Package 'HTqPCR'

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Type Package

Title Automated analysis of high-throughput qPCR data

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Description Analysis of Ct values from high throughput quantitative real-time PCR (qPCR) assays across multiple conditions or replicates. The input data can be from spatially-defined formats such ABI TaqMan Low Density Arrays or OpenArray; LightCycler from Roche Applied Science; the CFX plates from Bio-Rad Laboratories; conventional 96- or 384-well plates; or microfluidic devices such as the Dynamic Arrays from Fluidigm Corporation. HTqPCR handles data loading, quality assessment, normalization, visualization and parametric or non-parametric testing for statistical significance in Ct values between features (e.g. genes, microRNAs).

Reference Heidi Dvinge and Paul Bertone (2009). HTqPCR: High-throughput analysis and visualization of quantitative real-time PCR data in R Bioinformatics.

License Artistic-2.0

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HTqPCR-package *Analysis of High-Throughput qPCR data (HTqPCR)*

Description

This package is for analysing high-throughput qPCR data. Focus is on data from Taqman Low Density Arrays, but any kind of qPCR performed across several samples is applicable. Cycle threshold (Ct) data from different cards (samples) is read in, normalised, processed and the genes are tested for differential expression across different samples. Results are visualised in various ways.

Details

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Author(s)

Maintainer: Heidi Dvinge <hdvinge@fredhutch.org> Maintainer: Paul Bertone <bertone@ebi.ac.uk>

cbind *Combine qPCRset objects*

Description

Functions for combining multiple `qPCRset` objects into one, by either adding columns (samples) or rows (features).

Usage

```
## S3 method for class 'qPCRset'
cbind(..., deparse.level = 1)
## S3 method for class 'qPCRset'
rbind(..., deparse.level = 1)
```

Arguments

`...`	`qPCRset` objects that are to be combined.
`deparse.level`	not implemented currently. See `cbind`.

Details

In some cases it might be desirable to merge multiple qPCRset objects, that have been read into R or processed individually. This can be done for either identical samples across multiple different cards (such as a 384 well plate), or if more samples have been run on cards with the same layout.

cbind combines data assuming that all experiments have been carried out on identical cards, i.e. that featureNames, featureType, featurePos and featureClass is identical across all the qPCRset objects. rbind combines data assuming that the same samples have been analysed using different qPCR cards.

For both functions, the getCtHistory of all the individual objects will be added to the combined qPCRset.

Value

A combined qPCRset object.

Author(s)

Heidi Dvinge

See Also

cbind

---

changeCtLayout *Changing the dimensions (rows x columns) of qPCRset objects*

Description

A function for splitting up the individual qPCR cards, in case there are multiple samples present on each card. I.e. for cases where the layout isn't 1 sample x 384 features, but for example 4 samples x 96 features on each 384 well card.

Usage

```
changeCtLayout(q, sample.order)
```

Arguments

q	a qPCRset object.
sample.order	vector, same length as number of features on each card (e.g. 384). See details.

Details

The result from each qPCR run of a given card typically gets presented together, such as in a file with 384 lines, one per feature, for 384 well plates. However, some cards may contain multiple samples, such as commercial cards that are designed to be loaded with two separate samples and then include 192 individual features.

Per default, each card is read into the `qPCRset` object as consisting of a single sample, and hence one column in the Ct data matrix. When this is not the case, the data can subsequently be split into the correct features x samples (rows x columns) dimensions using this function. The parameter `sample.order` is a vector, that for each feature in the `qPCRset` indicates what sample it actually belongs to.

In the new `qPCRset` the samples (Ct columns) are ordered first by `sample.order` then by the original `sampleNames`, as shown in the examples below.

Value

A qPCRset object like the input, but with the dimensions changed according to the new layout.

Note

Since the actual biological samples are likely to differ on each card, after applying `changeCtLayout` renaming of the samples in `qPCRset` using `sampleNames` is advisable.

The features are assumed to be identical for all samples on a given card! I.e. if for example `sample.order=rep(c("A", "B"), each=192)`, then feature number 1 (the first for sample A) should be the same as feature number 193 (the first for sample B). The new `featureNames` are taken for those features listed as belonging to the first sample in `sample.order`.

