

# Package ‘lumi’

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**Type** Package

**Title** BeadArray Specific Methods for Illumina Methylation and Expression Microarrays

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**Suggests** beadarray, limma, vsn, lumiBarnes, lumiHumanAll.db, lumiHumanIDMapping, gene-filter, RColorBrewer

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**Description** The lumi package provides an integrated solution for the Illumina microarray data analysis. It includes functions of Illumina BeadStudio (GenomeStudio) data input, quality control, BeadArray-specific variance stabilization, normalization and gene annotation at the probe level. It also includes the functions of processing Illumina methylation microarrays, especially Illumina Infinium methylation microarrays.

**License** LGPL (>= 2)

**LazyLoad** yes

**biocViews** Microarray, OneChannel, Preprocessing, DNAMethylation, QualityControl, TwoChannel

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lumi-package

*A package for preprocessing Illumina microarray data***Description**

lumi R package is designed to preprocess the Illumina microarray (BeadArray) data. It includes functions of Illumina data input, quality control, variance stabilization, normalization and gene annotation.

**Details**

Package: lumi  
 Type: Package  
 Version: 1.1.0  
 Date: 2007-03-23  
 License: LGPL version 2 or newer

**Author(s)**

Pan Du, Simon Lin Maintainer: Pan Du <dupan@northwestern.edu>

**References**

1. Du, P., Kibbe, W.A. and Lin, S.M., (2008) 'lumi: a pipeline for processing Illumina microarray', *Bioinformatics* 24(13):1547-1548
2. Lin, S.M., Du, P., Kibbe, W.A., (2008) 'Model-based Variance-stabilizing Transformation for Illumina Microarray Data', *Nucleic Acids Res.* 36, e11
3. Du, P., Kibbe, W.A. and Lin, S.M., (2007) 'nuID: A universal naming schema of oligonucleotides for Illumina, Affymetrix, and other microarrays', *Biology Direct*, 2, 16

---

addAnnotationInfo	<i>Add probe color channel and basic annotation information based on the annotation library of Illumina methylation microarray</i>
-------------------	--

---

**Description**

Add probe color channel and basic annotation information based on the annotation library of Illumina methylation microarray

**Usage**

```
addAnnotationInfo(methyLumiM, lib = FDb.InfiniumMethylation.hg19, annotationColumn=c(COLOR_CHANNEL, C
```

**Arguments**

methyLumiM	a MethyLumiM object includes Illumina Infinium methylation data
lib	Annotation library of Illumina methylation microarray.
annotationColumn	only include 'COLOR_CHANNEL', 'CHROMOSOME' and 'POSITION' information

**Details**

The "lib" parameter supports both FeatureDb based annotation libraries and old array-based annotation libraries. 'FDb.InfiniumMethylation.hg19' is the FeatureDb based annotation library, which includes both 450k and 27k data. "IlluminaHumanMethylation27k.db" (for 27k array) and "IlluminaHumanMethylation450k.db" (450k infinium array) are old types of annotation libraries.

**Value**

return the MethyLumiM object with COLOR\_CHANNEL, CHROMOSOME and chromome POSITION information added to the featureData.

**Author(s)**

Pan DU

**See Also**

[lumiMethyR](#)

**Examples**

```
data(example.lumiMethy)
head(pData(featureData(example.lumiMethy)))
## removing color channel information
# testData = example.lumiMethy
# pData(featureData(testData))$COLOR_CHANNEL = NULL
# testData = addAnnotationInfo(testData, lib="IlluminaHumanMethylation27k.db")
## check whether the color channel information is added
# head(pData(featureData(testData)))
```

---

addControlData2lumi     *Add the control probe data into the controlData slot of LumiBatch object*

---

**Description**

Add the control probe profile data, outputted by BeadStudio, into the controlData slot of LumiBatch object.

**Usage**

```
addControlData2lumi(controlData, x.lumi)
```

**Arguments**

controlData	the control data can be a data.frame or the control probe filename outputted by BeadStudio
x.lumi	a LumiBatch object, to which controlData will be added.

**Details**

The controlData slot in LumiBatch object is a data.frame with first two columns as "controlType" and "ProbeID". The rest columns are the expression amplitudes for individual samples.

**Value**

Return the LumiBatch object with controlData slot filled.

**Author(s)**

Pan Du

**See Also**

[getControlData](#), [plotControlData](#)

**Examples**

```
## Not runnable
# controlFile <- Control_Probe_Profile.txt
# x.lumi <- addControlData2lumi(controlFile, x.lumi)
```

---

addControlData2methyLumiM

*Add methylation control data to a MethyLumiM object*

---

**Description**

Add methylation control data to a MethyLumiM object

**Usage**

```
addControlData2methyLumiM(controlData, methyLumiM, checkConsistency = TRUE, ...)
```

**Arguments**

controlData	a methylation control data file (output by GenomeStudio), or a MethyLumiQC object
methyLumiM	a MethyLumiM object to add control data
checkConsistency	whether to check the sample names consistency between methyLumiM and controlData
...	other parameters for reading controlData

**Details**

This function aims to add the controlData (MethyLumiQC object) to the controlData slot of a methyLumiM object. For control data, methylated data matrix in assayData slot corresponds to green channel, and unmethylated data matrix in assayData slot corresponds to red channel.

**Value**

Return the methyLumiM object with the controlData added

**Author(s)**

Pan DU

**See Also**

[lumiMethyR](#)

---

addNuID2lumi

*Add the nuID information to the LumiBatch object*

---

**Description**

Replace the Illumina Id (Target ID or Probe Id) as nuID (nucleotide universal identifier) for indexing genes in the LumiBatch object

**Usage**

```
addNuID2lumi(x.lumi, annotationFile=NULL, sep = NULL, lib.mapping = NULL, annotationColName = c(sequence
```

**Arguments**

x.lumi	a LumiBatch object
annotationFile	a annotation file, which includes the Illumina ID (target or probe ids) and probe sequence information
sep	the separation used in the annotation file. Automatically detect the separator if it is ",", or "\t".
lib.mapping	a Illumina ID mapping package, e.g, lumiHumanIDMapping
annotationColName	the annotation column name in the annotation file used for the probe sequence and TargetID and ProbeID
verbose	a boolean to decide whether to print out some messages

## Details

Since the default Illumina IDs (TargetID (ILMN\\_Gene ID) and ProbeId (Probe\\_Id)) are not consistent between different arrays and batches, we invented a nuID, which is one-to-one matching with the probe sequence. This function is to replace the Illumina ID with the nuID. If the annotation library (the unzipped manifest file (.bgx)) is provided, the function will automatically check whether the Illumina ID is provided for the microarray data. We recommend output the data using ProbeID when using Illumina BeadStudio software, because the TargetID (ILMN\\_Gene ID) are not unique.

## Value

a LumiBatch object with Illumina ID replaced by nuID.

## Author(s)

Pan Du

## References

Du, P., Kibbe, W.A., Lin, S.M., "nuID: A universal naming schema of oligonucleotides for Illumina, Affymetrix, and other microarrays", submitted.

## See Also

[IlluminaID2nuID](#), [lumiR](#)

## Examples

```
## load example data
# data(example.lumi)

## specify the annotation file for the Illumina chip
# annotationFile <- Human_RefSeq-8.csv
## Replace the Target ID with nuID
# lumi.nuID <- addNuID2lumi(example.lumi, annotationFile)

## An alternative way is to load the Annotation library and match the targetID (or Probe Id) with nuID
# lumi.nuID <- addNuID2lumi(example.lumi, lib.mapping=lumiHumanIDMapping)
```

---

adjColorBias.quantile *Color bias adjustment of Illumina Infinium methylaton microarrays using smooth quantile normalization*

---

## Description

Color bias adjustment of Illumina Infinium methylaton microarrays using smooth quantile normalization [smoothQuantileNormalization](#)



**Usage**

```
adjColorBias.quantile(methyLumiM, refChannel = c("green", "red"), logMode = TRUE, verbose = TRUE, ...)
```

**Arguments**

methyLumiM	a MethyLumiM object or any eSet object with "methylated" and "unmethylated" data matrix element in the assayData slot
refChannel	the reference color channel for color bias adjustment
logMode	whether perform the adjustment in log scale or not
verbose	whether print extra information during processing
...	other parameters used by <a href="#">smoothQuantileNormalization</a>

**Details**

Perform color bias adjustment of Illumina Infinium methylaton microarrays. It requires the input methyLumiM object includes the color channel information in the featureData. Basically, there should be a "COLOR\_CHANNEL" column in the data.frame returned by pData(featureData(methyLumiM)).

The basic idea of color bias adjustment is to treat it as the normalization between two color channels. It uses smooth quantile normalization [smoothQuantileNormalization](#) to normalize two color channels.

**Value**

Return an object (same class as input methyLumiM) with updated "methylated" and "unmethylated" data matrix after color bias adjustment.

**Author(s)**

Pan DU

**See Also**

See Also [lumiMethyC](#), [smoothQuantileNormalization](#) and [adjColorBias.ssn](#)

**Examples**

```
data(example.lumiMethy)
# before adjustment
plotColorBias1D(example.lumiMethy)
lumiMethy.adj = adjColorBias.quantile(example.lumiMethy)
# after adjustment
plotColorBias1D(lumiMethy.adj)
```

---

adjColorBias.ssn	<i>Color bias adjustment of Illumina Infinium methylaton microarrays using simple shift and scaling normalization</i>
------------------	---

---

### Description

Color bias adjustment of Illumina Infinium methylaton microarrays using simple shift and scaling normalization

### Usage

```
adjColorBias.ssn(methyLumiM, refChannel = c("green", "red", "mean"))
```

### Arguments

methyLumiM	a MethyLumiM object or any eSet object with "methylated" and "unmethylated" data matrix element in the assayData slot
refChannel	the reference color channel for color bias adjustment

### Details

Perform color bias adjustment of Illumina Infinium methylaton microarrays. It requires the input methyLumiM object includes the color channel information in the featureData. Basically, there should be a "COLOR\_CHANNEL" column in the data.frame returned by pData(featureData(methyLumiM)).

The basic idea of color bias adjustment is to treat it as the normalization between two color channels. It uses simple scaling normalization to normalize two color channels. The background levels are estimated using function [estimateMethylationBG](#).

### Value

Return an object (same class as input methyLumiM) with updated "methylated" and "unmethylated" data matrix after color bias adjustment.

### Author(s)

Pan DU

### See Also

See Also [lumiMethyC](#), [estimateMethylationBG](#) and [adjColorBias.quantile](#)

### Examples

```
data(example.lumiMethy)
# before adjustment
plotColorBias1D(example.lumiMethy)
lumiMethy.adj = adjColorBias.ssn(example.lumiMethy)
# after adjustment
plotColorBias1D(lumiMethy.adj)
```

---

asBigMatrix-methods     *convert the data matrix in the assayData of a ExpressionSet as BigMatrix*

---

### Description

convert the data matrix in the assayData of a ExpressionSet as BigMatrix

### Usage

```
## S4 method for signature ExpressionSet
asBigMatrix(object, rowInd=NULL, colInd=NULL, nCol=NULL, dimNames=NULL, saveDir=., savePrefix=NULL, ...)
```

### Arguments

object	an object of <a href="#">ExpressionSet</a> or its inherited class
rowInd	the subset of row index
colInd	the subset of column index
nCol	the number of columns of the data, which can be larger than the real data dimension. It is designed for adding future data.
dimNames	the dimension names, which is a list of two character vectors (rownames and colnames)
saveDir	the parent directory to save the BigMatrix data files
savePrefix	the folder name prefix of the directory to save the BigMatrix data files. The folder name will be like this: <code>paste(savePrefix, '_bigmat', sep='')</code>
...	optional arguments to <a href="#">BigMatrix</a>

### Details

This function does not work in Windows because the dependent package `bigmemoryExtras` does not support it. In order to make `lumi` package still compilation under Windows, I deliberately remove the dependency of `bigmemoryExtras` package. As a result, users need to manually load the `bigmemoryExtras` function before using this function.

The BigMatrix data files will be save in the directory `file.path(saveDir, paste(savePrefix, '_bigmat', sep=''))`

### See Also

[BigMatrix](#)

---

beta2m

*Convert methylation Beta-value to M-value*

---

### **Description**

Convert methylation Beta-value to M-value through a logistic transformation

### **Usage**

```
beta2m(beta)
```

### **Arguments**

beta            a matrix or vector of methylation Beta-value

### **Details**

Convert methylation Beta-value to M-value through a logistic transformation

### **Value**

return methylation M-value with the same size of input Beta-value

### **Author(s)**

Pan Du

### **References**

Du, P., Zhang, X., Huang, C.C., Jafari, N., Kibbe, W.A., Hou, L., and Lin, S.M., (2010) 'Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis', (under review)

### **See Also**

See Also as [m2beta](#)

---

`bgAdjust`*Background adjustment for Illumina data*

---

### Description

The method adjusts the data by subtracting an offset, which is estimated based on the quantile of the control probes

### Usage

```
bgAdjust(lumiBatch, probs = 0.5, ...)
```

### Arguments

<code>lumiBatch</code>	A LumiBatch object with controlData slot include control probe information
<code>probs</code>	The quantile used to estimate the background
<code>...</code>	other parameters used by <a href="#">quantile</a> method

### Details

The method adjusts the data by subtracting an offset, which is estimated based on the quantile of the control probes. The control probe information is kept in the controlData slot of the LumiBatch object. If no control data information, the method will do nothing.

### Value

It returns a LumiBatch object with background adjusted.

### Author(s)

Pan Du

### See Also

[lumiB](#)

### Examples

```
data(example.lumi)
## Here will assume the minimum of the control probe as the background,
## because there is no negative control (blank beads) information for the Barnes data.
example.lumi.b <- bgAdjust(example.lumi, probs=0)
```

---

bgAdjustMethylation     *Estimate and adjust the background levels of Illumina Infinium methylation microarrays*

---

### Description

Estimate and adjust the background levels of Illumina Infinium methylation microarrays

### Usage

```
bgAdjustMethylation(methyLumiM, separateColor = FALSE, targetBGLevel = 300, negPercTh = 0.25)
```

### Arguments

methyLumiM	a MethyLumiM object or any eSet object with "methylated" and "unmethylated" data matrix element in the assayData slot
separateColor	determine whether separately process two color channels
targetBGLevel	adjust background level to a non-zero target background level
negPercTh	the threshold of the percentage of negative values after subtract estimated background levels. A warning will be given if too many probes having intensities lower than background levels.

### Details

The estimation of background level of Infinium methylation microarray is based on the assumption that the lots of CpG sites are unmethylated, which results in a density mode of the intensities measured by methylated probes. The position of this mode represents the background level.

### Value

Return an object (same class as input methyLumiM) with updated "methylated" and "unmethylated" data matrix after background level adjustment. The estimated background level was kept in the attribute, "EstimatedBG", of the returned methyLumiM object.

### Author(s)

Pan DU

### See Also

See Also [lumiMethyB](#) and [estimateMethylationBG](#)

### Examples

```
data(example.lumiMethy)
lumiMethy.bgAdj = bgAdjustMethylation(example.lumiMethy)
attr(lumiMethy.bgAdj, "EstimatedBG")
```

---

boxplot, MethyLumiM-method  
*boxplot of a MethyLumiM object*

---

### Description

Creating a `hdr.boxplot` of the M-value in a MethyLumiM object

### Usage

```
## S4 method for signature MethyLumiM
boxplot(x, main, logMode = TRUE, ...)
```

### Arguments

<code>x</code>	a <code>MethyLumiM-class</code> object
<code>main</code>	title of the boxplot
<code>logMode</code>	only works when the <code>dataType</code> of <code>x</code> is "Intensity"
<code>...</code>	optional arguments to <code>bwplot</code> .

