
res <- basicPLM(pms, pns, TRUE, TRUE)
res[['Estimates']][1:4, 1:3]
res[['StdErrors']][1:4, 1:3]
res[['Residuals']][1:20, 1:3]
```

basicRMA

Simplified interface to RMA.

Description

Simple interface to RMA.

Usage

```
basicRMA(pmMat, pnVec, normalize = TRUE, background = TRUE, bgversion = 2, destructive = FALSE, verbose = FALSE)
```

Arguments

pmMat	Matrix of intensities to be processed.
pnVec	Probeset names.
normalize	Logical flag: normalize?
background	Logical flag: background adjustment?
bgversion	Version of background correction.
destructive	Logical flag: use destructive methods?
verbose	Logical flag: verbose.
...	Not currently used.

Value

Matrix.

Examples

```
set.seed(1)
pms <- 2^matrix(rnorm(1000), nc=20)
colnames(pms) <- paste("sample", 1:20, sep="")
pns <- rep(letters[1:10], each=5)
res <- basicRMA(pms, pns, TRUE, TRUE)
res[, 1:3]
```

boxplot

Boxplot

Description

Boxplot for observed (log-)intensities in a FeatureSet-like object (ExpressionFeatureSet, ExonFeatureSet, SnpFeatureSet, TilingFeatureSet) and ExpressionSet.

Usage

```
## S4 method for signature 'FeatureSet'
boxplot(x, which=c("pm", "mm", "bg", "both",
"all"), transfo=log2, nsample=10000, target = "mps1", ...)

## S4 method for signature 'ExpressionSet'
boxplot(x, which, transfo=identity, nsample=10000, ...)
```

Arguments

<code>x</code>	a FeatureSet-like object or ExpressionSet object.
<code>which</code>	character defining what probe types are to be used in the plot.
<code>transfo</code>	a function to transform the data before plotting. See 'Details'.
<code>nsample</code>	number of units to sample and build the plot.
<code>...</code>	arguments to be passed to the default boxplot method.

Details

The 'transfo' argument will set the transformation to be used. For raw data, 'transfo=log2' is a common practice. For summarized data (which are often in log2-scale), no transformation is needed (therefore 'transfo=identity').

Note

The boxplot methods for FeatureSet and Expression use a sample (via `sample`) of the probes/probesets to produce the plot. Therefore, the user interested in reproducibility is advised to use `set.seed`.

See Also

[hist](#), [image](#), [sample](#), [set.seed](#)

chromosome	<i>Accessor for chromosome information</i>
------------	--

Description

Returns chromosome information.

Usage

```
pmChr(object)
```

Arguments

object TilingFeatureSet or SnpCallSet object

Details

chromosome() returns the chromosomal information for all probes and pmChr() subsets the output to the PM probes only (if a TilingFeatureSet object).

Value

Vector with chromosome information.

crlmm	<i>Genotype Calls</i>
-------	-----------------------

Description

Performs genotype calls via CRLMM (Corrected Robust Linear Model with Maximum-likelihood based distances).

Usage

```
crlmm(filenamees, outdir, batch_size=40000, balance=1.5,
       minLLRforCalls=c(5, 1, 5), recalibrate=TRUE,
       verbose=TRUE, pkgname, reference=TRUE)
justCRLMM(filenamees, batch_size = 40000, minLLRforCalls = c(5, 1, 5),
recalibrate = TRUE, balance = 1.5, phenoData = NULL, verbose = TRUE,
pkgname = NULL, tmpdir=tmpdir())
```

Arguments

filenamees	character vector with the filenamees.
outdir	directory where the output (and some tmp files) files will be saved.
batch_size	integer defining how many SNPs should be processed at a time.
recalibrate	Logical - should recalibration be performed?
balance	Control parameter to balance homozygotes and heterozygotes calls.

minLLRforCalls	Minimum thresholds for genotype calls.
verbose	Logical.
phenoData	phenoData object or NULL
pkgname	alt. pdInfo package to be used
reference	logical, defaulting to TRUE ...
tmpdir	Directory where temporary files are going to be stored at.

Value

SnpCallSetPlus object.

darkColors	<i>Create set of colors, interpolating through a set of preferred colors.</i>
------------	---

Description

Create set of colors, interpolating through a set of preferred colors.

Usage

```
darkColors(n)
seqColors(n)
seqColors2(n)
divColors(n)
```

Arguments

n integer determining number of colors to be generated

Details

darkColors is based on the Dark2 palette in RColorBrewer, therefore useful to describe qualitative features of the data.

seqColors is based on Blues and generates a gradient of blues, therefore useful to describe quantitative features of the data. seqColors2 behaves similarly, but it is based on OrRd (white-orange-red).

divColors is based on the RdBu palette in RColorBrewer, therefore useful to describe quantitative features ranging on two extremes.

Examples

```
x <- 1:10
y <- 1:10
cols1 <- darkColors(10)
cols2 <- seqColors(10)
cols3 <- divColors(10)
cols4 <- seqColors2(10)
plot(x, y, col=cols1, xlim=c(1, 13), pch=19, cex=3)
points(x+1, y, col=cols2, pch=19, cex=3)
points(x+2, y, col=cols3, pch=19, cex=3)
points(x+3, y, col=cols4, pch=19, cex=3)
abline(0, 1, lty=2)
```



```
abline(-1, 1, lty=2)
abline(-2, 1, lty=2)
abline(-3, 1, lty=2)
```

fitProbeLevelModel *Tool to fit Probe Level Models.*

Description

Fits robust Probe Level linear Models to all the (meta)probesets in an [FeatureSet](#). This is carried out on a (meta)probeset by (meta)probeset basis.

Usage