Author(s)

Heidi Dvinge

Examples

```
# Example data
data(qPCRraw)
# With e.g. 2 or 4 samples per 384 well card.
sample2.order <- rep(c("subSampleA", "subSampleB"), each=192)
sample4.order <- rep(c("subA", "subB", "subC", "subD"), each=96)
# Splitting the data into all individual samples
qPCRnew2 <- changeCtLayout(qPCRraw, sample.order=sample2.order)
show(qPCRnew2)
qPCRnew4 <- changeCtLayout(qPCRraw, sample.order=sample4.order)
show(qPCRnew4)
sampleNames(qPCRnew4)
```

clusterCt *Clustering of qPCR Ct values*

Description

Hierarchical clustering of samples or genes from high-throughput qPCR experiments, such as the TaqMan Low Density Array platform. Individual clusters can be selected, and the features within them listed in the given order.

Usage

```
clusterCt(q, main = NULL, type = "genes", dist = "pearson", xlab = "Cluster dendrogram", n.cluster, h.cl
```

Arguments

q	object of class qPCRset.
main	character string, plot title.
type	character string, either "genes" (default) or "samples", indicating what is to be clustered.
dist	character string, specifying whether to use "pearson" correlation (default) or "euclidean" distance for the clustering.
xlab	character string, label for the x-axis.
n.cluster	integer, the number of cluster to divide the dendrogram into. See details.
h.cluster	numeric, the height at which to cut the dendrogram into clusters. See details.
select.cluster	logical, whether to select clusters interactively. See details.
...	any other arguments will be passed to the plot function.

Details

This function may be used to cluster the Ct values and present the result as a dendrogram.

The n.cluster and h.cluster parameters are from the rect.hclust function and can be used to divide the dendrogram into subclusters based on either number of clusters or height of branch, drawing boxes around subclusters. The members of each cluster can be returned (see value). If n.cluster is specified h.cluster will be ignored.

If select.cluster is chosen individual subclusters can be selected and marked by a box by clicking on their highest comment branch with the (first) mouse button. Multiple clusters can be selected until any mouse button other than the first is pressed, and the function can be used in conjunction with either n.cluster or h.cluster. The members of each cluster will likewise be returned, in the order they were selected.

Value

A plot is created on the current graphics device. If any subclusters are marked, these will be returned invisibly in a list, with one component for each subcluster. The individual slots in the list contain the names of the genes, and their position in the original input data (row number).

Author(s)

Heidi Dvinge

See Also

hclust, dist, rect.hclust, identify.hclust

Examples

```
# Load example data
data(qPCRraw)
# Clustering samples
clusterCt(qPCRraw, type="samples")
clusterCt(qPCRraw, type="samples", dist="euclidean")
# Clustering genes
clusterCt(qPCRraw, type="genes", cex=0.5)
clusterCt(qPCRraw, type="genes", h.cluster=1.5, cex=0.5)
cluster.list <- clusterCt(qPCRraw, type="genes", n.cluster=6, cex=0.5)
cluster.list[[1]]
```

---

filterCategory *Filter Ct values based on their feature categories.*

Description

Ct values corresponding to selected feature categories will be replaced by NA. Generally, the feature categories indicate how reliable the values are.

Usage

```
filterCategory(q, na.categories = c("Unreliable", "Undetermined"))
```

Arguments

q	a qPCRset object.
na.categories	character vector, with the name(s) of the feature categories where Ct values will be considered NA.

Value

A qPCRset object like the input, but with the selected Ct values replaced by NAs

Author(s)

Heidi Dvinge

See Also

setCategory for adjusting the categories.

Examples

```
data(qPCRraw)
qPCRraw2 <- setCategory(qPCRraw, groups=NULL)
x <- filterCategory(qPCRraw2)
summary(qPCRraw)
summary(x)
```

`filterCtData` *Filter out features (genes) from qPCR data.*

Description

This function is for filtering Ct data from high-throughput qPCR platforms like the TaqMan Low Density Arrays. This can for example be done prior to analysing the statistical significance of the data, to remove genes where the results are of low quality, or that are not of interest to the analysis in question.

Usage

```
filterCtData(q, remove.type, remove.name, remove.class, remove.category, n.category = 3, remove.IQR, v
```

Arguments

`q`	object of class qPCRset.
`remove.type`	character vector, the feature type(s) to be removed from the data object.
`remove.name`	character vector, the feature name(s) to be removed from the data object.
`remove.class`	character vector, the feature class(es) to be removed from the data object.
`remove.category`	character vector, the features categories(s) to be assessed across samples.
`n.category`	numeric, all features with more than this number of `remove.category` across samples are removed.
`remove.IQR`	numeric, all features with an interquartile range (IQR) below this limit across samples will be removed.
`verbose`	boolean, should some information be printed to the prompt.