### Details

Because the density plot of M-values usually includes two modes, using the traditional boxplot cannot accurately represent the distribution of the data. Here we use violin plot to show the density of M-values by samples

### See Also

[MethyLumiM-class](#), [panel.violin](#) and [boxplot, ExpressionSet-method](#)

### Examples

```
## load example data
data(example.lumiMethy)
boxplot(example.lumiMethy)
```

---

boxplot-methods      *boxplot of a ExpressionSet object*

---

## Description

Creating [boxplot](#) of sample intensities in a ExpressionSet object

## Usage

```
## S4 method for signature ExpressionSet
boxplot(x, range = 0, main, logMode = TRUE, subset = NULL, xlab = "", ylab = "Amplitude", ...)
```

## Arguments

x	a <a href="#">ExpressionSet</a> object
range	parameter of <a href="#">boxplot</a>
main	title of the boxplot
logMode	whether plot the data in log2 scale or not
subset	subset of rows used to plot. It can be an index vector, or the length of a random subset
xlab	xlab of the plot
ylab	ylab of the plot
...	optional arguments to <a href="#">boxplot</a> .

## Details

The boxplot function has a "subset" parameter. By default, it is set as 5000, i.e., randomly selected 5000 probes to plot the boxplot. The purpose of this is to plot the picture faster, but it will also make the boxplot has slightly different each time. If the user wants to make sure the boxplot is the same each time, you can set the "subset" parameter as NULL.

## See Also

[LumiBatch-class](#), [boxplot](#) and [boxplot](#), [MethyLumiM-method](#)

## Examples

```
## load example data
data(example.lumi)

boxplot(example.lumi)
```



---

boxplotColorBias      *Plot the Illumina Infinium methylation color bias in terms of boxplot*

---

### Description

Plot the Illumina Infinium methylation color bias in terms of boxplot. boxplot of red and green color channel will be plotted side by side

### Usage

```
boxplotColorBias(methyLumiM, logMode = TRUE, channel = c("both", "unmethy", "methy", "sum"), grid = TRUE)
```

### Arguments

methyLumiM	MethyLumiM-class object or eSet-class object, which include methylated and unmethylated probe intensities
logMode	whether plot the intensities in log-scale
channel	estimate the intensity in different methods
grid	whether to add grid on the plot
main	title of the plot
mar	margin of the plot
verbose	whether print verbose information during plot
subset	plot subset of randomly selected rows. All data will be plotted if it is NULL.
...	other parameters of <a href="#">boxplot</a>

### Details

Plot the Illumina Infinium methylation color bias in terms of boxplot. boxplot of red and green color channel will be plotted side by side

### Value

Invisibly return TRUE if plot successfully.

### Author(s)

Pan DU

### See Also

See Also as [boxplot](#) and [plotColorBias1D](#)

### Examples

```
data(example.lumiMethy)
boxplotColorBias(example.lumiMethy)
```

---

colorBiasSummary	<i>A summary of colorBias information</i>
------------------	---

---

### Description

A summary of colorBias information, which is a data.frame summarizing the intensities of individual samples

### Usage

```
colorBiasSummary(methyLumiM, logMode = TRUE, channel = c("both", "unmethy", "methy", "sum"))
```

### Arguments

methyLumiM	MethyLumiM-class object or eSet-class object, which include methylated and unmethylated probe intensities
logMode	Whether plot the intensities in log-scale
channel	estimate the intensity in different methods

### Details

A summary of colorBias information. There are four options using "channel" parameter to plot the density plot. "both": estimate the density by pooling together methylated and unmethylated probe intensities. "unmethy" and "methy": plot either unmethylated or methylated probe density. "sum" plot the density of the sum of methylated and unmethylated probe intensities.

### Value

A data.frame summarizing the intensities of individual samples

### Author(s)

Pan DU

---

density-methods	<i>Density plot of a ExpressionSet object</i>
-----------------	---

---

### Description

Creating density plot of sample intensities in a ExpressionSet object. It is equivalent to [hist-methods](#).

**Usage**

```
## S4 method for signature ExpressionSet
density(x, logMode=TRUE, xlab = NULL, ylab = "density", type = "l",
col=1:dim(x)[2], lty=1:dim(x)[2], lwd=1, xlim = NULL, index.highlight = NULL, color.highlight = 2,
symmetry = NULL, addLegend = TRUE, legendPos="topright", subset = NULL, main="", ...)
```

**Arguments**

x	a <a href="#">ExpressionSet</a> object
logMode	determine whether the density plot is based on a log2 scale
xlab	xlab of the density plot
ylab	ylab of the density plot
type	parameter of plot function
col	line colors of the density plot
lty	line types of the density plot
lwd	line width of plot function
xlim	parameter of the plot function
index.highlight	the column index of the highlighted density curve
color.highlight	color of highlighted density curve
symmetry	the boundary position suppose to be symmetric distributed
addLegend	whether add legend to the plot or not
legendPos	the legend position. It can be a string specifying the position, or a length two vector specifying the x and y position. Please check <a href="#">legend</a> for more details.
subset	subset of rows used to plot. It can be an index vector, or the length of a random subset
main	title for the plot
...	additional parameters for <a href="#">density</a> function

**See Also**

[LumiBatch-class](#), [hist-methods](#), [density](#)

**Examples**

```
## load example data
data(example.lumi)

density(example.lumi)
```

---

detectionCall	<i>Estimate the detectable probe ratio</i>
---------------	--

---

**Description**

Estimate the detectable probe ratio of each probe, sample or just return an AP matrix

**Usage**

```
detectionCall(x.lumi, Th = 0.01, type = c(probe, sample, matrix))
```

**Arguments**

x.lumi	a LumiBatch or MethyLumiM object
Th	the threshold. By default, when the detection p-value is less than 0.01, we suppose it is detectable. For the old version of BeadStudio output (version 2 or earlier), the threshold will automatically transferred as 1 - Th, because in the old format, value close to 1 is suppose to be detectable.
type	determine to calculate the detection count by probe or by sample

**Value**

If the type is 'probe', then returns the presentCount of each probe. If the type is 'sample', then return the detectable probe ratio of each sample. If the type is 'matrix', then return the AP matrix, in which 'A' represents absent (the detect p-value less than threshold) and 'P' represents present.

**Author(s)**

Pan Du

**See Also**

[lumiQ](#)

**Examples**

```
## load example data
data(example.lumi)
## load example data
data(example.lumi)

## estimate the detect call (percentage of expressed genes) of each sample
temp <- detectionCall(example.lumi, type=sample)
print(temp)

## estimate the present count of each gene (probe)
temp <- detectionCall(example.lumi, type=probe)
hist(temp)
```

---

detectOutlier	<i>Detect the outlier sample (or gene)</i>
---------------	--

---

### Description

Detect the outlier sample (or gene) based on distance to the cluster center

### Usage

```
detectOutlier(x, metric = "euclidean", standardize = TRUE, Th = 2, ifPlot = FALSE)
```

### Arguments

x	a LumiBatch object, ExpressionSet object or a matrix with each column corresponding to a sample or other profile
metric	the distance metric
standardize	standardize the profile or not
Th	the threshold of outlier,
ifPlot	to plot the result (as a hierarchical tree) or not

### Details

The current outlier detection is based on the distance from the sample to the center (average of all samples after removing 10 percent samples farthest away from the center). The assumption of the outlier detection is that there is only one single cluster and the distance from the sample to the center is Gaussian distributed.

The outlier is detected when its distance to the center is larger than a certain threshold. The threshold is calculated as  $Th * \text{median distances to the center}$ .

The profile relations can be visualized as a hierarchical tree.

### Value

Plot the results or return the outlier (a logic vector) with the distance matrix and threshold as attributes.

### Author(s)

Pan Du

### See Also

[lumiQ](#)

## Examples

```
## load example data
data(example.lumi)

## detect the outlier (Further improvement needed.)
temp <- detectOutlier(example.lumi, ifPlot=TRUE)
```

---

estimateBeta

*Estimate methylation Beta-value matrix*

---

## Description

Estimate methylation Beta-value matrix from MethyLumiM-class object or eSet-class object, which include methylated and unmethylated probe intensities

## Usage

```
estimateBeta(methyLumiM, returnType=c("ExpressionSet", "matrix"), offset = 100)
```

## Arguments

methyLumiM	MethyLumiM-class object or eSet-class object, which include methylated and unmethylated probe intensities
returnType	determine whether return an ExpressionSet or matrix object
offset	An offset value added to the denominator to avoid close to zero intensities

## Details

Beta-value is ratio between Illumina methylated probe intensity and total probe intensities (sum of methylated and unmethylated probe intensities, see [estimateIntensity](#)). An offset value added to the denominator to avoid close to zero intensities in the denominator. Beta-value is in the range of 0 and 1. If we assume the probe intensity follows Gamma distribution, then the Beta-value follows a Beta distribution.

## Value

An ExpressionSet or matrix object of methylation Beta-value

## Author(s)

Pan DU

## See Also

See Also as [estimateIntensity](#) and [estimateM](#)

**Examples**

```
data(example.lumiMethy)
methyLumiBeta = estimateBeta(example.lumiMethy)
density(methyLumiBeta)
```

---

estimateIntensity	<i>Estimate intensity of Illumina methylation data</i>
-------------------	--

---

**Description**

Estimate intensity of Illumina methylation data, which is the sum of Illumina methylated and unmethylated probe intensities

**Usage**

```
estimateIntensity(methyLumiM, returnType=c("ExpressionSet", "matrix"))
```

**Arguments**

methyLumiM	MethyLumiM-class object or eSet-class object, which include methylated and unmethylated probe intensities
returnType	determine whether return an ExpressionSet or matrix object

**Details**

The Intensity basically is the sum of Illumina methylated and unmethylated probe intensities.

**Value**

An ExpressionSet or matrix object of methylation Intensity-value

**Author(s)**

Pan DU

**See Also**

See Also as [estimateBeta](#) and [estimateM](#)

**Examples**

```
data(example.lumiMethy)
methyLumiIntensity = estimateIntensity(example.lumiMethy)
boxplot(methyLumiIntensity)
```

---

`estimateLumiCV`*Estimate the coefficient of variance matrix of LumiBatch object*

---

**Description**

Estimate the coefficient of variance matrix of LumiBatch object for each measurement or probe.

**Usage**

```
estimateLumiCV(x.lumi, type = c("measurement", "probe"), ifPlot = FALSE, ...)
```

**Arguments**

<code>x.lumi</code>	a LumiBatch object
<code>type</code>	estimate the coefficient of variance of each measurement or each probe
<code>ifPlot</code>	determine whether to plot the density plot or not
<code>...</code>	optional arguments to <a href="#">plot</a> .

**Details**

By default, the coefficient of variance is the ratio of the mean and variance of the bead expression values. Basically, it is the ratio of `exprs` and `se.exprs` element of LumiBatch object. If the type is "probe", it is the ratio of the mean and variance of probe expression profile.

**Value**

A matrix of coefficient of variance

**Author(s)**

Pan Du

**See Also**

[lumiQ](#)

**Examples**

```
## load example data
data(example.lumi)

## estimate the coefficient of variance and plot the density plot of it
cv <- estimateLumiCV(example.lumi, ifPlot = TRUE)
```



---

`estimateM`*Estimate methylation M-value matrix*

---

**Description**

Estimate methylation M-value matrix from MethyLumiM-class object or eSet-class object, which include methylated and unmethylated probe intensities

**Usage**

```
estimateM(methyLumiM, returnType=c("ExpressionSet", "matrix"), offset=100)
```

**Arguments**

<code>methyLumiM</code>	MethyLumiM-class object or eSet-class object, which include methylated and unmethylated probe intensities
<code>returnType</code>	determine whether return an ExpressionSet (MethyLumiM in this case) or matrix object
<code>offset</code>	offset added to the methylated and unmethylated probe intensities when estimating the M-value

**Details**

M-value is the log<sub>2</sub> ratio between Illumina methylated and unmethylated probe intensities. As variations of small intensities can cause big changes in the ratio estimation, so an offset is added to methylated and unmethylated probe intensities when estimating the M-value.

**Value**

A MethyLumiM or matrix object of methylation M-value

**Author(s)**

Pan DU

**References**

Du, P., Zhang, X., Huang, C.C., Jafari, N., Kibbe, W.A., Hou, L., and Lin, S.M., (2010) 'Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis', (under review)

**See Also**

See Also as [estimateBeta](#), [estimateIntensity](#)

## Examples

```
data(example.lumiMethy)
methyLumiM = estimateM(example.lumiMethy)
boxplot(methyLumiM)
```

---

estimateMethylationBG *Estimate the background levels of Illumina Infinium methylaton microarrays*

---

## Description

Estimate the background levels of Illumina Infinium methylaton microarrays. It is called by function [bgAdjustMethylation](#)

## Usage

```
estimateMethylationBG(methyLumiM, separateColor = FALSE, nbin = 1000)
```

## Arguments

methyLumiM	a MethyLumiM object or any eSet object with "methylated" and "unmethylated" data matrix element in the assayData slot
separateColor	determine whether to separately process two color channels
nbin	the number of bins in the histogram used to estimate the mode position of the density

## Details

When the controlData includes the negative control probe information, the background estimation will be the median of the negative control probes. Red and Green color channels will be estimated separately.

In the case the negative control data is not available, the background will be estimated based on the mode positions of unmethylated or methylated distribution (the smaller one). The assumption is that the lots of CpG sites are unmethylated, which results in a density mode of the intensities measured by methylated probes. The position of this mode represents the background level.

## Value

a vector of estimated background levels for individual samples.

## Author(s)

Pan DU

**See Also**

See Also [lumiMethyB](#) and [bgAdjustMethylation](#)

**Examples**

```
data(example.lumiMethy)
estimatedBG = estimateMethylationBG(example.lumiMethy)
estimatedBG
```

---

example.lumi

*Example Illumina Expression data in LumiBatch class*

---

**Description**

Example data as a LumiBatch object which is a subset of Barnes data (Barnes, 2005)

**Usage**

```
data(example.lumi)
```

**Format**

A 'LumiBatch' object

**Details**

The data is from (Barnes, 2005). It used Sentrix HumanRef-8 Expression BeadChip. Two samples "100US" and "95US:5P" (each has two technique replicates) were selected. In order to save space, 8000 genes were randomly selected. As a result, the example data includes 8000 genes, each has 4 measurements. The full data set was included in the Bioconductor Experiment data package lumiBarnes.

The entire data set has been built as a lumiBarnes data object and can be downloaded from Bioconductor Experiment Data.

**References**

Barnes, M., Freudenberg, J., Thompson, S., Aronow, B. and Pavlidis, P. (2005) Ex-perimental comparison and cross-validation of the Affymetrix and Illumina gene expression analysis platforms, Nucleic Acids Res, 33, 5914-5923.

The detailed data information can be found at: <http://www.bioinformatics.ubc.ca/pavlidis/lab/platformCompare/>

**Examples**

```
## load the data
data(example.lumi)

## summary of the data
example.lumi
```

---

example.lumiMethy      *Example Illumina Infinium Methylation data in MethyLumiM class*

---

### Description

An example Illumina Infinium Methylation27k dataset, which includes a control and treatment dataset with both technique and biological replicates

### Usage

```
data(example.lumiMethy)
```

### Details

The example dataset includes four control and four treatment samples together with their technique replicates. The original samples and technique replicates were measured in two batches. Here are the names of sixteen samples: Treat1, Treat2, Treat3, Treat4, Ctrl1, Ctrl2, Ctrl3, Ctrl4, Treat1.rep, Treat2.rep, Treat3.rep, Treat4.rep, Ctrl1.rep, Ctrl2.rep, Ctrl3.rep, Ctrl4.rep.