```
fitProbeLevelModel(object, background=TRUE, normalize=TRUE, target="core", method="plm", verbose=
```

Arguments

object	FeatureSet object.
background	Do background correction?
normalize	Do normalization?
target	character vector describing the summarization target. Valid values are: 'probeset', 'core' (Gene/Exon), 'full' (Exon), 'extended' (Exon).
method	summarization method to be used.
verbose	verbosity flag.
S4	return final value as an S4 object (oligoPLM) if TRUE. If FALSE, final value is returned as a list.
...	subset to be passed down to getProbeInfo for subsetting. See subset for details.

Value

fitProbeLevelModel returns an [oligoPLM](#) object, if S4=TRUE; otherwise, it will return a list.

Note

This is the initial port of fitPLM to oligo. Some features found on the original work by Ben Bolstad (in the affyPLM package) may not be yet available. If you found one of this missing characteristics, please contact Benilton Carvalho.

Author(s)

This is a simplified port from Ben Bolstad's work implemented in the affyPLM package. Problems with the implementation in oligo should be reported to Benilton Carvalho.

References

Bolstad, BM (2004) *Low Level Analysis of High-density Oligonucleotide Array Data: Background, Normalization and Summarization*. PhD Dissertation. University of California, Berkeley.

See Also

[rma](#), [summarizationMethods](#), [subset](#)

Examples

```
if (require(oligoData)){
  data(nimbleExpressionFS)
  fit <- fitProbeLevelModel(nimbleExpressionFS)
  image(fit)
  NUSE(fit)
  RLE(fit)
}
```

getAffinitySplineCoefficients

Estimate affinity coefficients.

Description

Estimate affinity coefficients using sequence information and splines.

Usage

```
getAffinitySplineCoefficients(intensities, sequences)
```

Arguments

intensities	Intensity matrix
sequences	Probe sequences

Value

Matrix with estimated coefficients.

See Also

[getBaseProfile](#)

getBaseProfile

Compute and plot nucleotide profile.

Description

Computes and, optionally, plots nucleotide profile, describing the sequence effect on intensities.

Usage

```
getBaseProfile(coefs, probeLength = 25, plot = FALSE, ...)
```

Arguments

coefs	affinity spline coefficients.
probeLength	length of probes
plot	logical. Plots profile?
...	arguments to be passed to <code>matplot</code> .

Value

Invisibly returns a matrix with estimated effects.

getContainer	<i>Get container information for NimbleGen Tiling Arrays.</i>
--------------	---

Description

Get container information for NimbleGen Tiling Arrays. This is useful for better identification of control probes.

Usage

```
getContainer(object, probeType)
```

Arguments

object	A <code>TilingFeatureSet</code> or <code>TilingFeatureSet</code> object.
probeType	String describing which probes to query ('pm', 'bg')

Value

'character' vector with container information.

getCrlmmSummaries	<i>Function to get CRLMM summaries saved to disk</i>
-------------------	--

Description

This will read the summaries written to disk and return them to the user as a `SnpCallSetPlus` or `SnpCnvCallSetPlus` object.

Usage

```
getCrlmmSummaries(tmpdir)
```

Arguments

tmpdir	directory where CRLMM saved the results to.
--------	---

Value

If the data were from SNP 5.0 or 6.0 arrays, the function will return a `SnpCnvCallSetPlus` object. It will return a `SnpCallSetPlus` object, otherwise.

 getNetAffx

NetAffx Biological Annotations

Description

Gets NetAffx Biological Annotations saved in the annotation package (Exon and Gene ST Affymetrix arrays).

Usage

```
getNetAffx(object, type = "probeset")
```

Arguments

object	'ExpressionSet' object (eg., result of rma())
type	Either 'probeset' or 'transcript', depending on what type of summaries were obtained.

Details

This retrieves NetAffx annotation saved in the (pd) annotation package - annotation(object). It is only available for Exon ST and Gene ST arrays.

The 'type' argument should match the summarization target used to generate 'object'. The 'rma' method allows for two targets: 'probeset' (target='probeset') and 'transcript' (target='core', target='full', target='extended').

Value

'AnnotatedDataFrame' that can be used as featureData(object)

Author(s)

Benilton Carvalho

 getNgsColorsInfo

Helper function to extract color information for filenames on Nimble-Gen arrays.

Description

This function will (try to) extract the color information for NimbleGen arrays. This is useful when using read.xlsfiles2 to parse XYS files for Tiling applications.

Usage

```
getNgsColorsInfo(path = ".", pattern1 = "_532", pattern2 = "_635", ...)
```

Arguments

path	path where to look for files
pattern1	pattern to match files supposed to go to the first channel
pattern2	pattern to match files supposed to go to the second channel
...	extra arguments for list.xlsfiles

Details

Many NimbleGen samples are identified following the pattern sampleID_532.XYS / sampleID_635.XYS. The function suggests sample names if all the filenames follow the standard above.

Value

A data.frame with, at least, two columns: 'channel1' and 'channel2'. A third column, 'sample-Names', is returned if the filenames follow the sampleID_532.XYS / sampleID_635.XYS standard.

Author(s)

Benilton Carvalho <bcarvalh@jhsp.edu>

getPlatformDesign *Retrieve Platform Design object*

Description

Retrieve platform design object.

Usage

```
getPlatformDesign(object)
getPD(object)
```

Arguments

object FeatureSet object

Details

Retrieve platform design object.

Value

platformDesign or PDInfo object.

getProbeInfo *Probe information selector.*

Description

A tool to simplify the selection of probe information, so user does not need to use the SQL approaches.

Usage

```
getProbeInfo(object, field, probeType = "pm", target = "core", sortBy = c("fid", "man_fsetid", "none"))
```

Arguments

object	FeatureSet object.
field	character string with names of field(s) of interest to be obtained from database.
probeType	character string: 'pm' or 'mm'
target	Used only for Exon or Gene ST arrays: 'core', 'full', 'extended', 'probeset'.
sortBy	Field to be used for sorting.
...	Arguments to be passed to subset

Value

A data.frame with the probe level information.

Note

The code allows for querying info on MM probes, however it has been used mostly on PM probes.

Author(s)

Benilton Carvalho

Examples