Details

This function may be used to exclude individual or small groups of features that are irrelevant to a given analysis. However, it can also be used on a more general basis, to for example split the data into separate `qPCRset` objects based on features with different characteristics, such as groups of markers or other gene classes present in `featureClass`.

`remove.IQR` can be used to exclude features that show only little variation across the samples. These are unlikely to be differentially expressed, so including them in downstream analysis such as `limmaCtData` or `ttestCtData` would result in a slight loss of power caused by the adjustment of p-values required due to multiple testing across all features.

Value

An object of class qPCRset like the input, but with the required features removed.

Note

After removing features the function `plotCtCard` will no longer work, since the number of features is now smaller than the card dimensions.

When using `remove.category` or `remove.IQR` and there are replicated features present on the array, it might no longer be possible to use the `ndups` parameter of `limmaCtData`, since the number of replicates isn't identical for each feature.

Filtering can be performed either before or after normalization, but in some cases normalization might be affected by this, for example if many features are removed, making it difficult to identify rank-invariant genes.

Author(s)

Heidi Dvinge

Examples

```
# Load some example data
data(qPCRpros)
show(qPCRpros)
# Filter based on different feature type
qFilt <- filterCtData(qPCRpros, remove.type=c("Endogenous Control"))
# Filter based on feature type and name
qFilt <- filterCtData(qPCRpros, remove.type=c("Endogenous Control"), remove.name=c("Gene1", "Gene20", "Gene30"))
# Filter based on feature class
qFilt <- filterCtData(qPCRpros, remove.class="Kinase")
# Filter based on feature categories, using two different cut-offs
qFilt <- filterCtData(qPCRpros, remove.category="Undetermined")
qFilt <- filterCtData(qPCRpros, remove.category="Undetermined", n.category=5)
# Remove features without much variation across samples
iqr <- apply(exprs(qPCRpros), 1, IQR, na.rm=TRUE)
hist(iqr, n=20)
qFilt <- filterCtData(qPCRpros, remove.IQR=2)
```

---

heatmapSig *Heatmap of deltadeltaCt values from qPCR data.*

Description

Heatmap and clustering of deltadeltaCt values from different sample comparisons using qPCR data.

Usage

```
heatmapSig(qDE, comparison = "all", col, zero.center = TRUE, mar, dist = "pearson", ...)
```

Arguments

qDE	data.frame or list, as created by ttestCtData or limmaCtData.
comparison	integers or the names of the comparisons to include in the plot. Defaults to all results in the qDE data, but a minimum of two is required.
col	colour scheme to use for the plot.
zero.center	logical, should the colour scale be centered around 0.
mar	vector of length two, the bottom and right side margins mof the heatmap.
dist	character string, either "pearson" (default) or "euclidean" indicating what type of distance is used for the clustering.
...	further arguments passed to heatmap.2.

Details

This function can be useful if multiple conditions are compared, for detecting features with similar behaviour in comparisons, and look at the general level of up and down regulation.

Value

A plot if produced in the current graphics device.

Author(s)

Heidi Dvinge

See Also

heatmap.2 for modifying the plot, and ttestCtData or limmaCtData for generating the data used for the plotting.

---

limmaCtData *Differentially expressed features with qPCR: limma*

Description

Function for detecting differentially expressed genes from high-throughput qPCR Ct values, based on the framework from the limma package. Multiple comparisons can be performed, and across more than two groups of samples.

Usage

```
limmaCtData(q, design = NULL, contrasts, sort = TRUE, stringent = TRUE, ndups = 1, spacing = NULL, dupcor
```

Arguments

q	object of class qPCRset.
design	matrix, design of the experiment rows corresponding to cards and columns to coefficients to be estimated. See details.
contrasts	matrix, with columns containing contrasts. See details
sort	boolean, should the output be sorted by adjusted p-values.
stringent	boolean, for flagging results as "Undetermined". See details.
ndups	integer, the number of times each feature is present on the card.
spacing	integer, the spacing between duplicate spots, spacing=1 for consecutive spots
dupcor	list, the output from duplicateCorrelation. See details.
...	any other arguments are passed to lmFit, contrasts.fit, eBayes or decideTests.

Details

This function is a wrapper for the functions lmFit, contrasts.fit (if a contrast matrix is supplied) and eBayes from the limma package. See the help pages for these functions for more information about setting up the design and contrast matrices.