To save storage space, we randomly subset 5000 CpG sites among about 27000 measured CpG sites.

### Examples

```
data(example.lumiMethy)
sampleNames(example.lumiMethy)
```

---

example.methyTitration      *Example Illumina Infinium Methylation titration data in MethyLumiM class*

---

### Description

An example Illumina Infinium Methylation27k dataset, which includes a titration dataset

### Usage

```
data(example.methyTitration)
```

### Details

The example dataset is a titration dataset. It includes 8 samples: "A1", "A2", "B1", "B2", "C1", "C2", "D" and "E". They are mixtures of Sample A (a B-lymphocyte sample) and Sample B (is a colon cancer sample from a female donor) at five different titration ratios: 100:0 (A), 90:10 (C), 75:25 (D), 50:50 (E) and 0:100 (B).

To save storage space, we randomly subset 10000 CpG sites among about 27000 measured CpG sites.

**Examples**

```
data(example.methyTitration)
sampleNames(example.methyTitration)
```

---

gammaFitEM	<i>Estimate the methylation status by fitting a Gamma mixture model using EM algorithm</i>
------------	--

---

**Description**

Estimate the methylation status by fitting a two component Gamma mixture model using EM algorithm based on the all M-values of a particular sample

**Usage**

```
gammaFitEM(M, initialFit = NULL, fix.k = NULL, weighted = TRUE, maxIteration = 50, tol = 1e-04, plotMode
```

**Arguments**

M	a vector of M-values covering the whole genome
initialFit	the initial estimation of the gamma parameters returned by .initialGammaEstimation function
fix.k	the k parameter of the gamma function which is fixed during estimation
weighted	determine whether to down-weight the long tails of two component densities beyond their modes
maxIteration	maximum iterations allowed before converging
tol	the difference threshold used to determine convergence
plotMode	determine whether plot the histogram and density plot estimation
truncate	determine whether to truncate the tails beyond the modes during parameter estimation
verbose	determine whether plot intermediate messages during iterations

**Details**

The assumption of this function is that the M-value distribution is composed of the mixture of two shifted gamma distributions, which are defined as:  $d\text{gamma}(x-s[1], \text{shape}=k[1], \text{scale}=\text{theta}[1])$  and  $d\text{gamma}(s[2]-x, \text{shape}=k[2], \text{scale}=\text{theta}[2])$ . Here s represents the shift.

NOTE: the methylation status modeling algorithm was developed based on 27K methylation array. It has not been tested for 450K array. Considering 450K array covers both promoter and gene body, the two component Gamma mixture model assumption may not be valid any more.

**Value**

The return is a list with "gammaFit" class attribute, which includes the following items:

logLikelihood	the log-likelihood of the fitting model
k	parameter k of gamma distribution
theta	parameter theta of gamma distribution
shift	parameter shift of gamma distribution
proportion	the proportion of two components (gamma distributions)
mode	the mode positions of the gamma distributions
probability	the estimated methylation status posterior probability of each CpG site

**Author(s)**

Pan Du

**See Also**

[methylationCall](#) and [plotGammaFit](#)

**Examples**

```
data(example.lumiMethy)
M <- exprs(example.lumiMethy)
fittedGamma <- gammaFitEM(M[,1], initialFit=NULL, maxIteration=50, tol=0.0001, plotMode=TRUE, verbose=FALSE)
```

---

getChipInfo

*Get Illumina Chip Information based on probe identifiers*

---

**Description**

Retrieve the matched Illumina chip information by searching the provided probe identifiers through the Illumina identifiers in all manifest files.

**Usage**

```
getChipInfo(x, lib.mapping = NULL, species = c("Human", "Mouse", "Rat", "Unknown"), chipVersion = NULL,
```

**Arguments**

x	a vector of probe identifiers, ExpressionSet object or a matrix with probe identifiers as row names
lib.mapping	the ID mapping library. If it is provided, the parameter "species" will be ignored.
species	species of the chip designed for. If users do not know it, it can be set as "Unknown".
chipVersion	chipVersion information returned by function <a href="#">getChipInfo</a>
idMapping	determine whether return the idMapping information (between Illumina ID and nuID)
returnAllMatches	determine whether return all matches or just the best match
verbose	determine whether print some warning information

**Details**

The function searches the provided probe Identifiers (Illumina IDs or nuIDs) through all the manifest file ID information kept in the IDMapping libraries (lumiHumanIDMapping, lumiMouseIDMapping, lumiRatIDMapping). The Illumina IDs kept in the library include "Search\_key" ("Search\_Key"), "Target" ("ILMN\_Gene"), "Accession", "Symbol", "ProbeId" ("Probe\_Id"). To determine the best match, the function calculate the number of matched probes. The higher "matchedProbeNumber" is claimed as better. When the "matchedProbeNumber" is the same, the manifest file with fewer probes is claimed as better. If x is NULL and chipVersion is provided, it will return the entire mapping table of the chip.

**Value**

The function returns a list with following items:

chipVersion	the file name of the manifest file for the corresponding version and release
species	the species of the chip designed for
IDType	the type of probe identifier
chipProbeNumber	the number of probes in the manifest file
matchedProbeNumber	the number of input probes matching the manifest file
idMapping	id mapping information between Illumina ID and nuID

When parameter "returnAllMatches" is TRUE, the items of "chipVersion", "IDType", "chipProbeNumber", "inputProbeNumber", "matchedProbeNumber" will be a vector corresponding to the matched manifest files, whose "matchedProbeNumber" is larger than zero, and the "idMapping" will be a matrix with each column corresponding to one matched manifest file. All of the items are sorted from the best match to worst (The higher "matchedProbeNumber" is claimed as better. When the "matchedProbeNumber" is the same, the manifest file with fewer probes is claimed as better.).

**Author(s)**

Pan Du

**See Also**

[nuID2IlluminaID](#), [IlluminaID2nuID](#)

**Examples**

```
## load example data
data(example.lumi)
if (require(lumiHumanIDMapping)) {
  chipInfo <- getChipInfo(example.lumi, species=Human)
  chipInfo
}
```

---

getChrInfo

*get the chromosome location information of methylation probes*

---

**Description**

get the chromosome location information of methylation probes

**Usage**

```
getChrInfo(methyData, lib = NULL, ...)
```

**Arguments**

methyData	a MethyLumiM object
lib	Methylation annotation library
...	optional arguments to <a href="#">addAnnotationInfo</a> .

**Value**

a data.frame

**Author(s)**

Pan Du



---

getControlData	<i>Get control probe information</i>
----------------	--------------------------------------

---

### Description

Get control probe information from Bead Studio output or a LumiBatch object.

### Usage

```
getControlData(x, type = c(data.frame, LumiBatch), ...)
```

### Arguments

x	the control data can be a LumiBatch object or the Control Probe Profile file outputted by BeadStudio
type	determine the return data type
...	other parameters used by <a href="#">lumiR</a> function

### Value

By default, it returns a data.frame with first two columns as "controlType" and "ProbeID". The rest columns are the expression amplitudes for individual samples. When type is 'LumiBatch', it returns a LumiBatch object, which basically is the return of lumiR without combining duplicated TargetIDs. As the return is a LumiBatch object, it includes more information, like probe number, detection p-value and standard error of the measurement.

### Author(s)

Pan Du

### See Also

[addControlData2lumi](#)

### Examples

```
controlFile <- system.file(doc, Control_Probe_Profile.txt, package=lumi)
## return a data.frame
controlData <- getControlData(controlFile)
class(controlData)
names(controlData)

## return a LumiBatch object
controlData <- getControlData(controlFile, type=LumiBatch)
summary(controlData)
```

---

getControlProbe	<i>Get the control probe Ids</i>
-----------------	----------------------------------

---

### Description

Get the control probe Ids corresponding to the control probe type provided. The control probe ids are kept in the second column of controlData data.frame.

### Usage

```
getControlProbe(controlData, type = NULL)
```

### Arguments

controlData	a LumiBatch object including control data or a control data data.frame
type	the type of control probe (case insensitive), which can be get by using <a href="#">getControlType</a> function

### Value

returns the corresponding probe Ids for the control type.

### Author(s)

Pan Du

### See Also

[addControlData2lumi](#)

### Examples

```
controlFile <- system.file(doc, Control_Probe_Profile.txt, package=lumi)
## return a data.frame
controlData <- getControlData(controlFile)
getControlType(controlData)
getControlProbe(controlData, type=housekeeping)
```

---

getControlType	<i>Get the types of the control probes</i>
----------------	--

---

**Description**

Get the types of the control probes, which is in the first column of the controlData data.frame for LumiBatch objects. For methylation data, it is the return of controlTypes function

**Usage**

```
getControlType(controlData)
```

**Arguments**

controlData     a LumiBatch object including control data, a control data data.frame, or a Methy-LumiQC object for methylation data

**Value**

return the unique type of control probe type.

**Author(s)**

Pan Du

**See Also**

[addControlData2lumi](#), [controlTypes](#) for methylation data

**Examples**

```
controlFile <- system.file(doc, Control_Probe_Profile.txt, package=lumi)
## return a data.frame
controlData <- getControlData(controlFile)
getControlType(controlData)
```

---

getNuIDMappingInfo      *get the mapping information from nuID to RefSeq ID*

---

### Description

Get the mapping information (including mapping quality information) of nuIDs to the most recent RefSeq release. These information was kept in the IDMapping libraries.

### Usage

```
getNuIDMappingInfo(nuID = NULL, lib.mapping)
```

### Arguments

nuID                    a vector of nuIDs. If it is NULL, all mappings will be returned.  
lib.mapping            the ID mapping library

### Details

The function basically return the nuID mapping information kept in the "nuID\\_MappingInfo" table of IDMapping libraries (lumiHumanIDMapping, lumiMouseIDMapping, lumiRatIDMapping). For more details of nuID mapping, please refer to the help of corresponding IDMapping library.

### Value

It returns a data.frame with each row corresponding to an input nuID.

### Author(s)

Warren Kibbe, Pan Du, Simon Lin

### Examples

```
## load example data
data(example.lumi)
if (require(lumiHumanIDMapping)) {
  nuIDs <- featureNames(example.lumi)
  mappingInfo <- getNuIDMappingInfo(nuIDs, lib.mapping=lumiHumanIDMapping)
  head(mappingInfo)
}
```

---

hist-methods	<i>Density plot of a ExpressionSet object</i>
--------------	---

---

**Description**

Creating density plot of sample intensities in a ExpressionSet object. It is equivalent to [density-methods](#).

**Usage**

```
## S4 method for signature ExpressionSet
hist(x, ...)
```

**Arguments**

x	a <a href="#">ExpressionSet</a> object
...	other parameters for <a href="#">density-methods</a> function

**See Also**

[LumiBatch-class](#), [density-methods](#), [hist](#)

**Examples**

```
## load example data
data(example.lumi)

hist(example.lumi)
```

---

id2seq	<i>Transfer a nuID as a nucleotide sequence</i>
--------	---

---

**Description**

The nuID (nucleotide universal identifier) is uniquely corresponding to probe sequence. The nuID is also self-identification and error checking

**Usage**

```
id2seq(id)
```

**Arguments**

id	a nuID (nucleotide universal identifier)
----	--

**Details**

A reverse of [seq2id](#). Please refer to reference for more details.

**Value**

a string of nucleotide sequence

**Author(s)**

Pan Du

**References**

Du, P., Kibbe, W.A. and Lin, S.M., "nuID: A universal naming schema of oligonucleotides for Illumina, Affymetrix, and other microarrays", *Biology Direct* 2007, 2:16 (31May2007).

**See Also**

[seq2id](#)

**Examples**

```
seq <- ACGTAAATTTTCAGTTTAAACCCCGG
id <- seq2id(seq)
id
id2seq(id)
```

---

IlluminaID2nuID

---

*Matching Illumina IDs to nuID based on Illumina ID mapping library*


---

**Description**

Matching Illumina IDs to nuID based on Illumina ID mapping libraries.

**Usage**

```
IlluminaID2nuID(IlluminaID, lib.mapping=NULL, species = c("Human", "Mouse", "Rat", "Unknown"), chipVer
```

**Arguments**

IlluminaID	a vector of Illumina IDs
lib.mapping	the ID mapping library. If it is provided, the parameter "species" will be ignored.
species	the species of the chip designed for. If users do not know it, it can be set as "Unknown".
chipVersion	chipVersion information returned by function <a href="#">getChipInfo</a>
...	other parameters of <a href="#">getChipInfo</a>

**Details**

When the parameter "chipVersion" is not provided, this function basically returned the "idMapping" item returned by function [getChipInfo](#).

**Value**

The mapping information from Illumina ID to nuID. It will be a matrix with each column corresponding to one matched manifest file when parameter "returnAllMatches" is TRUE. In this case, the columns are sorted from the best match to worst. If IlluminaID is NULL and chipVersion is provided, it will return all mapping information of the chip.

**Author(s)**

Pan Du

**See Also**

[getChipInfo](#), [nuID2IlluminaID](#)

---

importMethyIDAT

---

*Import Illumina methylation .idat files as an MethyLumiM object*


---

**Description**

Import Illumina methylation .idat files as an MethyLumiM object. An extension of [lumIDAT](#) function

**Usage**

```
importMethyIDAT(sampleInfo, dataPath = getwd(), lib = NULL, bigMatrix=FALSE, dir.bigMatrix=., savePref
```

**Arguments**

sampleInfo	A data.frame of sample information or a character vector of barcodes.
dataPath	The path of .idat files
lib	Annotation library
bigMatrix	whether to save the data as BigMatrix (designed for very large dataset)
dir.bigMatrix	the parent directory to save the BigMatrix data files
savePrefix.bigMatrix	the folder name prefix of the directory to save the BigMatrix data files. The fold name will be like this: paste(savePrefix.bigMatrix, '_bigmat', sep='')
...	other parameters used by <a href="#">lumIDAT</a> function

**Details**

This function is an extension of [lumIDAT](#). It adds sample information and probe annotation information to the data. As Illumina organizes the output .idat files by barcodes, the function will automatically check the sub-folders in the names of barcodes for .idat files. The "sampleInfo" parameter can be either a barcode vector, e.g., "7310440039\_R04C02" "7310440039\_R05C02". Or a data.frame with required columns of 'Sentrix\_Barcode' and 'Sentrix\_Position'. If "sampleInfo" is a data.frame, it will be added as the pData of the output MethyLumiM object.

**Value**

A MethyLumiM object

**Author(s)**

Pan Du, Tim Triche

**See Also**

[lumIDAT](#), [lumiMethyR](#), [addAnnotationInfo](#)

---

inverseVST

*Inverse VST transform*

---

**Description**

Inverse transform of VST (variance stabilizing transform), see [vst](#).

**Usage**

```
inverseVST(x, fun = c(asinh, log), parameter)
```

**Arguments**

x	a VST transformed LumiBatch object or a numeric matrix or vector
fun	function used in VST transform
parameter	parameter of VST function

**Details**

Recover the raw data from VST transformed data returned by [vst](#). This function can be directly applied to the VST transformed or VST + RSN normalized LumiBatch object to reverse transform the data to the original scale.