```
if (require(oligoData)){
  data(affyGeneFS)
  availProbeInfo(affyGeneFS)
  probeInfo <- getProbeInfo(affyGeneFS, c('fid', 'x', 'y', 'chrom'))
  head(probeInfo)
  ## Selecting antigenomic background probes
  agenGene <- getProbeInfo(affyGeneFS, field=c('fid', 'fsetid', 'type'), target='probeset', subset= type == 'c')
  head(agenGene)
}
```

getX	<i>Accessors for physical array coordinates.</i>
------	--

Description

Accessors for physical array coordinates.

Usage

```
getX(object, type)
getY(object, type)
```

Arguments

object	FeatureSet object
type	'character' defining the type of the probes to be queried. Valid options are 'pm', 'mm', 'bg'

Value

A vector with the requested coordinates.

Examples

```
## Not run:
x <- read.celfiles(list.celfiles())
theXpm <- getX(x, "pm")
theYpm <- getY(x, "pm")

## End(Not run)
```

hist	<i>Density estimate</i>
------	-------------------------

Description

Plot the density estimates for each sample

Usage

```
## S4 method for signature 'FeatureSet'
hist(x, transfo=log2, which=c("pm", "mm", "bg", "both", "all"),
      nsample=10000, target = "mps1", ...)

## S4 method for signature 'ExpressionSet'
hist(x, transfo=identity, nsample=10000, ...)
```

Arguments

x	FeatureSet or ExpressionSet object
transfo	a function to transform the data before plotting. See 'Details'.
nsample	number of units to sample and build the plot.
which	set of probes to be plotted ("pm", "mm", "bg", "both", "all").
...	arguments to be passed to <code>matplot</code>

Details

The 'transfo' argument will set the transformation to be used. For raw data, 'transfo=log2' is a common practice. For summarized data (which are often in log2-scale), no transformation is needed (therefore 'transfo=identity').

Note

The hist methods for FeatureSet and Expression use a sample (via `sample`) of the probes/probesets to produce the plot (unless `nsample > nrow(x)`). Therefore, the user interested in reproducibility is advised to use `set.seed`.

 image

Display a pseudo-image of a microarray chip

Description

Produces a pseudo-image (`graphics::image`) for each sample.

Usage

```
## S4 method for signature 'FeatureSet'
image(x, which, transfo=log2, ...)

## S4 method for signature 'PLMset'
image(x, which=0,
      type=c("weights", "resids", "pos.resids", "neg.resids", "sign.resids"),
      use.log=TRUE, add.legend=FALSE, standardize=FALSE,
      col=NULL, main, ...)
```

Arguments

x	FeatureSet object
which	integer indices of samples to be plotted (optional).
transfo	function to be applied to the data prior to plotting.
type	Type of statistics to be used.
use.log	Use log.
add.legend	Add legend.
standardize	Standardize residuals.
col	Colors to be used.
main	Main title.
...	parameters to be passed to <code>image</code>

Examples

```
if(require(oligoData) & require(pd.hg18.60mer.expr)){
  data(nimbleExpressionFS)
  par(mfrow=c(1, 2))
  image(nimbleExpressionFS, which=4)
  ## fit <- fitPLM(nimbleExpressionFS)
  ## image(fit, which=4)
  plot(1) ## while fixing fitPLM TODO
}
```

justSNPRMA

Summarization of SNP data

Description

This function implements the SNPRMA method for summarization of SNP data. It works directly with the CEL files, saving memory.

Usage

```
justSNPRMA(filenamees, verbose = TRUE, phenoData = NULL, normalizeToHapmap = TRUE)
```

Arguments

filenamees	character vector with the filenames.
verbose	logical flag for verbosity.
phenoData	a phenoData object or NULL
normalizeToHapmap	Normalize to Hapmap? Should always be TRUE, but it's kept here for future use.

Value

SnqQSet or a SnpCnvQSet, depending on the array type.

Examples

```
## snprmaResults <- justSNPRMA(list.celfiles())
```

<code>list.xysfiles</code>	<i>List XYS files</i>
----------------------------	-----------------------

Description

Lists the XYS files.

Usage

```
list.xysfiles(...)
```

Arguments

... parameters to be passed to [list.files](#)

Details

The functions interface [list.files](#) and the user is asked to check that function for further details.

Value

Character vector with the filenames.

See Also

[list.files](#)

Examples

```
list.xysfiles()
```

MAplot	<i>MA plots</i>
--------	-----------------

Description

Create MA plots using a reference array (if one channel) or using channel2 as reference (if two channel).

Usage

```
MAplot(object, ...)
```

```
## S4 method for signature 'FeatureSet'
MAplot(object, what=pm, transfo=log2, groups,
        refSamples, which, pch=".", summaryFun=rowMedians,
        plotFun=smoothScatter, main="vs pseudo-median reference chip",
        pairs=FALSE, ...)
```

```
## S4 method for signature 'TilingFeatureSet'
```

```

MAplot(object, what=pm, transfo=log2, groups,
        refSamples, which, pch=".", summaryFun=rowMedians,
        plotFun=smoothScatter, main="vs pseudo-median reference chip",
        pairs=FALSE, ...)

## S4 method for signature 'PLMset'
MAplot(object, what=coefs, transfo=identity, groups,
        refSamples, which, pch=".", summaryFun=rowMedians,
        plotFun=smoothScatter, main="vs pseudo-median reference chip",
        pairs=FALSE, ...)

## S4 method for signature 'matrix'
MAplot(object, what=identity, transfo=identity,
        groups, refSamples, which, pch=".", summaryFun=rowMedians,
        plotFun=smoothScatter, main="vs pseudo-median reference chip",
        pairs=FALSE, ...)