All results are assigned to a category, either "OK" or "Unreliable" depending on the input Ct values. If stringent=TRUE any unreliable or undetermined measurements among technical and biological replicates will result in the final result being "Undetermined". For stringent=FALSE the result will be "OK" unless at least half of the Ct values for a given gene are unreliable/undetermined.

Note that when there are replicated features in the samples, each feature is assumed to be present the same number of times, and with regular spacing between replicates. Reordering the sample by featureNames and setting spacing=1 is recommendable.

If technical sample replicates are available, dupcor can be used. It is a list containing the estimated correlation between replicates. limmaCtData will then take this correlation into account when fitting a model for each gene. It can be calculate using the function duplicateCorrelation. Technical replicates and duplicated spots can't be assessed at the same time though, so if dupcor is used, ndups should be 1.

Value

A list of data.frames, one for each column in design, or for each comparison in contrasts if this matrix is supplied. Each component of the list contains the result of the given comparisons, with one row per gene and has the columns:

genes	Feature IDs.
feature.pos	The unique feature IDs from featurePos of the q object. Useful if replicates are not collapsed, in which case there might be several features with identical names.
t.test	The result of the t-test.
p.value	The corresponding p.values.
adj.p.value	P-values after correcting for multiple testing using the Benjamini-Holm method.
ddCt	The deltadeltaCt values.

FC	The fold change; 2^(-ddCt).
meanTest	The average Ct across the test samples for the given comparison.
meanReference	The average Ct across the reference samples for the given comparison.
categoryTest	The category of the Ct values ("OK", "Undetermined") across the test samples for the given comparison.
categoryReference	The category of the Ct values ("OK", "Undetermined") across the reference samples for the given comparison.

Also, the last item in the list is called "Summary", and it's the result of calling `decideTests` from limma on the fitted data. This is a data frame with one row per feature and one column per comparison, with down-regulation, no change and up-regulation marked by -1, 0 and 1.

Author(s)

Heidi Dvinge

References

Smyth, G. K. (2005). Limma: linear models for microarray data. In: Bioinformatics and Computational Biology Solutions using R and Bioconductor. R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds), Springer, New York, pages 397–420.

See Also

`lmFit`, `contrasts.fit` and `ebayes` for more information about the underlying limma functions. `mannwhitneyCtData` and `ttestCtData` for other functions calculating differential expression of Ct data. `plotCtRQ`, `heatmapSig` and `plotCtSignificance` can be used for visualising the results.

Examples

```
# Load example preprocessed data
data(qPCRpros)
samples <- read.delim(file.path(system.file("exData", package="HTqPCR"), "files.txt"))
# Define design and contrasts
design <- model.matrix(~0+samples$Treatment)
colnames(design) <- c("Control", "LongStarve", "Starve")
contrasts <- makeContrasts(LongStarve-Control, LongStarve-Starve, Starve-Control, levels=design)
# The actual test
diff.exp <- limmaCtData(qPCRpros, design=design, contrasts=contrasts)
# Some of the results
diff.exp[["LongStarve - Control"]][1:10,]
```

mannwhitneyCtData *Differentially expressed features with qPCR: Mann-Whitney*

Description

Function for calculating p-values across two groups for the features present in high-throughput qPCR data, such as from TaqMan Low Density Arrays. Also known as two sample Wilcoxon test.

Usage

```
mannwhitneyCtData(q, groups = NULL, calibrator, alternative = "two.sided", paired = FALSE, replicates =
```

Arguments

q	qPCRset object.
groups	factor, assigning each sample to one of two groups.
calibrator	which of the two groups is to be considered as the reference and not the test? Defaults to the first group in groups.
alternative	character string (first letter is enough), specifying the alternative hypothesis, "two.sided" (default), "greater" or "less".
paired	logical, should a paired t-test be used.
replicates	logical, if replicated genes are present on the array, the statistics will be calculated for all the replicates combined, rather than the individual wells.
sort	boolean, should the output be sorted by p-values.
stringent	boolean, for flagging results as "Undetermined". See details.
p.adjust	character string, which method to use for p-value adjustment for multiple testing. See details.
...	any other arguments will be passed to the wilcox.test function.

Details

Once the Ct values have been normalised, differential expression can be calculated. This function deals with just the simple case, where there are two types of samples to compare. For a parametric test see ttestCtData and limmaCtData for more complex studies.

The underlying statistics is calculated by wilcox.test. Due to the high possibility of ties for each feature between samples, the test is run with exact=FALSE.