**Value**

Return the raw data before VST transform



**Author(s)**

Pan Du

**References**

Lin, S.M., Du, P., Kibbe, W.A., "Model-based Variance-stabilizing Transformation for Illumina Mi-croarray Data", submitted

**See Also**[vst](#)**Examples**

```
## load example data
data(example.lumi)

## get the gene expression mean for one chip
u <- exprs(example.lumi)[,1]
## get the gene standard deviation for one chip
std <- se.exprs(example.lumi)[,1]

## do variance stabilizing transform
transformedU <- vst(u, std)

## do inverse transform and recover the raw data
parameter <- attr(transformedU, parameter)
transformFun <- attr(transformedU, transformFun)
recoveredU <- inverseVST(transformedU, fun=transformFun, parameter=parameter)

## compare with the raw data
print(u[1:5])
print(recoveredU[1:5])

## do inverse transform of the VST + RSN processed data
lumi.N <- lumiExpresso(example.lumi[,1:2])
## Inverse transform.
## Note: as the normalization is involved, the processed data will be different from the raw data.
lumi.N.raw <- inverseVST(lumi.N)
```

---

is.nuID

*nuID self-identification*

---

**Description**

Self-identify nuID (nucleotide universal identifier) by verify the check code value and the checksum value

**Usage**

```
is.nuID(id)
```

**Arguments**

```
id          nuId or other string
```

**Value**

Return TRUE if id is a nuID, or else return FALSE.

**Author(s)**

Pan Du

**References**

Du, P., Kibbe, W.A. and Lin, S.M., "nuID: A universal naming schema of oligonucleotides for Illumina, Affymetrix, and other microarrays", *Biology Direct* 2007, 2:16 (31May2007).

**See Also**

[seq2id](#), [id2seq](#)

**Examples**

```
## check the function using a random sequence
id <- adfasdfafd
is.nuID(id) # FALSE

## check the function using a read nuID
seq <- ACGTAAATTCAGTTAAAACCCCG
id <- seq2id(seq)
is.nuID(id) # TRUE
```

---

lumiB

*Background correction of Illumina Expression data*

---

**Description**

Background correction of Illumina Expression data

**Usage**

```
lumiB(x.lumi, method = c(none, bgAdjust, forcePositive, bgAdjust.affy), verbose = TRUE, ...)
```

## Arguments

x.lumi	an ExpressionSet inherited object or a data matrix with columns as samples and rows as genes. For 'bgAdjust' method, it should be a LumiBatch Object
method	the background correction method, it can be any function with a ExpressionSet Object or matrix as the first argument and return an processed object with the same class
verbose	a boolean to decide whether to print out some messages
...	other parameters used by the user provided background correction method

## Details

We assume the BeadStudio output data is background corrected. So by default, it will do nothing. The 'bgAdjust' method will estimate the background based on the control probe information, which is kept in the controlData slot of LumiBatch object. The 'forcePositive' method will force all expression values to be positive by adding an offset (minus minimum value plus one), it does nothing if all expression values are positive. The purpose of this is to avoid NA when do logarithm transformation. 'none' does not but return the LumiBatch object. 'bgAdjust.affy' will call the [bg.adjust](#) function in affy package. User can also provide their own function with a LumiBatch Object as the first argument and return a LumiBatch Object with background corrected.

Thanks Kevin Coombes (M.D. Anderson Cancer Center) suggested adding this function.

## Value

Return an object with background corrected. The class of the return object is the same as the input object x.lumi.

## Author(s)

Pan Du, Kevin Coombes

## See Also

[bgAdjust](#), [lumiExpresso](#)

## Examples

```
## load example data
data(example.lumi)

## Do the default background correction method
lumi.B <- lumiB(example.lumi, method=bgAdjust, probs=0)
```

LumiBatch-class

*Class LumiBatch: contain and describe Illumina microarray data*

## Description

This is a class representation for Illumina microarray data. It extends [ExpressionSet](#).

## Extends

Directly extends class [ExpressionSet](#).

## Creating Objects

```
new(LumiBatch, exprs = [matrix], se.exprs = [matrix], beadNum = [matrix], detection = [matrix], phenoData = [matrix])
```

LumiBatch instances are usually created through `new("LumiBatch", ...)`. The arguments to `new` should include `exprs` and `se.exprs`, others can be missing, in which case they are assigned default values.

Objects can be created using the function [lumiR](#).

## Slots

Slot specific to LumiBatch:

**history:** a data.frame recording the operation history of the LumiBatch object.

**controlData:** a data.frame with first two columns as "controlType" and "ProbeID". The rest columns are the control probe expression amplitudes for individual samples.

**QC:** a the quality control information of the LumiBatch object, returned by `lumiQ` function.

Slots inherited from [ExpressionSet](#):

**assayData** contains equal dimensional matrices: `exprs` (contains gene expression level, which is the mean of its bead replicates.), `se.exprs` (contains gene expression standard error, which is the standard error of its bead replicates.), `beadNum` (records the number of beads for the probe.), `detection` (records the detection p-value of the probe. The number is from [0,1]. By default, < 0.01 indicates good detection.). For more details of `assayData`, please see [ExpressionSet](#)

**phenoData:** See [eSet](#)

**experimentData:** See [eSet](#)

**annotation:** See [eSet](#)

**Methods****Class-specific methods:**

`se.exprs(LumiBatch)`, `se.exprs(LumiBatch,matrix)<-`: Access and set elements named `se.exprs` in the `AssayData-class` slot.

`beadNum(LumiBatch)`, `beadNum(LumiBatch)<-`: Access and set elements named `beadNum` in the `AssayData-class` slot. Use "`beadNum(LumiBatch) <- NULL`" to remove the `beadNum` element.

`detection(LumiBatch)`, `detection(LumiBatch)<-`: Access and set elements named `detection` in the `AssayData-class` slot. Use "`detection(LumiBatch) <- NULL`" to remove the `detection` element.

`getHistory(LumiBatch)`: Access the operation history of `LumiBatch` object.

**Derived from [ExpressionSet](#)** (For the directly inherited methods, please see [ExpressionSet](#) and [eSet](#)):

`combine(LumiBatch,missing)`: Combine two `LumiBatch` objects, including history slot. See [eSet](#)

`exprs(LumiBatch)`, `exprs(LumiBatch,matrix)<-`: Access and set elements named `exprs` in the `AssayData-class` slot.

`object[(i,j)]`: Conduct subsetting of the data in a `LumiBatch` object

**Standard generic methods** (For the directly inherited methods, please see [ExpressionSet](#) and [eSet](#)):

`initialize(LumiBatch)`: Object instantiation, used by `new`; not to be called directly by the user.

`validObject(LumiBatch)`: Validity-checking method, ensuring that `exprs` and `se.exprs` is a member of `assayData`. Other validity check is the same as `checkValidity(ExpressionSet)`.

`show(LumiBatch)` A summary of the `LumiBatch` object.

**Author(s)**

Pan Du, Simon Lin

**See Also**

[lumiR](#), [lumiT](#), [lumiN](#), [boxplot-methods](#), [pairs-methods](#), [MAplot-methods](#)

**Examples**

```
## load example data
data(example.lumi)

## show the summary of the data
# summary(example.lumi)
example.lumi

## get expression matrix
temp <- exprs(example.lumi)
```

```
## get a subset
temp <- example.lumi[,1] ## retrieve the first sample

## get the probe id
featureNames(example.lumi)[1:3]

## combine LumiBatch objects
temp <- combine(example.lumi[,1], example.lumi[,3])
temp
```

---

lumiExpresso

*From raw Illumina probe intensities to expression values*


---

## Description

Goes from raw Illumina probe intensities to expression values

## Usage

```
lumiExpresso(lumiBatch, bg.correct = TRUE, bgcorrect.param = list(method=bgAdjust), variance.stabilize
varianceStabilize.param = list(), normalize = TRUE, normalize.param = list(), QC.evaluation = TRUE,
QC.param = list(), verbose = TRUE)
```

## Arguments

lumiBatch	a LumiBatch object, which can be the return of <a href="#">lumiR</a>
bg.correct	a boolean to decide whether to do background correction or not
bgcorrect.param	a list of parameters of <a href="#">lumiB</a>
variance.stabilize	a boolean to decide whether to do variance stabilization or not
varianceStabilize.param	a list of parameters of <a href="#">lumiT</a>
normalize	a boolean to decide whether to do normalization or not
normalize.param	a list of parameters of <a href="#">lumiN</a>
QC.evaluation	a boolean to decide whether to do quality control estimation before and after preprocessing
QC.param	a list of parameters of <a href="#">lumiQ</a>
verbose	a boolean to decide whether to print out some messages

## Details

The function is to encapsulate the major functions of Illumina preprocessing. It is organized in a similar way as the [expresso](#) function in affy package.

**Value**

return a processed LumiBatch object. The operation history can be track in the history slot of the object.

**Author(s)**

Pan Du

**See Also**

[lumiB](#), [lumiT](#), [lumiN](#)

**Examples**

```
## load example data
data(example.lumi)

## Do all the default preprocessing in one step
lumi.N <- lumiExpresso(example.lumi)

## Do customized preprocessing. No variance stabilizing or log transform, use Quantile normalization.
lumi.N <- lumiExpresso(example.lumi, variance.stabilize=FALSE, normalize.param = list(method=quantile))
```

---

lumiMethyB

*Adjust background level of Illumina Infinium methylation data*


---

**Description**

Adjust background level of Illumina Infinium methylation data, which is an object in MethyLumiM class.

**Usage**

```
lumiMethyB(methyLumiM, method = c("bgAdjust2C", "forcePositive", "none"), separateColor = FALSE, verbose = FALSE)
```

**Arguments**

methyLumiM	a MethyLumiM object includes Illumina Infinium methylation data
method	background adjustment methods or user provided function, whose input and output should be a intensity matrix (pool of methylated and unmethylated probe intensities)
separateColor	determine whether to separately process two color channels
verbose	a boolean to decide whether to print out some messages
overwriteBigMatrix	whether to overwrite the result to the BigMatrix data, only valid when the input data is BigMatrix-based
...	other parameters used by corresponding method

**Value**

Return an object (same class as input `methyLumiM`) with updated "methylated" and "unmethylated" data matrix after background level adjustment.

**Author(s)**

Pan DU

**See Also**

See Also [bgAdjustMethylation](#) and [estimateMethylationBG](#)

**Examples**

```
data(example.lumiMethy)
lumiMethy.bgAdj = lumiB(example.lumiMethy)
attr(lumiMethy.bgAdj, "EstimatedBG")
```

---

lumiMethyC

*Color bias adjust of Illumina Infinium methylation data*

---

**Description**

Color bias adjust of Illumina Infinium methylation data, which is an object in `MethyLumiM` class.

**Usage**

```
lumiMethyC(methyLumiM, method = c("quantile", "ssn", "none"), verbose = TRUE, overwriteBigMatrix=FALSE)
```

**Arguments**

<code>methyLumiM</code>	a <code>MethyLumiM</code> object includes Illumina Infinium methylation data
<code>method</code>	color bias adjustment methods or user provided function, see "details" for more information of user defined function.
<code>verbose</code>	a boolean to decide whether to print out some messages
<code>overwriteBigMatrix</code>	whether to overwrite the result to the <code>BigMatrix</code> data, only valid when the input data is <code>BigMatrix</code> -based
<code>...</code>	other parameters used by corresponding method

**Details**

The first two arguments of the user defined function should be two intensity matrix (pool of methylated and unmethylated probe intensities) of red and green channel respectively. The return of the user defined function should be a list including color adjusted matrix of red and green channel. For example: `return(list(red=redData, green=grnData))`. "redData" and "grnData" are two color adjusted matrix.



**Value**

Return an object (same class as input `methyLumiM`) with updated "methylated" and "unmethylated" data matrix after background level adjustment.

**Author(s)**

Pan DU

**See Also**

See Also [adjColorBias.quantile](#) and [adjColorBias.ssn](#)

**Examples**

```
data(example.lumiMethy)
# before adjustment
plotColorBias1D(example.lumiMethy)
# plot in 2D plot of one selected sample
plotColorBias2D(example.lumiMethy, selSample = 1)
lumiMethy.adj = lumiMethyC(example.lumiMethy)
# after adjustment
plotColorBias1D(lumiMethy.adj)
# plot in 2D plot of one selected sample
plotColorBias2D(lumiMethy.adj, selSample = 1)
```

---

lumiMethyN

*Normalize the Illumina Infinium methylation data*


---

**Description**

Normalize the Illumina Infinium methylation data, which is an object in `MethyLumiM` class.

**Usage**

```
lumiMethyN(methyLumiM, method = c("quantile", "ssn", "none"), separateColor = FALSE, verbose = TRUE, ov
```

**Arguments**

<code>methyLumiM</code>	a <code>MethyLumiM</code> object includes Illumina Infinium methylation data
<code>method</code>	supported normalization methods or user provided function, whose input and output should be a intensity matrix (pool of methylated and unmethylated probe intensities)
<code>separateColor</code>	determine whether to separately process two color channels
<code>verbose</code>	a boolean to decide whether to print out some messages
<code>overwriteBigMatrix</code>	whether to overwrite the result to the <code>BigMatrix</code> data, only valid when the input data is <code>BigMatrix</code> -based
<code>...</code>	other parameters used by corresponding method

**Value**

Return an object (same class as input `methyLumiM`) with updated "methylated" and "unmethylated" data matrix after background level adjustment.

**Author(s)**

Pan DU

**See Also**

See Also [normalizeMethylation.ssn](#) and [normalizeMethylation.quantile](#)

**Examples**

```
data(example.lumiMethy)
lumiMethy.norm = lumiN(example.lumiMethy)
```

---

lumiMethyR

*Reading Illumina methylation microarray data*

---

**Description**

This function is a wrap of [methylumiR](#) function in `methylumi` package.

**Usage**

```
lumiMethyR(..., lib = NULL, controlData = NULL)
```

**Arguments**

<code>...</code>	parameters of <a href="#">methylumiR</a> function in <code>methylumi</code> package
<code>lib</code>	Annotation library of Illumina methylation microarray
<code>controlData</code>	the <code>controlData</code> file name or a <code>MethyLumiQC</code> object to be added to the "controlData" slot of the <code>MethyLumiM</code> object

**Details**

This function is a wrap of [methylumiR](#) function in `methylumi` package. It will coerce the returned object as `MethyLumiM` class. The methylated and unmethylated probe intensity information is required for color-bias adjustment and normalization. If users have the Illumina IDAT files, we suggest use [importMethyIDAT](#) function to import the data. The [importMethyIDAT](#) function will automatically retrieve the required information and return a `MethyLumiM` object.

**Value**

return a `MethyLumiM` object

**Author(s)**

Pan Du

**See Also**See Also [importMethyIDAT](#), [methyLumiR](#) and [addControlData2methyLumiM](#)

---

`lumiMethyStatus`*Estimate the methylation status of individual methylation sites*

---

**Description**

Estimate the methylation status of individual methylation sites by fitting a two component Gamma mixture model for each sample

**Usage**

```
lumiMethyStatus(methyLumiM, ...)
```

**Arguments**

<code>methyLumiM</code>	a <code>MethyLumiM</code> class object
<code>...</code>	Other parameters used by <a href="#">methylationCall</a>

**Details**

This function calls [methylationCall](#) and returns the methylation status of individual methylation sites. The methylation status includes: "Unmethy" (unmethylation probability > unmethylation threshold), "Methy" (methylation probability > methylation threshold), or "Margin". The methylation probability is returned as an attribute of "probability".

**Value**

return a methylation status matrix with "probability" attribute

**Author(s)**

Pan Du

**See Also**See Also [methylationCall](#) and [gammaFitEM](#)**Examples**

```
data(example.lumiMethy)
methyCall <- lumiMethyStatus(example.lumiMethy)
head(methyCall)
```

---

lumiN

*Between chip normalization of a LumiBatch object*


---

### Description

A main function of between chip normalization of a LumiBatch object. Currently, four methods ("rsn", "ssn", "quantile", "loess", "vsn") are supported.