## S4 method for signature 'ExpressionSet'
MAplot(object, what=exprs, transfo=identity,
        groups, refSamples, which, pch=".", summaryFun=rowMedians,
        plotFun=smoothScatter, main="vs pseudo-median reference chip",
        pairs=FALSE, ...)

```

Arguments

object	FeatureSet, PLMset or ExpressionSet object.
what	function to be applied on object that will extract the statistics of interest, from which log-ratios and average log-intensities will be computed.
transfo	function to transform the data prior to plotting.
groups	factor describing groups of samples that will be combined prior to plotting. If missing, MvA plots are done per sample.
refSamples	integers (indexing samples) to define which subjects will be used to compute the reference set. If missing, a pseudo-reference chip is estimated using summaryFun.
which	integer (indexing samples) describing which samples are to be plotted.
pch	same as pch in plot
summaryFun	function that operates on a matrix and returns a vector that will be used to summarize data belonging to the same group (or reference) on the computation of grouped-stats.
plotFun	function to be used for plotting. Usually smoothScatter, plot or points.
main	string to be used in title.
pairs	logical flag to determine if a matrix of MvA plots is to be generated
...	Other arguments to be passed downstream, like plot arguments.

Details

MAplot will take the following extra arguments:

1. subset: indices of elements to be plotted to reduce impact of plotting 100's thousands points (if pairs=FALSE only);

2. span: see [loess](#);
3. family.loess: see [loess](#);
4. addLoess: logical flag (default TRUE) to add a loess estimate;
5. parParams: list of params to be passed to par() (if pairs=TRUE only);

Value

Plot

Author(s)

Benilton Carvalho - based on Ben Bolstad's original MAplot function.

See Also

[plot](#), [smoothScatter](#)

Examples

```
if(require(oligoData) & require(pd.hg18.60mer.expr)){
  data(nimbleExpressionFS)
  nimbleExpressionFS
  groups <- factor(rep(c('brain', 'UnivRef'), each=3))
  data.frame(sampleNames(nimbleExpressionFS), groups)
  MAplot(nimbleExpressionFS, pairs=TRUE, ylim=c(-.5, .5), groups=groups)
}
```

mm

Accessors and replacement methods for the intensity/PM/MM/BG matrices.

Description

Accessors and replacement methods for the PM/MM/BG matrices.

Usage

```
intensity(object)
mm(object, subset = NULL, target='core')
pm(object, subset = NULL, target='core')
bg(object, subset = NULL)
mm(object, subset = NULL, target='core')<-value
pm(object, subset = NULL, target='core')<-value
bg(object)<-value
```

Arguments

object	FeatureSet object.
subset	Not implemented yet.
value	matrix object.
target	One of 'probeset', 'core', 'full', 'extended'. This is ignored if the array design is something other than Gene ST or Exon ST.

Details

For all objects but `TilingFeatureSet`, these methods will return matrices. In case of `TilingFeatureSet` objects, the value is a 3-dimensional array (probes x samples x channels).

`intensity` will return the whole intensity matrix associated to the object. `pm`, `mm`, `bg` will return the respective PM/MM/BG matrix.

When applied to `ExonFeatureSet` or `GeneFeatureSet` objects, `pm` will return the PM matrix at the transcript level ('core' probes) by default. The user should set the `target` argument accordingly if something else is desired. The valid values are: 'probeset' (Exon and Gene arrays), 'core' (Exon and Gene arrays), 'full' (Exon arrays) and 'extended' (Exon arrays).

The `target` argument has no effects when used on designs other than Gene and Exon ST.

Examples

```
if (require(maqcExpression4plex) & require(pd.hg18.60mer.expr)){
  xysPath <- system.file("extdata", package="maqcExpression4plex")
  xysFiles <- list.xysfiles(xysPath, full.name=TRUE)
  ngsExpressionFeatureSet <- read.xysfiles(xysFiles)
  pm(ngsExpressionFeatureSet)[1:10,]
}
```

mmindex

Accessors for PM, MM or background probes indices.

Description

Extracts the indexes for PM, MM or background probes.

Usage

```
mmindex(object, ...)
pmindex(object, ...)
bgindex(object, ...)
```

Arguments

<code>object</code>	FeatureSet or DBPDIInfo object
<code>...</code>	Extra arguments, not yet implemented

Details

The indices are ordered by 'fid', i.e. they follow the order that the probes appear in the CEL/XYS files.

Value

A vector of integers representing the rows of the intensity matrix that correspond to PM, MM or background probes.

Examples

```
## How pm() works
## Not run:
x <- read.celfiles(list.celfiles())
pms0 <- pm(x)
pmi <- pmindex(x)
pms1 <- exprs(x)[pmi,]
identical(pms0, pms1)

## End(Not run)
```

mmSequence

Probe Sequences

Description

Accessor to the (PM/MM/background) probe sequences.

Usage

```
mmSequence(object)
pmSequence(object, ...)
bgSequence(object, ...)
```

Arguments

```
object      FeatureSet, AffySNPPDInfo or DBPDInfo object
...         additional arguments
```

Value

A DNASTringSet containing the PM/MM/background probe sequence associated to the array.

oligo-defunct

Defunct Functions in Package 'oligo'

Description

The functions or variables listed here are no longer part of 'oligo'

Usage