All results are assigned to a category, either "OK" or "Undetermined" depending on the input Ct values. If stringent=TRUE any unreliable or undetermined measurements among technical and biological replicates will result in the final result being "Undetermined". For stringent=FALSE the result will be "OK" unless at least half of the Ct values for a given gene are unreliable/undetermined.

The argument p.adjust is passed on to the p.adjust function. Options include e.g. "BH" (Benjamini & Hochberg, the default), "fdr" and "bonferroni". See p.adjust for more information on the individual methods.

Value

A data.frame containing the following information:

genes	The names of the features on the card.
feature.pos	The featurePos of the genes. If replicated genes are used, the feature positions will be concatenated together.
MB.test	The name and value of the test statistic.
p.value	The corresponding p-value.
ddCt	The delta delta Ct values.
FC	The fold change; 2^(-ddCt).
meanCalibrator	The average expression level of each gene in the calibrator sample(s).
meanTarget	The average expression level of each gene in the target sample(s).
categoryCalibrator	The category of the Ct values ("OK", "Undetermined") across the calibrator.
categoryTarget	Ditto for the target.

Author(s)

Heidi Dvinge

See Also

wilcox.test, ttestCtData, limmaCtData. plotCtRQ and plotCtSignificance can be used for visualising the results.

---

normalizeCtData *Normalization of Ct values from qPCR data.*

Description

This function is for normalizing Ct data from high-throughput qPCR platforms like the TaqMan Low Density Arrays. Normalization can be either within or across different samples.

Usage

```
normalizeCtData(q, norm = "deltaCt", deltaCt.genes = NULL, scale.rank.samples, rank.type = "pseudo.medi
```

Arguments

q	object of class qPCRset.
norm	character string with partial match allowed, the normalisation method to use. "deltaCt" (default) , "scale.rankinvariant", "norm.rankinvariant", "quantile" and "geometric.mean" are implemented. See details.

`deltaCt.genes` character vector, the gene(s) to use for deltaCt normalization. Must correspond to some of the `featureNames` in `q` or `NULL`, in which case the endogenous controls from `featureType` are used.

`scale.rank.samples` integer, for the "scale.rankinvariant" method, how many samples should a feature be rank invariant across to be included. Defaults to number of samples-1.

`rank.type` string, the reference sample for the rank invariant normalisation. Either "pseudo.median" or "pseudo.mean" for using the median or mean across samples as a pseudo-reference sample.

`Ct.max` numeric, Ct values above this will be ignored when identifying rank invariant genes.

`geo.mean.ref` numeric, the reference sample to scale to for the "geometric.mean" method. Defaults to sample number 1.

`verbose` boolean, should some information be printed to the prompt.

Details

"quantile" will make the expression distributions across all cards more or less identical. "deltaCt" calculates the standard deltaCt values, i.e. subtracts the mean of the chosen controls from all other values on the array. "scale.rankinvariant" sorts features from each sample based on Ct values, and identifies a set of features that remain rank invariant, i.e. whose ordering is constant. The average of these rank invariant features is then used to scale the Ct values on each array individually. "norm.rankinvariant" also identifies rank invariant features between each sample and a reference, and then uses these features to generate a normalisation curve individually for each sample by smoothing. "geometric.mean" calculates the geometric mean of all Ct values below Ct.max in each sample, and scales the Ct values accordingly.

For the rank invariant methods it can make a significant difference whether high Ct values, such as "40" or something else being used for undetermined Ct values is removed during the normalisation using the Ct.max parameter. "norm.rankinvariant" also depends on having enough rank invariant genes for generating a robust smoothing curve.

"quantile" is base on `normalizeQuantiles` from `limma`, and the rank invariant normalisations implement methods from `normalize.invariantset` in package `affy`.

The distribution of Ct values before/after normalisation can be assessed with the function `plotCtDensity`.

Value

An object of class qPCRset like the input.

Author(s)

Heidi Dvinge

See Also

`normalize.invariantset` for the rank invariant normalisations, `normalizequantiles` and `plotCtDensity`