### Usage

```
lumiN(x.lumi, method = c("quantile", "rsn", "ssn", "loess", "vsn", "rankinvariant"), verbose = TRUE, ..
```

### Arguments

x.lumi	an ExpressionSet inherited object or a data matrix with columns as samples and rows as genes
method	five different between chips normalization methods ("quantile", "rsn", "ssn", "loess", "vsn", "rankinvariant") are supported
verbose	a boolean to decide whether to print out some messages
...	other parameters used by corresponding method

### Details

lumiN is an interface for different normalization methods. Currently it supports "RSN" (See [rsn](#)), "SSN" (See [ssn](#)), "loess" (See [normalize.loess](#)), "quantile" (See [normalize.quantiles](#)), "VSN" (See [vsn](#)) and "rankinvariant" (See [rankinvariant](#)). See details in individual functions. Note: the "VSN" normalization should be directly applied to the raw data instead of the lumiT processed data.

### Value

Return an object with expression values normalized. The class of the return object is the same as the input object x.lumi. If it is a LumiBatch object, it also includes the VST transform function and its parameters as attributes: "transformFun", "parameter". See [inverseVST](#) for details.

### Author(s)

Pan Du, Simon Lin

### See Also

[rsn](#), [ssn](#), [rankinvariant](#)

## Examples

```
## load example data
data(example.lumi)

## Do lumi transform
lumi.T <- lumiT(example.lumi)

## Do lumi between chip normaliazation
lumi.N <- lumiN(lumi.T, method=rsn, ifPlot=TRUE)
```

---

lumiQ

*Quality control evaluation of the LumiBatch object*

---

## Description

Quality control evaluation of the LumiBatch object and returns a summary of the data

## Usage

```
lumiQ(x.lumi, logMode = TRUE, detectionTh = 0.01, verbose = TRUE)
```

## Arguments

x.lumi	a LumiBatch object
logMode	transform as log2 or not (the function can check whether it is already log transformed.)
detectionTh	the detection threshold used by <a href="#">detectionCall</a>
verbose	a boolean to decide whether to print out some messages

## Details

Quality control of a LumiBatch object includes estimating the mean and standard deviation of the chips, detectable probe ratio of each chip, sample (chip) relations, detecting outliers of samples (chips). The produced QC information is kept in the QC slot of LumiBatch class. The summary function will provide a summary of the QC information (See example).

## Value

a LumiBatch object with QC slot keeping the QC information

## Author(s)

Pan Du

## See Also

[LumiBatch](#), [plot](#), [ExpressionSet-method](#)

## Examples

```
## load example data
data(example.lumi)

## Do quality control estimation
lumi.Q <- lumiQ(example.lumi)

## A summary of the QC
summary(lumi.Q, QC)

## Plot the results
## plot the pairwise sample correlation
plot(lumi.Q, what=pair)

## see more examples in "plot,ExpressionSet-method" help documents
```

---

lumiR

*Read in Illumina expression data*


---

## Description

Read in Illumina expression data. We assume the data was saved in a comma or tab separated text file.

## Usage

```
lumiR(fileName, sep = NULL, detectionTh = 0.01, na.rm = TRUE, convertNuID = TRUE, lib.mapping = NULL, dec = TRUE, columnNameGrepPattern = list(exprs=AVG_SIGNAL, se.exprs=BEAD_STD, detection=DETECTION, bead.inputAnnotation=TRUE, annotationColumn=c(ACCESSION, SYMBOL, PROBE_SEQUENCE, PROBE_START, CHROMOSOME,
```

## Arguments

fileName	fileName of the data file
sep	the separation character used in the text file.
detectionTh	the p-value threshold of determining detectability of the expression. See more details in <a href="#">lumiQ</a>
na.rm	determine whether to remove NA
convertNuID	determine whether convert the probe identifier as nuID
lib.mapping	a Illumina ID mapping package, e.g, <a href="#">lumiHumanIDMapping</a> , used by <a href="#">addNuID2lumi</a>
dec	the character used in the file for decimal points.
parseColumnName	determine whether to parse the column names and retrieve the sample information (Assume the sample information is separated by "\\_".)
checkDupId	determine whether to check duplicated TargetIDs or ProbeIds. The duplicated ones will be averaged.

QC	determine whether to do quality control assessment after read in the data.
columnNameGrepPattern	the string grep patterns used to determine the slot corresponding columns.
inputAnnotation	determine whether input the annotation information outputted by BeadStudio if exists.
annotationColumn	the column names of the annotation information outputted by BeadStudio
verbose	a boolean to decide whether to print out some messages
...	other parameters used by <code>read.table</code> function

## Details

The function can automatically determine the separation character if it is Tab or comma. Otherwise, the user should specify the separator manually. If the annotation library is provided, the Illumina Id will be replaced with nuID, which is used as the index Id for the lumi annotation packages. If the annotation library is not provided, it will try to directly convert the probe sequence (if provided in the BeadStudio output file) as nuIDs.

The parameter "columnNameGrepPattern" is designed for some advanced users. It defines the string grep patterns used to determine the slot corresponding columns. For example, for the "exprs" slot in LumiBatch object, it is composed of the columns whose name includes "AVG\_SIGNAL". In some cases, the user may not want to read the "detection" and "beadNum" related columns to save memory. The user can set the "detection" and "beadNum" as NA in "columnNameGrepPattern". If the 'se.exprs' is set as NA or the corresponding columns are not available, then lumiR will create a ExpressionSet object instead of LumiBatch object.

The parameter "parseColumnName" is designed to parse the column names and retrieve the sample information. We assume the sample information is separated by "\_" and the last element after "\_" is the sample label (sample names of the LumiBatch object). If the parsed sample labels are not unique, then the entire string will be used as the sample label. For example: "1881436055\_A\_STA 27aR" is included in one of the column names of BeadStudio output file. Here, the program will first treat "STA 27aR" as the sample label. If it is not unique across the samples, "1881436055\_A\_STA 27aR" will be the sample label. If it is still not unique, the program will report warning messages. All the parsed information is kept in the phenoData slot. By default, "parseColumnName" is FALSE. We suggest the users use it only when they know what they are doing.

Current version of lumiR can adaptively read the output of BeadStudio Version 1 and 3. The format Version 3 made quite a few changes comparing with previous versions. One change is the detection value. It was called detectable when the detection value is close to one for Version 1 format. However, the detection value became a p-value in the Version 3. As a result, the detectionTh is automatically changed based on the version. The detectionTh 0.01 for the Version 3 will be changed as the detectionTh 0.99 for Version 1. Another big change is that Version 3 separately output the control probe (gene) information and a "Samples Table". As a result, the controlData slot in LumiBatch class was added to keep the control probe (gene) information, and a QC slot to keep the quality control information, including the "Sample Table" output by BeadStudio version 3.

The recent version of BeadStudio can also output the annotation information together with the expression data. In the users also want to input the annotation information, they can set the parameter "inputAnnotation" as TRUE. At the same time, they can also specify which columns to be inputted

by setting parameter "annotationColumn". The BeadStudio annotation columns include: SPECIES, TRANSCRIPT, ILMN\\_GENE, UNIGENE\\_ID, GI, ACCESSION, SYMBOL, PROBE\\_ID, ARRAY\\_ADDRESS\\_ID, PROBE\\_TYPE, PROBE\\_START, PROBE\\_SEQUENCE, CHROMOSOME, PROBE\\_CHR\\_ORIENTATION, PROBE\\_COORDINATES, DEFINITION, ONTOLOGY\\_COMPONENT, ONTOLOGY\\_PROCESS, ONTOLOGY\\_FUNCTION, SYNONYMS, OBSOLETE\\_PROBE\\_ID. As the annotation data is huge, by default, we only input: ACCESSION, SYMBOL, PROBE\\_START, CHROMOSOME, PROBE\\_CHR\\_ORIENTATION, PROBE\\_COORDINATES, DEFINITION. This annotation information is kept in the featureData slot of ExpressionSet, which can be retrieved using `pData(featureData(x.lumi))`, suppose `x.lumi` is the LumiBatch object. As some annotation information may be outdated. We recommend using Bioconductor annotation packages to retrieve the annotation information.

The BeadStudio may output either STDEV or STDERR (standard error of the mean) columns. As the variance stabilization (see `vst` function) requires the information of the standard deviation instead of the standard error of the mean, the value correction is required. The `lumiR` function will automatically check whether the BeadStudio output file includes STDEV or STDERR columns. If it is STDERR columns, it will correct STDERR as STDEV. The corrected value will be  $x * \sqrt{N}$ , where  $x$  is the STDERR value (standard error of the mean),  $N$  is the number of beads corresponding to the probe. (Thanks Sebastian Balbach and Gordon Smyth kindly provided this information.). This correction was previous implemented in the `lumiT` function.

### Value

return a LumiBatch object

### Author(s)

Simon Lin, Pan Du

### See Also

[LumiBatch](#), [addNuID2lumi](#)

### Examples

```
## specify the file name
# fileName <- Barnes_gene_profile.txt # Not Run
## load the data
# x.lumi <- lumiR(fileName)

## load the data with empty detection and beadNum slots
# x.lumi <- lumiR(fileName, columnNameGrepPattern=list(detection=NA, beadNum=NA))
```

---

lumiR.batch

*Read BeadStudio output files in batch*

---

### Description

Read BeadStudio output files in batch and combine them as a single LumiBatch object



**Usage**

```
lumiR.batch(fileList, convertNuID = TRUE, lib.mapping = NULL, detectionTh = 0.01, QC = TRUE, transform =
```

**Arguments**

fileList	a vector of file names or a directory keeping the data files in the format of .csv
convertNuID	determine whether convert the probe identifier as nuID
lib.mapping	same as <a href="#">lumiR</a> parameter lib.mapping (optional)
detectionTh	the p-value threshold of determining detectability of the expression. See more details in <a href="#">lumiQ</a>
QC	determine whether to do quality control assessment after read in the data.
transform	determine whether to do transform after input each file
sampleInfoFile	a Tab-separated text file or a data.frame keeping the sample information (optional)
verbose	a boolean to decide whether to print out some messages
...	other parameters used by <a href="#">lumiR</a>

**Details**

The function basically call lumiR for individual files and then combine the returns. The sampleInfoFile parameter is optional. It provides the sample information (for phenoData slot in LumiBatch object), it is a Tab-separated text file. ID column is required. It represents sample ID, which is defined based on the column names of BeadStudio output file. For example, sample ID of column "1881436070\\_A\\_STA.AVG\\_Signal" is "1881436070\\_A\\_STA". The sample ID column can also be found in the "Samples Table.txt" file output by BeadStudio. Another "Label" column (if provided) will be used as the sampleNames of LumiBatch object. All information of sampleInfoFile will be directly added in the phenoData slot in LumiBatch object.

To save memory space in the case of reading large data set, we can do transformation using lumiT function right after input the data, and the information like se.exprs, beadNum will be removed from the LumiBatch object after transformation.

**Value**

A LumiBatch object which combines the individual LumiBatch object corresponding to each file

**Author(s)**

Pan Du

**See Also**

[lumiR](#)

**Examples**

```
## fileList <- c(file1.csv, file2.csv)
## x.lumi <- lumiR.batch(fileList, sampleInfoFile=sampleInfo.txt)
```

---

`lumiT`*Transfer the Illumina data to stabilize the variance*

---

**Description**

Transfer the Illumina data to stabilize the variance.

**Usage**

```
lumiT(x.lumi, method = c("vst", log2, cubicRoot), ifPlot = FALSE, simpleOutput = TRUE, verbose = TRUE, .
```

**Arguments**

<code>x.lumi</code>	LumiBatch object
<code>method</code>	four methods are supported: "vst", "log2", "cubicRoot"
<code>ifPlot</code>	determine whether to plot the intermediate results
<code>simpleOutput</code>	determine whether to simplify the output LumiBatch object, which will set the <code>se.exprs</code> , <code>detection</code> and <code>beadNum</code> slots as NULL.
<code>verbose</code>	a boolean to decide whether to print out some messages
<code>...</code>	other parameters used by <a href="#">vst</a>

**Details**

`lumiT` is an interface of difference variance stabilizing transformation. See [vst](#) for details of VST (Variance Stabilizing Transform) of Illumina data.

NOTE: This correction of STDERR as STDEV was moved to the `lumiR` function.

**Value**

Return a LumiBatch object with transformed expression values. It also includes the VST transform function and its parameters as attributes: "transformFun", "parameter". See [inverseVST](#) for details.

**Author(s)**

Pan Du, Simon Lin

**References**

Lin, S.M., Du, P., Kibbe, W.A., (2008) 'Model-based Variance-stabilizing Transformation for Illumina Microarray Data', *Nucleic Acids Res.* 36, e11

**See Also**

[vst](#)

## Examples

```
## load example data
data(example.lumi)

## Do default VST variance stabilizing transform
lumi.T <- lumiT(example.lumi, ifPlot=TRUE)
```

---

m2beta	<i>Convert methylation M-value to Beta-value</i>
--------	--

---

## Description

Convert methylation M-value to Beta-value

## Usage

```
m2beta(m)
```

## Arguments

m                    a matrix or vector of methylation M-value

## Details

Convert methylation M-value to Beta-value

## Value

return methylation Beta-value with the same size of input M-value

## Author(s)

Pan Du

## References

Du, P., Zhang, X., Huang, C.C., Jafari, N., Kibbe, W.A., Hou, L., and Lin, S.M., (2010) 'Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis', (under review)

## See Also

See Also as [beta2m](#)

**Description**

Creating pairwise MAplot of sample intensities in a ExpressionSet object

**Usage**

```
## S4 method for signature ExpressionSet
MAplot(object, ..., smoothScatter = FALSE, logMode = TRUE, subset = 5000, main = NULL)
```

**Arguments**

object	an <a href="#">ExpressionSet</a> object
...	optional arguments to <a href="#">MAplot</a> .
smoothScatter	whether use <a href="#">smoothScatter</a> function to plot points
logMode	whether plot the data in log2 scale or not
subset	subset of rows used to plot. It can be an index vector, or the length of a random subset
main	title of the plot

**Details**

To increase the plot efficiency, by default, we only plot RANDOMLY selected subset of points (based on parameter "subset"). If users want to plot all the points, they can set the parameter "subset = NULL". When smoothScatter is set as TRUE, the subsetting will be suppressed because [smoothScatter](#) function has good plot efficiency for large number of points.

**See Also**

[LumiBatch-class](#), [MAplot](#)

**Examples**

```
## load example data
data(example.lumi)

MAplot(example.lumi)

MAplot(example.lumi, smoothScatter=TRUE)
```

---

methylationCall	<i>Estimated methylation call</i>
-----------------	-----------------------------------

---

### Description

Estimated methylation call based on the fitting results of [gammaFitEM](#)

### Usage

```
methylationCall(x, threshold = 0.95, ...)
```

### Arguments

x	a vector of M-values covering the whole genome or a "gammaFit" class object returned by <a href="#">gammaFitEM</a>
threshold	the probability threshold to make a methylation call. The threshold can be a vector of two: unmethylation threshold and methylation threshold
...	other parameters used by <a href="#">gammaFitEM</a>

### Details

Retrieve the probability element returned by [gammaFitEM](#), and convert it as three status calls based on probability threshold

NOTE: the methylation status modeling algorithm was developed based on 27K methylation array. It has not been tested for 450K array. Considering 450K array covers both promoter and gene body, the two component Gamma mixture model assumption may not be valid any more.

### Value

A vector of three methylation status: "Unmethy" (unmethylation posterior probability > unmethylation threshold), "Methy" (methylation posterior probability > methylation threshold), or "Margin". The sum of unmethylation posterior probability and methylation posterior probability equals one. The methylation probability is returned as an attribute of "probability".