```
fitPLM(...)
coefs(...)
resids(...)
```

Arguments

```
...         Arguments.
```

Details

fitPLM was replaced by fitProbeLevelModel, allowing faster execution and providing more specific models. fitPLM was based in the code written by Ben Bolstad in the affyPLM package. However, all the model-fitting functions are now in the package preprocessCore, on which fitProbeLevelModel depends.

coefs and resids, like fitPLM, were inherited from the affyPLM package. They were replaced respectively by coef and residuals, because this is how these statistics are called everywhere else in R.

oligoPLM-class	Class "oligoPLM"
----------------	------------------

Description

A class to represent Probe Level Models.

Objects from the Class

Objects can be created by calls of the form fitProbeLevelModel(FeatureSetObject), where FeatureSetObject is an object obtained through read.celfiles or read.xysfiles, representing intensities observed for different probes (which are grouped in probesets or meta-probesets) across distinct samples.

Slots

chip.coefs: "matrix" with chip/sample effects - probeset-level
 description: "MIAME" compliant description information.
 phenoData: "AnnotatedDataFrame" with phenotypic data.
 protocolData: "AnnotatedDataFrame" with protocol data.
 probe.coefs: "numeric" vector with probe effects
 weights: "matrix" with weights - probe-level
 residuals: "matrix" with residuals - probe-level
 se.chip.coefs: "matrix" with standard errors for chip/sample coefficients
 se.probe.coefs: "numeric" vector with standard errors for probe effects
 residualSE: scale - residual standard error
 geometry: array geometry used for plots
 method: "character" string describing method used for PLM
 manufacturer: "character" string with manufacturer name
 annotation: "character" string with the name of the annotation package
 narrays: "integer" describing the number of arrays
 nprobes: "integer" describing the number of probes before summarization
 nprobesets: "integer" describing the number of probesets after summarization

Methods

annotation signature(object = "oligoPLM"): accessor/replacement method to annotation slot

boxplot signature(x = "oligoPLM"): boxplot method

coef signature(object = "oligoPLM"): accessor/replacement method to coef slot

coefs.probe signature(object = "oligoPLM"): accessor/replacement method to coefs.probe slot

geometry signature(object = "oligoPLM"): accessor/replacement method to geometry slot

image signature(x = "oligoPLM"): image method

manufacturer signature(object = "oligoPLM"): accessor/replacement method to manufacturer slot

method signature(object = "oligoPLM"): accessor/replacement method to method slot

ncol signature(x = "oligoPLM"): accessor/replacement method to ncol slot

nprobes signature(object = "oligoPLM"): accessor/replacement method to nprobes slot

nprobesets signature(object = "oligoPLM"): accessor/replacement method to nprobesets slot

residuals signature(object = "oligoPLM"): accessor/replacement method to residuals slot

residualSE signature(object = "oligoPLM"): accessor/replacement method to residualSE slot

se signature(object = "oligoPLM"): accessor/replacement method to se slot

se.probe signature(object = "oligoPLM"): accessor/replacement method to se.probe slot

show signature(object = "oligoPLM"): show method

weights signature(object = "oligoPLM"): accessor/replacement method to weights slot

NUSE signature(x = "oligoPLM") : Boxplot of Normalized Unscaled Standard Errors (NUSE) or NUSE values.

RLE signature(x = "oligoPLM") : Relative Log Expression boxplot or values.

opset2eset signature(x = "oligoPLM") : Convert to ExpressionSet.

Author(s)

This is a port from Ben Bolstad's work implemented in the affyPLM package. Problems with the implementation in oligo should be reported to the package's maintainer.

References

Bolstad, BM (2004) *Low Level Analysis of High-density Oligonucleotide Array Data: Background, Normalization and Summarization*. PhD Dissertation. University of California, Berkeley.

See Also

[rma](#), [summarize](#)

Examples

```
## TODO: review code and fix broken
## Not run:
if (require(oligoData)){
  data(nimbleExpressionFS)
  fit <- fitProbeLevelModel(nimbleExpressionFS)
  image(fit)
  NUSE(fit)
  RLE(fit)
}
```



```

}
## End(Not run)

```

paCalls *Methods for P/A Calls*

Description

Methods for Present/Absent Calls are meant to provide means of assessing whether or not each of the (PM) intensities are compatible with observations generated by background probes.

Usage

```

paCalls(object, method, ..., verbose=TRUE)
## S4 method for signature 'ExonFeatureSet'
paCalls(object, method, verbose = TRUE)
## S4 method for signature 'GeneFeatureSet'
paCalls(object, method, verbose = TRUE)
## S4 method for signature 'ExpressionFeatureSet'
paCalls(object, method, ..., verbose = TRUE)

```

Arguments

object	Exon/Gene/Expression-FeatureSet object.
method	String defining what method to use. See 'Details'.
...	Additional arguments passed to MAS5. See 'Details'
verbose	Logical flag for verbosity.

Details

For Whole Transcript arrays (Exon/Gene) the valid options for method are 'DABG' (p-values for each probe) and 'PSDABG' (p-values for each probeset). For Expression arrays, the only option currently available for method is 'MAS5'.

ABOUT MAS5 CALLS:

The additional arguments that can be passed to MAS5 are:

1. alpha1: a significance threshold in (0, alpha2);
2. alpha2: a significance threshold in (alpha1, 0.5);
3. tau: a small positive constant;
4. ignore.saturated: if TRUE, do the saturation correction described in the paper, with a saturation level of 46000;

This function performs the hypothesis test:

H0: median(Ri) = tau, corresponding to absence of transcript
H1: median(Ri) > tau, corresponding to presence of transcript

where $R_i = (PM_i - MM_i) / (PM_i + MM_i)$ for each i a probe-pair in the probe-set represented by data.

The p-value that is returned estimates the usual quantity:

Pr(observing a more "present looking" probe-set than data | data is absent)

So that small p-values imply presence while large ones imply absence of transcript. The detection call is computed by thresholding the p-value as in:

call "P" if p-value < alpha1 call "M" if alpha1 <= p-value < alpha2 call "A" if alpha2 <= p-value

Value

A matrix (of dimension dim(PM) if method="DABG" or "MAS5"; of dimension length(unique(probeNames(object))) x ncol(object) if method="PSDABG") with p-values for P/A Calls.

Author(s)

Benilton Carvalho

References

Clark et al. Discovery of tissue-specific exons using comprehensive human exon microarrays. *Genome Biol* (2007) vol. 8 (4) pp. R64

Liu, W. M. and Mei, R. and Di, X. and Ryder, T. B. and Hubbell, E. and Dee, S. and Webster, T. A. and Harrington, C. A. and Ho, M. H. and Baid, J. and Smeekens, S. P. (2002) Analysis of high density expression microarrays with signed-rank call algorithms, *Bioinformatics*, 18(12), pp. 1593–1599.

Liu, W. and Mei, R. and Bartell, D. M. and Di, X. and Webster, T. A. and Ryder, T. (2001) Rank-based algorithms for analysis of microarrays, *Proceedings of SPIE, Microarrays: Optical Technologies and Informatics*, 4266.

Affymetrix (2002) Statistical Algorithms Description Document, Affymetrix Inc., Santa Clara, CA, whitepaper. http://www.affymetrix.com/support/technical/whitepapers/sadd_whitepaper.pdf

Examples