### Author(s)

Pan DU

### See Also

[gammaFitEM](#)

**Examples**

```

data(example.lumiMethy)
M <- exprs(example.lumiMethy)
fittedGamma <- gammaFitEM(M[,1], initialFit=NULL, maxIteration=50, tol=0.0001, plotMode=TRUE, verbose=FALSE)
methyCall <- methylationCall(fittedGamma)
table(methyCall)

```

monoSmu

*Monotonic smooth method***Description**

Fit the monotonic-constraint spline curve

**Usage**

```
monoSmu(x, y, newX = NULL, nSupport = min(200, length(x)), nKnots = 6, rotate = FALSE, ifPlot = FALSE, xlab, ylab, ...)
```

**Arguments**

x	a vector represents x values
y	a vector represents y values
newX	the new values to be transformed. If not provided, "x" will be used.
nSupport	downsampled data points
nKnots	parameter used by <a href="#">monoSpline</a>
rotate	determine whether to rotate the axis with 45 degrees in clockwise, i.e., fit the curve in the MA-plot.
ifPlot	determine whether to plot intermediate results
xlab	the xlab of the plot
ylab	the ylab of the plot
...	parameters used by <a href="#">supsmu</a> and <a href="#">plot</a>

**Details**

function called by lumiN.rsn. The function first fits a monotonic spline between vector x and y, then transforms the vector newX based on the fitted spline. (After transformation the fitted spline is supposed to be a diagonal line, i.e., x=y)

**Value**

Return the transformed "newX" based on the smoothed curve

**Author(s)**

Simon Lin, Pan Du

**References**

Lin, S.M., Du, P., Kibbe, W.A., (2008) 'Model-based Variance-stabilizing Transformation for Illumina Microarray Data', Nucleic Acids Res. 36, e11

**See Also**

[monoSpline](#)

---

monoSpline

*Fitting a curve with monotonic spline*

---

**Description**

Fitting a curve with monotonic spline

**Usage**

```
monoSpline(x, y, newX=NULL, nKnots = 6, ifPlot = FALSE)
```

**Arguments**

x	a vector represents x values
y	a vector represents y values
newX	the new values to be transformed. If not provided, "x" will be used.
nKnots	parameter used by function smoothCon in package mgcv
ifPlot	determine whether to plot intermediate results

**Details**

Function internally called by monoSmu

**Value**

return the transformed "newX" based on the smoothed curve

**Author(s)**

Simon Lin, Pan Du

**See Also**

[monoSmu](#)

---

normalizeMethylation.quantile

*Quantile normalization of Illumina Infinium methylation data at probe level*

---

### Description

Quantile normalization of Illumina Infinium methylation data at probe level. Input data is a MethyLumiM object

### Usage

```
normalizeMethylation.quantile(methyLumiM, separateColor = FALSE, reference = NULL, ...)
```

### Arguments

methyLumiM	a MethyLumiM object includes Illumina Infinium methylation data
separateColor	determine whether separately normalize two color channels
reference	the reference vector (same length as the number of matrix rows) for quantile normalization
...	other parameters used by normalize.quantiles.robust method

### Value

Return an object (same class as input methyLumiM) with updated "methylated" and "unmethylated" data matrix after background level adjustment.

### Author(s)

Pan DU

### See Also

See Also [lumiMethyN](#), and [normalizeMethylation.ssn](#)

### Examples

```
data(example.lumiMethy)
lumiMethy.norm = normalizeMethylation.quantile(example.lumiMethy)
```



---

```
normalizeMethylation.ssn
```

*Shift and scaling normalization of Illumina Infinium methylation data at probe level*

---

### Description

Shift and scaling normalization of Illumina Infinium methylation data at probe level

### Usage

```
normalizeMethylation.ssn(methyLumiM, separateColor = FALSE)
```

### Arguments

`methyLumiM` a MethyLumiM object includes Illumina Infinium methylation data  
`separateColor` determine whether separately normalize two color channels

### Value

Return an object (same class as input `methyLumiM`) with updated "methylated" and "unmethylated" data matrix after background level adjustment.

### Author(s)

Pan DU

### See Also

See Also [lumiMethyN](#), and [normalizeMethylation.quantile](#)

### Examples

```
data(example.lumiMethy)
lumiMethy.norm = normalizeMethylation.ssn(example.lumiMethy)
```

---

nuID2EntrezID	<i>Map nuID to Entrez ID</i>
---------------	------------------------------

---

### Description

Map nuID to EntrezID through RefSeq ID based on IDMapping libraries.

### Usage

```
nuID2EntrezID(nuID = NULL, lib.mapping, filterTh = c(Strength1 = 95, Uniqueness = 95), returnAllInfo = F
```

### Arguments

nuID	a vector of nuIDs. If it is NULL, all mappings will be returned.
lib.mapping	the ID mapping library
filterTh	the mapping quality filtering threshold used to filter the ID mapping.
returnAllInfo	determine to return the detailed mapping information or just the matched RefSeq IDs

### Details

This function is based on the return of [getNuIDMappingInfo](#) function. The mapping from nuID to EntrezID was based on the mapping from nuID to RefSeqID and RefSeqID to EntrezID. It uses mapping quality information to filter out the bad mappings from nuID to RefSeqID. The parameter "filterTh" is obsolete for lumi ID mapping package > version 1.3, which only keeps the perfect mapping. For the old version of ID mapping package (< 1.3), the names of "filterTh" are basically the field names of "nuID\\_MappingInfo" table, which include 'Strength1', 'Strength2', 'Uniqueness' and 'Total hits'. For the definition of these metrics, please refer to the IDMapping library or see the reference website.

### Value

returns the matched Entrez IDs or a data.frame with each row corresponding to an input nuID (when "returnAllInfo" is TRUE).

### Author(s)

Warren Kibbe, Pan Du, Simon Lin

### References

<https://prod.bioinformatics.northwestern.edu/nuID/>

### See Also

See Also [getNuIDMappingInfo](#)

**Examples**

```
## load example data
data(example.lumi)
if (require(lumiHumanIDMapping)) {
  nuIDs <- featureNames(example.lumi)
  mappingInfo <- nuID2EntrezID(nuIDs, lib.mapping=lumiHumanIDMapping)
  head(mappingInfo)
}
```

---

nuID2IlluminaID	<i>Matching nuIDs to Illumina IDs based on Illumina ID mapping library</i>
-----------------	--

---

**Description**

Matching nuIDs to Illumina IDs based on Illumina ID mapping library

**Usage**

```
nuID2IlluminaID(nuID, lib.mapping=NULL, species = c("Human", "Mouse", "Rat", "Unknown"), idType=c(All,
```

**Arguments**

nuID	a vector of nuIDs
lib.mapping	the ID mapping library. If it is provided, the parameter "species" will be ignored.
species	the species of the chip designed for. If users do not know it, it can be set as "Unknown".
idType	the Illumina ID type
chipVersion	chipVersion information returned by function <a href="#">getChipInfo</a>
...	other parameters of <a href="#">getChipInfo</a>

**Details**

The parameter "idType" represents different types of Illumina IDs. It returns the entire table when idType = "All". When idType = 'Probe', it returns "ProbeId" or "Probe\\_Id". When idType = 'Gene', it returns "Target" or "ILMN\\_Gene" IDs.

This function basically returned the "idMapping" item returned by function [getChipInfo](#). If nuID is NULL and chipVersion is provided, it will return all mapping information of the chip.

**Value**

The mapping information from nuID to Illumina ID. It will be a matrix with each column corresponding to one matched manifest file when parameter "returnAllMatches" is TRUE. In this case, the columns are sorted from the best match to worst.

**Author(s)**

Pan Du

**See Also**

[getChipInfo, IlluminaID2nuID](#)

**Examples**

```
## load example data
data(example.lumi)
nuIDs <- featureNames(example.lumi)
if (require(lumiHumanIDMapping)) {
  illuminaID <- nuID2IlluminaID(nuIDs[1:5], lib=lumiHumanIDMapping)
  illuminaID
}
```

---

nuID2probeID

*Mapping nuID into Illumina ProbeID*

---

**Description**

Mapping nuID into Illumina ProbeID.

**Usage**

```
nuID2probeID(nuID, lib.mapping = "lumiHumanIDMapping", ...)
```

**Arguments**

nuID	a vector of nuID
lib.mapping	an Illumina ID mapping library
...	other parameters of <a href="#">nuID2IlluminaID</a>

**Details**

The function will call [nuID2IlluminaID](#) when ID mapping library were provided.

**Value**

see function [nuID2IlluminaID](#)

**Author(s)**

Pan Du

**References**

Du, P., Kibbe, W.A. and Lin, S.M., "nuID: A universal naming schema of oligonucleotides for Illumina, Affymetrix, and other microarrays", *Biology Direct* 2007, 2:16 (31May2007).

**See Also**

[probeID2nuID](#), [nuID2IlluminaID](#)

**Examples**

```
if (require(lumiHumanIDMapping)) {
  nuID2probeID("B2J6WGhV.RevOJYff4", lib.mapping = "lumiHumanIDMapping")
}
```

---

nuID2RefSeqID

*Map nuID to RefSeq ID*


---

**Description**

Map nuID to RefSeq ID based on IDMapping libraries.

**Usage**

```
nuID2RefSeqID(nuID = NULL, lib.mapping, filterTh = c(Strength1 = 95, Uniqueness = 95), returnAllInfo = F
```

**Arguments**

nuID	a vector of nuIDs. If it is NULL, all mappings will be returned.
lib.mapping	the ID mapping library
filterTh	the mapping quality filtering threshold used to filter the ID mapping. Obsolete for lumi ID mapping package > version 1.3!
returnAllInfo	determine to return the detailed mapping information or just the matched RefSeq IDs

**Details**

This function is based on the return of [getNuIDMappingInfo](#) function. It uses mapping quality information to filter out the bad mappings. The parameter "filterTh" is obsolete for lumi ID mapping package > version 1.3, which only keeps the perfect mapping. For the old version of ID mapping package (< 1.3), the names of "filterTh" are basically the field names of "nuID\_MappingInfo" table, which include 'Strength1', 'Strength2', 'Uniqueness' and 'Total hits'. For the definition of these metrics, please refer to the IDMapping library or see the reference website.

**Value**

returns the matched RefSeq IDs or a data.frame with each row corresponding to an input nuID (when "returnAllInfo" is TRUE).

**Author(s)**

Warren Kibbe, Pan Du, Simon Lin

## References

<https://prod.bioinformatics.northwestern.edu/nuID/>

## See Also

See Also [getNuIDMappingInfo](#)

## Examples

```
## load example data
data(example.lumi)
if (require(lumiHumanIDMapping)) {
  nuIDs <- featureNames(example.lumi)
  mappingInfo <- nuID2RefSeqID(nuIDs, lib.mapping=lumiHumanIDMapping)
  head(mappingInfo)
}
```

---

nuID2targetID

*Mapping nuID into Illumina TargetID*

---

## Description

Mapping nuID into Illumina TargetID or GeneID.

## Usage

```
nuID2targetID(nuID, lib.mapping = "lumiHumanIDMapping", ...)
```

## Arguments

nuID	a vector of nuID
lib.mapping	an Illumina ID mapping library
...	other parameters of <a href="#">nuID2IlluminaID</a>

## Details

The function will call [nuID2IlluminaID](#) when ID mapping library were provided.

## Value

see function [nuID2IlluminaID](#)

## Author(s)

Pan Du

**References**

Du, P., Kibbe, W.A. and Lin, S.M., "nuID: A universal naming schema of oligonucleotides for Illumina, Affymetrix, and other microarrays", *Biology Direct* 2007, 2:16 (31May2007).

**See Also**

[targetID2nuID](#), [nuID2IlluminaID](#)

**Examples**

```
if (require(lumiHumanIDMapping)) {
  nuID2targetID("B2J6WGHV.Rev0JYff4", lib.mapping = "lumiHumanIDMapping")
}
```

---

pairs-methods

*Pair plot of an ExpressionSet object*

---

**Description**

Creating [pairs](#) plot of sample intensities in an ExpressionSet object

**Usage**

```
## S4 method for signature ExpressionSet
pairs(x, ..., smoothScatter = FALSE, logMode = TRUE, subset = 5000, fold=2, dotColor=1,
highlight = NULL, highlightColor = 2, main = NULL, checkTransform = TRUE)
```

**Arguments**

x	a <a href="#">ExpressionSet</a> object
...	optional arguments to <a href="#">pairs</a> .
smoothScatter	whether use <a href="#">smoothScatter</a> function to plot points
logMode	whether plot the data in log2 scale
subset	subset of rows used to plot. It can be an index vector, or the length of a random subset
fold	The fold-change threshold used to estimate the number of probes having high fold-changes
dotColor	color of points in the scatter plot
highlight	the subset dots need to be highlighted
highlightColor	the color for those highlighted dots
main	title of the plot
checkTransform	whether to check the data is log2-transformed or not

**Details**

To increase the plot efficiency, by default, we only plot RANDOMLY selected subset of points (based on parameter "subset"). If users want to plot all the points, they can set the parameter "subset = NULL". When smoothScatter is set as TRUE, the subsetting will be suppressed because [smoothScatter](#) function has good plot efficiency for large number of points.

**See Also**

[LumiBatch-class](#), [pairs](#)

**Examples**

```
## load example data
data(example.lumi)

pairs(example.lumi)

pairs(example.lumi, smoothScatter=TRUE)
```

---

plot-methods

*Plot of a ExpressionSet object*

---

**Description**

Creating quality control plots of a ExpressionSet object

**Usage**

```
## S4 method for signature ExpressionSet,missing
plot(x, what = c("density", "boxplot", "pair", "MAplot", "sampleRelation", "outlier", "cv"), main, ...)
```

**Arguments**

x	a ExpressionSet object returned by <a href="#">lumiQ</a>
what	one of the six kinds of QC plots
main	the title of the QC plot
...	additional parameters for the corresponding QC plots

**Details**

The parameter "what" of plot function controls the type of QC plots, which includes:

- **density**: the density plot of the chips, see [hist-methods](#)
- **boxplot**: box plot of the chip intensities, see [boxplot-methods](#)
- **pair**: the correlation among chips, plot as a hierarchical tree, see [pairs-methods](#)
- **MAplot**: the MAplot between chips, see [MAplot-methods](#)



- **sampleRelation**: plot the sample relations. See [plotSampleRelation](#)
- **outlier**: detect the outliers based on the sample distance to the center. See [detectOutlier](#)
- **cv**: the density plot of the coefficients of variance of the chips. See [estimateLumiCV](#)

### See Also

[LumiBatch-class](#), [hist-methods](#), [boxplot-methods](#), [MAplot-methods](#), [pairs-methods](#), [plotSampleRelation](#), [estimateLumiCV](#), [detectOutlier](#)

### Examples

```
## load example data
data(example.lumi)

## Quality control estimation
lumi.Q <- lumiQ(example.lumi)

## summary
summary(lumi.Q)

## plot the density
plot(lumi.Q, what=density)

## plot the pairwise sample correlation
plot(lumi.Q, what=pair)

## plot the pairwise MAplot
plot(lumi.Q, what=MAplot)

## sample relations
plot(lumi.Q, what=sampleRelation, method=mds, color=c(100US, 95US:5P, 100US, 95US:5P))

## detect outlier based on the distance to the mean profile
plot(lumi.Q, what=outlier)

## Density plot of coefficient of variance
plot(lumi.Q, what=cv)
```

---

plotCDF

*plot the cumulative distribution function of a ExpressionSet object*

---

### Description

plot the cumulative distribution function of a ExpressionSet object from high to low value or in reverse

**Usage**

```
plotCDF(x, reverse=TRUE, logMode=TRUE, xlab = NULL, ylab = "Cumulative density",
col=1:dim(x)[2], lwd=1, xlim = NULL, index.highlight = NULL, color.highlight = 2,
addLegend = TRUE, main="", ...)
```

**Arguments**

x	a numeric or <a href="#">ExpressionSet</a> object
reverse	determine whether plot the CDF from high to low value or not
logMode	determine whether the CDF plot is based on a log2 scale
xlab	xlab of the plotCDF plot
ylab	ylab of the plotCDF plot
col	line colors of the plotCDF plot
lwd	line width of plot function
xlim	parameter of the plot function
index.highlight	the column index of the highlighted plotCDF curve
color.highlight	color of highlighted plotCDF curve
addLegend	whether add legend to the plot or not
main	title for the plot
...	additional parameters for <a href="#">plot.ecdf</a> function

**See Also**

[LumiBatch-class](#), [ecdf](#)

**Examples**

```
## load example data
data(example.lumi)

plotCDF(example.lumi)
```

---

plotColorBias1D

*Plot the color bias density plot of Illumina Infinium Methylation data*

---

**Description**

Plot the color bias density plot of Illumina Infinium Methylation data in one dimension (comparing with 2D scatter plot)

**Usage**

```
plotColorBias1D(methyLumiM, channel = c("both", "unmethy", "methy", "sum"), colorMode=TRUE, removeGend
```

**Arguments**

methyLumiM	MethyLumiM-class object or eSet-class object, which include methylated and unmethylated probe intensities
channel	estimate the intensity in different methods
colorMode	whether separate two color channels or not
removeGenderProbes	determine whether exclude probes on X and Y chromosomes if the chromosome information is provided in the methyLumiM object.
logMode	Whether plot the intensities in log-scale
subset	plot subset of randomly selected rows. All data will be plotted if it is NULL.
...	other parameters used by <a href="#">density</a> and <a href="#">plot</a>

**Details**

Plot the color bias density plot of Illumina Infinium Methylation data. There are four options using "channel" parameter to plot the density plot. "both": estimate the density by pooling together methylated and unmethylated probe intensities. "unmethy" and "methy": plot either unmethylated or methylated probe density. "sum" plot the density of the sum of methylated and unmethylated probe intensities.

**Value**

Invisibly return TRUE if plot successfully.

**Author(s)**

Pan DU

**See Also**

See Also as [plotColorBias2D](#) and [boxplotColorBias](#)

**Examples**

```
data(example.lumiMethy)
# before adjustment
plotColorBias1D(example.lumiMethy)
```

---

plotColorBias2D	<i>Plot the color bias of Illumina Infinium Methylation data in two dimensions</i>
-----------------	--

---

### Description

Plot the color bias (red and green channel) of Illumina Infinium Methylation data of one selected sample in two dimensions (methylated and unmethylated probe intensities)

### Usage

```
plotColorBias2D(methyLumiM, selSample = 1, combineMode = F, layoutRatioWidth = c(0.75, 0.25), layoutRat
```

### Arguments

methyLumiM	MethyLumiM-class object or eSet-class object, which include methylated and unmethylated probe intensities
selSample	The index of sample name of the selected sample to plot color bias
combineMode	Whether combine two color channels together and plot as one color
layoutRatioWidth	the plot figure ratio between scatter plot and density plot
layoutRatioHeight	the plot figure ratio between scatter plot and density plot
margins	margin of the plot
cex	A numerical value giving the amount by which plotting text and symbols should be magnified relative to the default. See <a href="#">par</a>
logMode	Whether plot the intensities in log-scale
subset	plot subset of randomly selected rows. All data will be plotted if it is NULL.
...	other parameters used by <a href="#">plot</a>

### Details

The function basically plots the probe intensities in 2-dimension (methylated vs unmethylated), and colors the dots in Red and Green based on their color channel information. The related density plot will also be plotted at the right and top of the scatter plot.

### Value

Invisibly return TRUE if plot successfully.

### Author(s)

Pan DU

**See Also**

See Also as [plotColorBias1D](#)

**Examples**

```
data(example.lumiMethy)
# plot in 2D plot of one selected sample
plotColorBias2D(example.lumiMethy, selSample = 1)
```

---

plotControlData	<i>Plot the mean expression (with standard deviation bar) of different type of control probes</i>
-----------------	---

---

**Description**

Plot the mean intensity (with standard deviation bar) of different type of control probes. Multiple control types can be plotted in a single plot. The available control types can be get by running `getControlType(controlData)`.

**Usage**

```
plotControlData(controlData, type = NULL, slideIndex = NULL, logMode = FALSE, new = TRUE, ...)
```

**Arguments**

controlData	a LumiBatch object including control data, a control data data.frame, a MethyLumiQC object or a MethyLumiM object including MethyLumiQC control data
type	the control probe type (case insensitive), which can be get by running <code>getControlType(controlData)</code>
slideIndex	the slide index or ID corresponding to each sample
logMode	whether show the data in log2 scale
new	whether refresh the new plot or add it on the old one
...	other parameters used by default plot function

**Details**

When multiple control types are selected, they will be plotted in a two-column plot. For methylation data, the red and green channels will be plotted respectively in red and green colors.

**Value**

plot the picture and invisibly return TRUE if everything is OK

**Author(s)**

Pan Du

**See Also**

[addControlData2lumi](#) and [addControlData2methyLumiM](#)

**Examples**

```
controlFile <- system.file(doc, Control_Probe_Profile.txt, package=lumi)
controlData <- getControlData(controlFile)
getControlType(controlData)
plotControlData(controlData, type=NEGATIVE)
```

---

<code>plotDensity</code>	<i>plot the density distribution</i>
--------------------------	--------------------------------------

---

**Description**

plot the density distribution of a dataMatrix or ExpressionSet object

**Usage**

```
plotDensity(dataMatrix, logMode=TRUE, addLegend=TRUE, legendPos="topright", subset = NULL, ...)
```

**Arguments**

<code>dataMatrix</code>	a data matrix or <a href="#">ExpressionSet</a> object
<code>logMode</code>	determine whether the CDF plot is based on a log2 scale
<code>addLegend</code>	whether add legend to the plot or not
<code>legendPos</code>	the position of the legend. If its length is two, then it specifies the x and y location of legend.
<code>subset</code>	plot subset of randomly selected rows. All data will be plotted if it is NULL.
<code>...</code>	additional parameters for <a href="#">matplot</a> function

**See Also**

[LumiBatch-class](#), [density](#)

**Examples**

```
## load example data
data(example.lumi)

plotDensity(example.lumi)
```

---

plotGammaFit                    *plot the fitting results of [gammaFitEM](#)*

---

### Description

plot the fitting results of [gammaFitEM](#). It basically plot the histogram of M-values plus the estimated gamma density plots and their mixture.

### Usage

```
plotGammaFit(x, gammaFit = NULL, k = NULL, theta = NULL, shift = NULL, proportion = NULL, plotType = c("h
```

### Arguments

x	a vector of M-values covering the whole genome
gammaFit	a "gammaFit" class object returned by <a href="#">gammaFitEM</a>
k	parameter k of gamma distribution
theta	parameter theta of gamma distribution
shift	parameter shift of gamma distribution
proportion	the proportion of two components (gamma distributions)
plotType	determine the way to show the distribution of the input data, either histogram or density plot
...	Other parameters used by <a href="#">hist</a> or <a href="#">plot</a> (for "density" plotType) function.

### Details

This function is to visualize the fitting results, which helps us understand how well the fitting is.

### Value

Invisibly return TRUE if the plot is successful.

### Author(s)

Pan DU

### See Also

[gammaFitEM](#)

### Examples

```
data(example.lumiMethy)
M <- exprs(example.lumiMethy)
fittedGamma <- gammaFitEM(M[,1], initialFit=NULL, maxIteration=50, tol=0.0001, plotMode=FALSE, verbose=FALSE)
plotGammaFit(M[,1], gammaFit=fittedGamma)
```

---

plotHousekeepingGene *Plot the housekeeping gene expression profile*

---

**Description**

Plot the housekeeping gene expression profile

**Usage**

```
plotHousekeepingGene(controlData, lib = NULL, slideIndex = NULL, addLegend = TRUE, logMode = TRUE, ...)
```

**Arguments**

controlData	a LumiBatch object including control data or a control data data.frame
lib	the annotation library (for retrieving the gene name)
slideIndex	the slide index or ID corresponding to each sample
addLegend	whether add legend or not
logMode	whether show the data in log2 scale
...	other parameters used by default matplot function

**Value**

plot the picture and return TRUE if everything is OK

**Author(s)**

Pan Du

**See Also**

[addControlData2lumi](#), [plotControlData](#)

**Examples**

```
controlFile <- system.file(doc, Control_Probe_Profile.txt, package=lumi)
controlData <- getControlData(controlFile)
plotHousekeepingGene(controlData)
```



---

plotSampleRelation     *visualize the sample relations*

---

**Description**

plot the sample relations based on MDS or hierarchical clustering

**Usage**

```
plotSampleRelation(x, subset = NULL, cv.Th = 0.1, standardize = TRUE, method = c("cluster", "mds"), dimension = 2, color = "black", main = "", ...)
```

**Arguments**

x	a LumiBatch object, ExpressionSet object or a matrix with each column corresponding to a sample
subset	the subset probes used to determine the sample relations. If it is one number, then randomly selected "number" of probes will be used. If not provide, all the probes will be used.
cv.Th	the threshold of the coefficient of variance of probes used to select probes to estimate sample relations
standardize	standardize the expression profiles or not
method	"MDS" or "hierarchical clustering"
dimension	the principle components to visualize the MDS plot
color	the color for each sample during plot. Only support the "mds" method
main	the title of the plot
...	Other parameters used by plot function.

**Details**

Estimate the sample relations based on selected probes (based on large coefficient of variance (mean / standard variance)). Two methods can be used: MDS (Multi-Dimensional Scaling) or hierarchical clustering methods.

**Value**

Invisibly return the hierarchical clustering results (if 'cluster' method used) or coordinates of the mds plot (if 'mds' method used) .

**Author(s)**

Pan Du

**See Also**

[lumiQ](#), [LumiBatch](#), [plot, ExpressionSet-method](#)

## Examples

```
## load example data
data(example.lumi)

## plot the sample relations with MDS
## the color of sample is automatically set based on the sample type
plotSampleRelation(example.lumi, col=c(100US, 95US:5P, 100US, 95US:5P))

## plot the sample relations with hierarchical clustering
plotSampleRelation(example.lumi, method=cluster)
```

---

plotStringencyGene      *plot the Stringency related control probe profiles*

---

## Description

Plot the Stringency related control probe (Low-Stringency, Medium-Stringency and High-Stringency) profiles. Using getControlType function to view available stringency types.

## Usage

```
plotStringencyGene(controlData, lib = NULL, slideIndex = NULL, addLegend = TRUE, logMode = TRUE, ...)
```

## Arguments

controlData	a LumiBatch object including control data or a control data data.frame
lib	the annotation library (for retrieving the gene name)
slideIndex	the slide index or ID corresponding to each sample
addLegend	whether add legend or not
logMode	whether show the data in log2 scale
...	other parameters used by default matplot function

## Value

plot the picture and return TRUE if everything is OK

## Author(s)

Pan Du

## See Also

[addControlData2lumi](#), [plotControlData](#)

**Examples**

```
controlFile <- system.file(doc, Control_Probe_Profile.txt, package=lumi)
controlData <- getControlData(controlFile)
plotStringencyGene(controlData)
```

---

plotVST

*plot the VST (Variance Stabilizing Transform) function*

---

**Description**

plot the VST (Variance Stabilizing Transform) function of VST transformed LumiBatch object or parameters of VST function.

**Usage**

```
plotVST(x, transFun = NULL, plotRange = NULL, addLegend = TRUE, ...)
```

**Arguments**

x	a LumiBatch object after lumiT transform, or a matrix or data.frame with VST parameter
transFun	a character vector of transformation function (asinh or log2)
plotRange	the plot range of untransformed data
addLegend	add legend or not
...	other parameter used by <a href="#">plot</a> function

**Value**

invisibly return the untransformed and transformed values.

**Author(s)**

Pan Du

**See Also**

[vst](#)

**Examples**

```
## load example data
data(example.lumi)

## Do default VST variance stabilizing transform
lumi.T <- lumiT(example.lumi, ifPlot=TRUE)
```

```
## plot the transform function
plotVST(lumi.T)
```

---

probeID2nuID	<i>Mapping Illumina ProbeID as nuID</i>
--------------	---

---

### Description

Mapping Illumina ProbeID as nuID.

### Usage

```
probeID2nuID(probeID, lib.mapping = "lumiHumanIDMapping", ...)
```

### Arguments

probeID	a vector of Illumina ProbeID
lib.mapping	an Illumina ID mapping library
...	other parameters of <a href="#">IlluminaID2nuID</a>

### Details

The function will call [IlluminaID2nuID](#) when ID mapping library were provided.

### Value

see function [IlluminaID2nuID](#)

### Author(s)

Pan Du

### References

Du, P., Kibbe, W.A. and Lin, S.M., "nuID: A universal naming schema of oligonucleotides for Illumina, Affymetrix, and other microarrays", *Biology Direct* 2007, 2:16 (31May2007).

### See Also

[nuID2probeID](#), [IlluminaID2nuID](#)

### Examples

```
if (require(lumiHumanIDMapping)) {
  probeID2nuID(0001240020, lib=lumiHumanIDMapping)
}
```

---

`produceGEOPlatformFile`*Produce GEO Platform Submission File in SOFT format*

---

**Description**

Produce GEO Sample Submission File in SOFT format based on the provided LumiBatch object and Illumina ID Mapping library

**Usage**

```
produceGEOPlatformFile(x.lumi, lib.mapping = NULL, nuIDMode = TRUE, includeAllChipProbe=FALSE, fileName
```

**Arguments**

<code>x.lumi</code>	The LumiBatch object keeping all probes
<code>lib.mapping</code>	The Illumina ID Mapping library, e.g., "lumiHumanIDMapping"
<code>nuIDMode</code>	Determine whether producing the platform indexed by nuID
<code>includeAllChipProbe</code>	Determine whether including all probes in the Manifest file or just the probes used in the x.lumi object
<code>fileName</code>	Filename of the GEO Platform File name

**Details**

The function produces the GEO platform submission file based on the chip information kept in the Illumina ID Mapping library (specified by `lib.mapping` parameter). The determination of chip type will be automatically done by selecting the best matching of the probe IDs with individual chips.

**Value**

Save the result as a text file in SOFT platform submission format.

**Author(s)**

Pan Du

**References**

<http://www.ncbi.nlm.nih.gov/projects/geo/info/soft2.html>

**See Also**

[produceGEOSubmissionFile](#)

## Examples

```
# data(example.lumi)
# produceGEOPlatformFile(example.lumi, lib.mapping=lumiHumanIDMapping)
```

---

```
produceGEOSampleInfoTemplate
```

*Produce the template of GEO sample information*

---

## Description

Produce the template of GEO sample information, which is used for function [produceGEOSubmissionFile](#).

## Usage

```
produceGEOSampleInfoTemplate(lumiNormalized, lib.mapping = NULL, fileName = "GEOsampleInfo.txt")
```

## Arguments

lumiNormalized	The normalized data (LumiBatch object)
lib.mapping	The Illumina ID Mapping library, e.g., "lumiHumanIDMapping"
fileName	The file name of Tab separated sample information file

## Details

This function just produces a template of sample information with some default fillings. Users need to fill in the detailed sample descriptions, especially the Sample\\_title, Sample\\_description and some protocols. No blank fields are allowed. Function [produceGEOSubmissionFile](#) will produce the file GEO submission file based on this sample information. The users should not use "\#" in the description as it is a reserved character.