```
## Not run:
if (require(oligoData) & require(pd.huex.1.0.st.v2)){
  data(affyExonFS)
  ## Get only 2 samples for example
  dabgP = paCalls(affyExonFS[, 1:2])
  dabgPS = paCalls(affyExonFS[, 1:2], "PSDABG")
  head(dabgP) ## for probe
  head(dabgPS) ## for probeset
}

## End(Not run)
```

Description

The plotM methods are meant to plot log-ratios for different classes of data.

Methods

object = "SnpQSet", i = "character" Plot log-ratio for SNP data for sample i.
object = "SnpQSet", i = "integer" Plot log-ratio for SNP data for sample i.
object = "SnpQSet", i = "numeric" Plot log-ratio for SNP data for sample i.
object = "TilingQSet", i = "missing" Plot log-ratio for Tiling data for sample i.

pmAllele *Access the allele information for PM probes.*

Description

Accessor to the allelic information for PM probes.

Usage

```
pmAllele(object)
```

Arguments

object SnpFeatureSet or PDInfo object.

pmFragmentLength *Access the fragment length for PM probes.*

Description

Accessor to the fragment length for PM probes.

Usage

```
pmFragmentLength(object, enzyme, type=c('snp', 'cn'))
```

Arguments

object PDInfo or SnpFeatureSet object.
enzyme Enzyme to be used for query. If missing, all enzymes are used.
type Type of probes to be used: 'snp' for SNP probes; 'cn' for Copy Number probes.

Value

A list of length equal to the number of enzymes used for digestion. Each element of the list is a data.frame containing:

- row: the row used to link to the PM matrix;
- length: expected fragment length.

Note

There is not a 1:1 relationship between probes and expected fragment length. For one enzyme, a given probe may be associated to multiple fragment lengths. Therefore, the number of rows in the data.frame may not match the number of PM probes and the row column should be used to match the fragment length with the PM matrix.

pmPosition	<i>Accessor to position information</i>
------------	---

Description

pmPosition will return the genomic position for the (PM) probes.

Usage

```
pmPosition(object)  
pmOffset(object)
```

Arguments

object AffySNPPDInfo, TilingFeatureSet or SnpCallSet object

Details

pmPosition will return genomic position for PM probes on a tiling array.

pmOffset will return the offset information for PM probes on SNP arrays.

pmStrand	<i>Accessor to the strand information</i>
----------	---

Description

Returns the strand information for PM probes (0 - sense / 1 - antisense).

Usage

```
pmStrand(object)
```

Arguments

object AffySNPPDInfo or TilingFeatureSet object

probeNames	<i>Accessor to feature names</i>
------------	----------------------------------

Description

Accessors to featureset names.

Usage

```
probeNames(object, subset = NULL, ...)
probesetNames(object, ...)
```

Arguments

object	FeatureSet or DBPDInfo
subset	not implemented yet.
...	Arguments (like 'target') passed to downstream methods.

Value

probeNames returns a string with the probeset names for *each probe* on the array. probesetNames, on the other hand, returns the *unique probeset names*.

read.celfiles	<i>Parser to CEL files</i>
---------------	----------------------------

Description

Reads CEL files.

Usage

```
read.celfiles(..., filenames, pkgname, phenoData, featureData,
experimentData, protocolData, notes, verbose=TRUE, sampleNames,
rm.mask=FALSE, rm.outliers=FALSE, rm.extra=FALSE, checkType=TRUE)
```

```
read.celfiles2(channel1, channel2, pkgname, phenoData, featureData,
experimentData, protocolData, notes, verbose=TRUE, sampleNames,
rm.mask=FALSE, rm.outliers=FALSE, rm.extra=FALSE, checkType=TRUE)
```

Arguments

...	names of files to be read.
filenames	a character vector with the CEL filenames.
channel1	a character vector with the CEL filenames for the first 'channel' on a Tiling application
channel2	a character vector with the CEL filenames for the second 'channel' on a Tiling application

pkgname	alternative data package to be loaded.
phenoData	phenoData
featureData	featureData
experimentData	experimentData
protocolData	protocolData
notes	notes
verbose	logical
sampleNames	character vector with sample names (usually better descriptors than the file-names)
rm.mask	logical. Read masked?
rm.outliers	logical. Remove outliers?
rm.extra	logical. Remove extra?
checkType	logical. Check type of each file? This can be time consuming.

Details

When using 'affyio' to read in CEL files, the user can read compressed CEL files (CEL.gz). Additionally, 'affyio' is much faster than 'affxparser'.

The function guesses which annotation package to use from the header of the CEL file. The user can also provide the name of the annotation package to be used (via the pkgname argument). If the annotation package cannot be loaded, the function returns an error. If the annotation package is not available from BioConductor, one can use the pdInfoBuilder package to build one.

Value

ExpressionFeatureSet
if Expression arrays

ExonFeatureSet if Exon arrays

SnpFeatureSet if SNP arrays

TilingFeatureSet
if Tiling arrays

See Also

[list.celfiles](#), [read.xysfiles](#)

Examples

```
if(require(pd.mapping50k.xba240) & require(hapmap100kxba)){
  celPath <- system.file("celFiles", package="hapmap100kxba")
  celFiles <- list.celfiles(celPath, full.name=TRUE)
  affySnpFeatureSet <- read.celfiles(celFiles)
}
```

read.xysfiles	<i>Parser to XYS files</i>
---------------	----------------------------

Description

NimbleGen provides XYS files which are read by this function.

Usage

```
read.xysfiles(..., filenames, pkgname, phenoData, featureData,
experimentData, protocolData, notes, verbose=TRUE, sampleNames,
checkType=TRUE)
```

```
read.xysfiles2(channel1, channel2, pkgname, phenoData, featureData,
experimentData, protocolData, notes, verbose=TRUE, sampleNames,
checkType=TRUE)
```

Arguments

...	file names
filenames	character vector with filenames.
channel1	a character vector with the XYS filenames for the first 'channel' on a Tiling application
channel2	a character vector with the XYS filenames for the second 'channel' on a Tiling application
pkgname	character vector with alternative PD Info package name
phenoData	phenoData
featureData	featureData
experimentData	experimentData
protocolData	protocolData
notes	notes
verbose	verbose
sampleNames	character vector with sample names (usually better descriptors than the file-names)
checkType	logical. Check type of each file? This can be time consuming.

Details

The function will read the XYS files provided by NimbleGen Systems and return an object of class FeatureSet.

The function guesses which annotation package to use from the header of the XYS file. The user can also provide the name of the annotation package to be used (via the pkgname argument). If the annotation package cannot be loaded, the function returns an error. If the annotation package is not available from BioConductor, one can use the pdInfoBuilder package to build one.

Value

ExpressionFeatureSet
 if Expression arrays
 TilingFeatureSet
 if Tiling arrays

See Also

[list.xysfiles](#), [read.celfiles](#)

Examples

```
if (require(maqcExpression4plex) & require(pd.hg18.60mer.expr)){
  xysPath <- system.file("extdata", package="maqcExpression4plex")
  xysFiles <- list.xysfiles(xysPath, full.name=TRUE)
  ngsExpressionFeatureSet <- read.xysfiles(xysFiles)
}
```

readSummaries

Read summaries generated by crlmm

Description

This function read the different summaries generated by crlmm.

Usage

```
readSummaries(type, tmpdir)
```

Arguments

type type of summary of character class: 'alleleA', 'alleleB', 'alleleA-sense', 'alleleA-antisense', 'alleleB-sense', 'alleleB-antisense', 'calls', 'llr', 'conf'.
 tmpdir directory containing the output saved by crlmm

Details

On the 50K and 250K arrays, given a SNP, there are probes on both strands (sense and antisense). For this reason, the options 'alleleA-sense', 'alleleA-antisense', 'alleleB-sense' and 'alleleB-antisense' should be used ****only**** with such arrays (XBA, HIND, NSP or STY).

On the SNP 5.0 and SNP 6.0 platforms, this distinction does not exist in terms of algorithm (note that the actual strand could be queried from the annotation package). For these arrays, options 'alleleA', 'alleleB' are the ones to be used.

The options calls, llr and conf will return, respectively, the CRLMM calls, log-likelihood ratios (for devel purpose ****only****) and CRLMM confidence calls matrices.

Value

Matrix with values of summaries.

Description

Robust Multichip Average preprocessing methodology. This strategy allows background subtraction, quantile normalization and summarization (via median-polish).

Usage

```
## S4 method for signature 'ExonFeatureSet'
rma(object, background=TRUE, normalize=TRUE, subset=NULL, target="core")
## S4 method for signature 'HTAFeatureSet'
rma(object, background=TRUE, normalize=TRUE, subset=NULL, target="core")
## S4 method for signature 'ExpressionFeatureSet'
rma(object, background=TRUE, normalize=TRUE, subset=NULL)
## S4 method for signature 'GeneFeatureSet'
rma(object, background=TRUE, normalize=TRUE, subset=NULL, target="core")
## S4 method for signature 'SnpCnvFeatureSet'
rma(object, background=TRUE, normalize=TRUE, subset=NULL)
```

Arguments

object	Exon/HTA/Expression/Gene/SnpCnv-FeatureSet object.
background	Logical - perform RMA background correction?
normalize	Logical - perform quantile normalization?
subset	To be implemented.
target	Level of summarization (only for Exon/Gene arrays)

Methods

`signature(object = "ExonFeatureSet")` When applied to an `ExonFeatureSet` object, `rma` can produce summaries at different levels: probeset (as defined in the PGF), core genes (as defined in the `core.mps` file), full genes (as defined in the `full.mps` file) or extended genes (as defined in the `extended.mps` file). To determine the level for summarization, use the `target` argument.

`signature(object = "ExpressionFeatureSet")` When used on an `ExpressionFeatureSet` object, `rma` produces summaries at the probeset level (as defined in the CDF or NDF files, depending on the manufacturer).

`signature(object = "GeneFeatureSet")` When applied to a `GeneFeatureSet` object, `rma` can produce summaries at different levels: probeset (as defined in the PGF) and 'core genes' (as defined in the `core.mps` file). To determine the level for summarization, use the `target` argument.

`signature(object = "HTAFeatureSet")` When applied to a `HTAFeatureSet` object, `rma` can produce summaries at different levels: probeset (as defined in the PGF) and 'core genes' (as defined in the `core.mps` file). To determine the level for summarization, use the `target` argument.

signature(object = "SnpCnvFeatureSet") If used on a SnpCnvFeatureSet object (ie., SNP 5.0 or SNP 6.0 arrays), rma will produce summaries for the CNV probes. Note that this is an experimental feature for internal (and quick) assessment of CNV probes. We recommend the use of the 'crmm' package, which contains a Copy Number tool specifically designed for these data.

References

Rafael. A. Irizarry, Benjamin M. Bolstad, Francois Collin, Leslie M. Cope, Bridget Hobbs and Terence P. Speed (2003), Summaries of Affymetrix GeneChip probe level data Nucleic Acids Research 31(4):e15

Bolstad, B.M., Irizarry R. A., Astrand M., and Speed, T.P. (2003), A Comparison of Normalization Methods for High Density Oligonucleotide Array Data Based on Bias and Variance. Bioinformatics 19(2):185-193

Irizarry, RA, Hobbs, B, Collin, F, Beazer-Barclay, YD, Antonellis, KJ, Scherf, U, Speed, TP (2003) Exploration, Normalization, and Summaries of High Density Oligonucleotide Array Probe Level Data. Biostatistics. Vol. 4, Number 2: 249-264

See Also

[snprma](#)

Examples