## Value

Save the result as a Tab separated text file or return a data.frame if the fileName is NULL.

## Author(s)

Pan Du

## References

<http://www.ncbi.nlm.nih.gov/projects/geo/info/soft2.html>

## See Also

[produceGEOSubmissionFile](#)

---

 produceGEOSubmissionFile

*Produce GEO Sample Submission File in SOFT format*


---

### Description

Produce GEO Sample Submission File in the SOFT format based on the provided LumiBatch object and sample information

### Usage

```
produceGEOSubmissionFile(lumiNormalized, lumiRaw, lib.mapping = NULL, idType = Probe, sampleInfo = NULL)
```

### Arguments

lumiNormalized	The normalized data (LumiBatch object)
lumiRaw	The raw data (LumiBatch object), e.g., returned by <a href="#">lumiR</a>
lib.mapping	The Illumina ID Mapping library, e.g., "lumiHumanIDMapping"
idType	the idType parameter of function <a href="#">nuID2IlluminaID</a>
sampleInfo	The sample information filename or data.frame, which is returned by <a href="#">produceGEOSampleInfoTemplate</a>
fileName	The file name of GEO Submission file
supplementaryRdata	determine whether produce the Rdata supplement data, which include both lumiNormalized and lumiRaw R objects.
...	other parameters used by function <a href="#">nuID2IlluminaID</a>

### Details

The function produces the GEO sample submission file including both normalized and raw data information in the SOFT format. The sample information should be provided by the user as a data.frame or Tab separated text file following the format of the template, which can be produced by function [produceGEOSampleInfoTemplate](#). Users need to fill in the detailed sample descriptions in the template, especially the Sample\\_title, Sample\\_description and some protocols. Users are also required to fill in the "Sample\\_platform\\_id" by checking information of the GEO Illumina platform.

When the parameter "supplementaryRdata" is TRUE, the R objects, lumiNormalized, lumiRaw and sampleInfo, will be saved in a file named 'supplementaryData.Rdata'.

### Value

Save the result as a text file in SOFT sample submission format. The supplementary Rdata will be saved in a file 'supplementaryData.Rdata'.

### Author(s)

Pan Du

**References**

<http://www.ncbi.nlm.nih.gov/projects/geo/info/soft2.html>

**See Also**

[produceGEOSampleInfoTemplate](#), [produceGEOPlatformFile](#)

**Examples**

```
## Not run
## Produce the sample information template
# produceGEOSampleInfoTemplate(lumiNormalized, lib.mapping = NULL, fileName = "GEOsampleInfo.txt")
## After editing the GEOsampleInfo.txt by filling in sample information
# produceGEOSubmissionFile(lumiNormalized, lumiRaw, lib=lumiHumanIDMapping, sampleInfo=GEOsampleInfo.txt)
```

---

produceMethylationGEOSubmissionFile

*Produce GEO Sample Submission File of Illumina methylation microarray data in SOFT format*

---

**Description**

Produce GEO Sample Submission File in the SOFT format based on the provided MethyLumiM object and sample information

**Usage**

```
produceMethylationGEOSubmissionFile(methyLumiM, methyLumiM.raw = NULL, lib.mapping = NULL, idType = "P")
```

**Arguments**

methyLumiM	The normalized data in MethyLumiM class
methyLumiM.raw	The raw data in MethyLumiM class
lib.mapping	Currently not used for Illumina methylation data
idType	Currently no other options for Illumina methylation data
sampleInfo	The sample information filename or data.frame, which is returned by <a href="#">produceGEOSampleInfoTemplate</a>
fileName	The file name of GEO Submission file
supplementaryRdata	determine whether produce the Rdata supplement data, which include both methyLumiM and methyLumiM.raw R objects.
...	other parameters used by function <a href="#">nuID2IlluminaID</a> , but not implemented for methylation data



## Details

The function produces the GEO sample submission file including both normalized and raw data information in the SOFT format. The sample information should be provided by the user as a data.frame or Tab separated text file following the format of the template, which can be produced by function [produceGEOSampleInfoTemplate](#). Users need to fill in the detailed sample descriptions in the template, especially the Sample\\_title, Sample\\_description and some protocols. Users are also required to fill in the "Sample\\_platform\\_id" by checking information of the GEO Illumina platform.

When the parameter "supplementaryRdata" is TRUE, the R objects, methyLumiM, methyLumiM.raw and sampleInfo, will be saved in a file named 'supplementaryData.Rdata'.

## Value

Save the result as a text file in SOFT sample submission format. The supplementary Rdata will be saved in a file 'supplementaryData.Rdata'.

## Author(s)

Pan Du

## References

<http://www.ncbi.nlm.nih.gov/projects/geo/info/soft2.html>

## See Also

[produceGEOSampleInfoTemplate](#), [produceGEOPlatformFile](#)

## Examples

```
## Not run
## Produce the sample information template
# produceGEOSampleInfoTemplate(methyLumiM, fileName = "GEOsampleInfo.txt")
## After editing the GEOsampleInfo.txt by filling in sample information
# produceMethylationGEOSubmissionFile(methyLumiM, methyLumiM.raw, sampleInfo=GEOsampleInfo.txt)
```

---

rankinvariant

*Rank Invariant Normalization*

---

## Description

This function basically adjusts the samples to the same background level and then optionally scales to the same foreground level.

## Usage

```
rankinvariant(x.lumi, targetArray = NULL, rrc = .05, lowRank = seq(.5, .25, -.05), highRank = .9, minSiz
```

## Arguments

<code>x.lumi</code>	an ExpressionSet inherited object or a data matrix with columns as samples and rows as genes
<code>targetArray</code>	A target chip is the model for other chips to normalize. It can be a column index, a vector or a LumiBatch object with one sample.
<code>rrc</code>	The relative rank change allowed for a gene to be selected as rank invariant
<code>lowRank</code>	A vector with, in decreasing order, the minimum ranks where candidate genes can be selected as rank invariant
<code>highRank</code>	The maximum rank where candidate genes can be selected as rank invariant
<code>minSize</code>	Fraction of genes required to be selected as rank invariant
<code>maxit</code>	Maximum number of iterations for <code>rlm</code> to reach convergence

## Details

Rank invariant normalization uses a set of genes that are rank invariant between a given sample and a target sample. The target sample can be predefined by setting the `targetArray` argument. If `targetArray` is NULL the average expression of all samples will be the target. Rank invariant genes are found for each sample separately by calculation the relative rank change for each gene. Furthermore, only genes with ranks between the `lowRank` and `highRank` are considered. If the number of probes is less than `minSize` multiplies by the number of genes the next `lowRank` value tried. If no rank invariant set can be found an error is thrown.

The default settings of this function are the same as used Genomstudio (Illumina). The results produced by this method are similar, but not identical to Genomstudio.

## Value

Return an object with expression values normalized. The class of the return object is the same as the input object `x.lumi`.

## Author(s)

Arno Velds (contact: a.velds (at) nki.nl)

## See Also

[lumiN](#)

---

rsn

*Robust Spline Normalization between chips*

---

## Description

Robust spline normalization (monotonic curves) between chips

## Usage

```
rsn(x.lumi, targetArray = NULL, excludeFold = 2, span = 0.03, ifPlot = FALSE, ...)
```

## Arguments

<code>x.lumi</code>	an ExpressionSet inherited object or a data matrix with columns as samples and rows as genes
<code>targetArray</code>	A target chip is the model for other chips to normalize. It can be a column index, a vector or a LumiBatch object with one sample.
<code>excludeFold</code>	exclude the genes with fold change larger than "excludeFold" during fitting the curve in normalization
<code>span</code>	the span parameter used by <a href="#">monoSmu</a>
<code>ifPlot</code>	determine whether to plot intermediate results
<code>...</code>	other parameters used by <a href="#">monoSmu</a>

## Details

The robust spline normalization (RSN) algorithm combines the features of quantile and loess normalization. It is designed to normalize the variance-stabilized data. The function will check whether the data is variance stabilized (vst or log2 transform), if not, it will automatically run `lumiT` before run `rsn`. For details of the algorithm, please see the reference.

The `targetArray` can be a column index, a vector or a LumiBatch object with one sample, which corresponds to an external sample to be normalized with. This is very useful for handling large data set or normalizing the data set with a common reference (`targetArray`).

## Value

Return an object with expression values normalized. The class of the return object is the same as the input object `x.lumi`. If it is a LumiBatch object, it also includes the VST transform function and its parameters as attributes: "transformFun", "parameter". See [inverseVST](#) for details.

## Author(s)

Pan Du, Simon Lin

## See Also

[lumiN](#), [monoSmu](#)

---

`seq2id`*Transfer a nucleotide sequence as a nuID*

---

**Description**

The nuID (nucleotide universal identifier) is uniquely corresponding to probe sequence. The nuID is also self-identification and error checking

**Usage**

```
seq2id(seq)
```

**Arguments**

`seq` a nucleotide sequence composed of A, C, G, T (U).

**Details**

The nuID is a exact mapping of nucleotide sequence based on Base64 encoding scheme. A character set A-Z, a-z, 0-9, "\\_" and "." is used to represent to the base-64 numbers of 0-63. The first character of nuID is a checking code, which provide information of both the number of padded "A"s at the nucleotide sequence and error checking. Please refer to reference for more details.

**Value**

A string represents nuID

**Author(s)**

Pan Du

**References**

Du, P., Kibbe, W.A. and Lin, S.M., "nuID: A universal naming schema of oligonucleotides for Illumina, Affymetrix, and other microarrays", *Biology Direct* 2007, 2:16 (31May2007).

**See Also**

[id2seq](#)

**Examples**

```
seq <- ACGTAAATTCAGTTTAAACCCCGG
id <- seq2id(seq)
id
id2seq(id)
```

---

smoothQuantileNormalization  
*Smooth quantile normalization*

---

**Description**

Smooth quantile normalization with a reference sample

**Usage**

```
smoothQuantileNormalization(dataMatrix, ref = NULL, adjData=NULL, logMode = TRUE, bandwidth = NULL, deg
```

**Arguments**

dataMatrix	a matrix of microarray intensity data
ref	a vector of reference sample intensity, which matches the dataMatrix
adjData	data to be adjusted based on the ref and dataMatrix distribution
logMode	whether perform the analysis in log2 scale
bandwidth	a parameter used by <a href="#">locpoly</a>
degree	a parameter used by <a href="#">locpoly</a>
verbose	whether print the processing sample names
...	other parameters used by <a href="#">locpoly</a>

**Value**

a data matrix with intensity normalized.

**Author(s)**

Pan DU

**See Also**

See Also [adjColorBias.quantile](#)

---

 ssn

*Simple Scaling Normalization*


---

**Description**

This function basically adjusts the samples to the same background level and then optionally scales to the same foreground level.

**Usage**

```
ssn(x.lumi, targetArray = NULL, scaling = TRUE, bgMethod=c(density, mean, median, none), fgMethod=c(mea
```

**Arguments**

<code>x.lumi</code>	an ExpressionSet inherited object or a data matrix with columns as samples and rows as genes
<code>targetArray</code>	A target chip is the model for other chips to normalize. It can be a column index, a vector or a LumiBatch object with one sample.
<code>scaling</code>	determine whether do scaling or just background shift
<code>bgMethod</code>	optional methods of determining the background level
<code>fgMethod</code>	optional methods of determining the foreground level
<code>...</code>	other parameters used by <a href="#">density</a> function

**Details**

This function basically adjusts the samples to the same background level and then optionally scales to the same foreground level. The adjustment is based on the raw scale data (For the transformed data, it still estimates the parameters in the raw scale by inverse transformation.).

Comparing with other normalization methods, like quantile and curve-fitting methods, SSN is a more conservative method. The only assumption is that each sample has the same background levels and the same scale (if do scaling). There are three methods ('density', 'mean' and 'median') for background estimation. If `bgMethod` is 'none', then the background level will be set as 0, i.e., no background adjustment. For the 'density' `bgMethod`, it estimates the background based on the mode of probe intensities based on the assumption that the background level intensity is the most frequent value across all the probes in the chip. For the foreground level estimation, it also provides three methods ('mean', 'density', 'median'). For the 'density' `fgMethod`, it assumes the background probe levels are symmetrically distributed. Then we estimate the foreground levels by taking the intensity mean of all other probes except from the background probes. For the 'mean' and 'median' methods (for both `bgMethod` and `fgMethod`), it basically estimates the level based on the mean or median of all probes of the sample. If the `fgMethod` is the same as `bgMethod` (except 'density' method), no scaling will be performed.

**Value**

Return an object with expression values normalized. The class of the return object is the same as the input object `x.lumi`.

**Author(s)**

Pan Du, Simon Lin

**See Also**

[lumiN](#)

---

targetID2nuID

*Mapping Illumina TargetID (GeneID) into nuID*

---

**Description**

Mapping Illumina TargetID (GeneID) into nuID.

**Usage**

```
targetID2nuID(targetID, lib.mapping = "lumiHumanIDMapping", ...)
```

**Arguments**

targetID	a vector of Illumina TargetID (GeneID)
lib.mapping	an Illumina ID mapping library
...	other parameters of <a href="#">IlluminaID2nuID</a>

**Details**

The function will call [IlluminaID2nuID](#) when ID mapping library were provided.

**Value**

see function [IlluminaID2nuID](#)

**Author(s)**

Pan Du

**References**

Du, P., Kibbe, W.A. and Lin, S.M., "nuID: A universal naming schema of oligonucleotides for Illumina, Affymetrix, and other microarrays", *Biology Direct* 2007, 2:16 (31May2007).

**See Also**

[nuID2targetID](#), [IlluminaID2nuID](#)

**Examples**

```
if (require(lumiHumanIDMapping)) {
  targetID2nuID(GI_21389350-S, lib=lumiHumanIDMapping)
}
```

vst

*Variance Stabilizing Transformation***Description**

Stabilizing the expression variance based on the bead level expression variance and mean relations

**Usage**

```
vst(u, std, nSupport = min(length(u), 500), backgroundStd=NULL, fitMethod = c(linear, quadratic), lowCu
```

**Arguments**

u	mean expression of the beads with same sequence
std	expression standard deviation of the beads with same sequence
nSupport	the number of down-sampling to speed processing
backgroundStd	pre-estimated background standard deviation level
fitMethod	methods of fitting the relations between expression variance and mean relations
lowCutoff	cutoff ratio to determine the low expression range. Do not change this until you now what you are doing.
ifPlot	plot intermediate results or not

**Details**

The variance-stabilizing transformation (VST) takes the advantage of larger number of technical replicates available on the Illumina microarray. It models the mean-variance relationship of the within-array technical replicates at the bead level of Illumina microarray. An arcsinh transform is then applied to stabilize the variance. See reference for more details.

For the methods of fitting the relations between expression variance and mean relations, the 'linear' method is more robust and provides detailed parameters for inverseVST.

**Value**

Return the transformed (variance stabilized) expression values.

**Author(s)**

Pan Du, Simon Lin



## References

Lin, S.M., Du, P., Kibbe, W.A., "Model-based Variance-stabilizing Transformation for Illumina Mi-croarray Data", submitted

## See Also

[lumiT](#), [inverseVST](#)

## Examples

```
## load example data
data(example.lumi)

## get the gene expression mean for one chip
u <- exprs(example.lumi)[,1]
## get the gene standard deviation for one chip
std <- se.exprs(example.lumi)[,1]

## do variance stabilizing transform
transformedU <- vst(u, std)

## do variance stabilizing transform with plotting intermediate result
transformedU <- vst(u, std, ifPlot=TRUE)
```

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