```
if (require(maqcExpression4plex) & require(pd.hg18.60mer.expr)){
  xysPath <- system.file("extdata", package="maqcExpression4plex")
  xysFiles <- list.xysfiles(xysPath, full.name=TRUE)
  ngsExpressionFeatureSet <- read.xysfiles(xysFiles)
  summarized <- rma(ngsExpressionFeatureSet)
  show(summarized)
}
```

runDate

Date of scan

Description

Retrieves date information in CEL/XYS files.

Usage

```
runDate(object)
```

Arguments

object 'FeatureSet' object.

sequenceDesignMatrix *Create design matrix for sequences*

Description

Creates design matrix for sequences.

Usage

```
sequenceDesignMatrix(seqs)
```

Arguments

seqs character vector of 25-mers.

Details

This assumes all sequences are 25bp long.

The design matrix is often used when the objective is to adjust intensities by sequence.

Value

Matrix with length(seqs) rows and 75 columns.

Examples

```
genSequence <- function(x)
  paste(sample(c("A", "T", "C", "G"), 25, rep=TRUE), collapse="", sep="")
seqs <- sapply(1:10, genSequence)
X <- sequenceDesignMatrix(seqs)
Y <- rnorm(10, mean=12, sd=2)
Ydemean <- Y-mean(Y)
X[1:10, 1:3]
fit <- lm(Ydemean~X)
coef(fit)
```

snprma

Preprocessing SNP Arrays

Description

This function preprocess SNP arrays.

Usage

```
snprma(object, verbose = TRUE, normalizeToHapmap = TRUE)
```

Arguments

object	SnpFeatureSet object
verbose	Verbosity flag. logical
normalizeToHapmap	internal

Value

A SnpQSet object.

summarize

Tools for microarray preprocessing.

Description

These are tools to preprocess microarray data. They include background correction, normalization and summarization methods.

Usage

```
backgroundCorrectionMethods()
normalizationMethods()
summarizationMethods()
backgroundCorrect(object, method=backgroundCorrectionMethods(), copy=TRUE, extra, subset=NULL, target=NULL, verbose=TRUE, ...)
summarize(object, probes=rownames(object), method="medianpolish", verbose=TRUE, ...)
## S4 method for signature 'FeatureSet'
normalize(object, method=normalizationMethods(), copy=TRUE, subset=NULL, target='core', verbose=TRUE, ...)
## S4 method for signature 'matrix'
normalize(object, method=normalizationMethods(), copy=TRUE, verbose=TRUE, ...)
## S4 method for signature 'ff_matrix'
normalize(object, method=normalizationMethods(), copy=TRUE, verbose=TRUE, ...)
normalizeToTarget(object, targetDist, method="quantile", copy=TRUE, verbose=TRUE)
```

Arguments

object	Object containing probe intensities to be preprocessed.
method	String determining which method to use at that preprocessing step.
targetDist	Vector with the target distribution
probes	Character vector that identifies the name of the probes represented by the rows of object.
copy	Logical flag determining if data must be copied before processing (TRUE), or if data can be overwritten (FALSE).
subset	Not yet implemented.
target	One of the following values: 'core', 'full', 'extended', 'probeset'. Used only with Gene ST and Exon ST designs.
extra	Extra arguments to be passed to other methods.
verbose	Logical flag for verbosity.
...	Arguments to be passed to methods.

Details

Number of rows of object must match the length of probes.

Value

backgroundCorrectionMethods and normalizationMethods will return a character vector with the methods implemented currently.

backgroundCorrect, normalize and normalizeToTarget will return a matrix with same dimensions as the input matrix. If they are applied to a FeatureSet object, the PM matrix will be used as input.

The summarize method will return a matrix with length(unique(probes)) rows and ncol(object) columns.

Examples

```

ns <- 100
nps <- 1000
np <- 10
intensities <- matrix(rnorm(ns*nps*np, 8000, 400), nc=ns)
ids <- rep(as.character(1:nps), each=np)
bgCorrected <- backgroundCorrect(intensities)
normalized <- normalize(bgCorrected)
summarizationMethods()
expression <- summarize(normalized, probes=ids)
intensities[1:20, 1:3]
expression[1:20, 1:3]
target <- rnorm(np*nps)
normalizedToTarget <- normalizeToTarget(intensities, target)

if (require(oligoData) & require(pd.hg18.60mer.expr)){
  ## Example of normalization with real data
  data(nimbleExpressionFS)
  boxplot(nimbleExpressionFS, main='Original')
  for (mtd in normalizationMethods()){
    message('Normalizing with ', mtd)
    res <- normalize(nimbleExpressionFS, method=mtd, verbose=FALSE)
    boxplot(res, main=mtd)
  }
}

```

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