Examples

```
# Load example data
data(qPCRraw)
# Perform different normalisations
dnorm <- normalizeCtData(qPCRraw, norm="deltaCt", deltaCt.genes="Gene1")
qnorm <- normalizeCtData(qPCRraw, norm="quantile")
nrnorm <- normalizeCtData(qPCRraw, norm="norm.rankinvariant")
srnorm <- normalizeCtData(qPCRraw, norm="scale.rankinvariant")
gnorm <- normalizeCtData(qPCRraw, norm="geometric.mean")
# Normalized versus raw data
cols <- rep(brewer.pal(6, "Spectral"), each=384)
plot(exprs(qPCRraw), exprs(dnorm), pch=20, col=cols, main="deltaCt normalization")
plot(exprs(qPCRraw), exprs(qnorm), pch=20, col=cols, main="Quantile normalization")
plot(exprs(qPCRraw), exprs(nrnorm), pch=20, col=cols, main="norm.rankinvariant")
plot(exprs(qPCRraw), exprs(srnorm), pch=20, col=cols, main="scale.rankinvariant")
plot(exprs(qPCRraw), exprs(gnorm), pch=20, col=cols, main="geometric.mean")
# With or without removing high Ct values
nrnorm <- normalizeCtData(qPCRraw, norm="norm.rankinvariant")
nrnorm2 <- normalizeCtData(qPCRraw, norm="norm.rankinvariant", Ct.max=40)
plot(exprs(nrnorm), exprs(nrnorm2), pch=20, col=cols, xlab="Ct.max = 35", ylab="Ct.max = 40")
# Distribution of the normalised data
par(mfrow=c(2,3), mar=c(3,3,2,1))
plotCtDensity(qPCRraw, main="Raw Ct values")
plotCtDensity(dnorm, main="deltaCt")
plotCtDensity(qnorm, main="quantile")
plotCtDensity(srnorm, main="scale.rankinvariant")
plotCtDensity(nrnorm, main="norm.rankinvariant")
plotCtDensity(gnorm, main="geometric.mean")
```

---

plotCtArray *Image plot of qPCR Ct values from an array format*

Description

Function for plotting high-throughput qPCR Ct values from a platform with a defined spatial layout, such as Fluidigm Dynamic Arrays (BioMark) or OpenArray from Applied Biosystems. The location of Ct values in the plot corresponds to the position of each well on the array.

Usage

```
plotCtArray(q, plot = "Ct", main, col, col.range, na.col = "grey", na.value = 40, chamber.size, ...)
```

Arguments

q	object of class qPCRset.
plot	character string indicating what type of plot to produce. Currently only "Ct" is implemented.
main	character string, the title of the plot. Per default "Ct values".

col	the name of a colour scheme.
col.range	vector, the range of colours to use.
na.col	the colour used for well with NA (undetermined) Ct values.
na.value	numeric, if NA has been replaced by an (arbitrary) high Ct value in the data.
chamber.size	numeric, for adjusting the size of the reaction chamber on the card.
...	any other arguments will be passed to the plot function.

Value

A plot is created on the current graphics device.

Author(s)

Heidi Dvinge

See Also

plotCtCard for plotting data from other high-throughput qPCR platforms.

Examples

```
# Locate example data
exPath <- system.file("exData", package="HTqPCR")
exFiles <- "BioMark_sample.csv"
# Create qPCRset object
raw <- readCtData(exFiles, path=exPath, n.features=48, n.data=48, format="BioMark")
# Plot
plotCtArray(raw)
# Change colour and range
plotCtArray(raw, col=brewer.pal(11, "Spectral"), col.range=c(10,35))
```

---

plotCtBoxes *Boxplots for qPCR Ct values.*

Description

Function for making boxplots of Ct values from high-throughput qPCR data. The boxes can be made either using all values on each card, or stratified by different feature information.

Usage

```
plotCtBoxes(q, cards = TRUE, xlab = "", col, main = NULL, names, stratify = "type", mar = c(7, 4, 3, 1), .
```

Arguments

q	object of class qPCRset.
cards	vector, the numbers of the cards to plot. Defaults to TRUE = all cards.
xlab	character string, label for the x-axis.
col	vector of colours to use, defaults to different colour for each card.
main	character string, plot title.
names	vector, names to plot under the boxes. Defaults to sample names.
stratify	character, specifying what to stratify the Ct values by. NULL, the default means no stratification, "type" is the feature types of the qPCRset, and "class" the feature class.
mar	vector, the size of the margins. See par for details.
...	any other arguments will be passed to the boxplot or parfunction.

Details

For the stratified plots all boxes with Ct values from the same card are plotted in identical colours. "type" and "class" are automatically extracted from the qPCRset using featureType and featureClass.

Value

A plot is created on the current graphics device.

Author(s)

Heidi Dvinge

See Also

boxplot

Examples

```
# Loading the data
data(qPCRraw)
# Make plot with all samples or just a few
plotCtBoxes(qPCRraw, stratify=NULL)
plotCtBoxes(qPCRraw, cards=c(1,4))
plotCtBoxes(qPCRraw, stratify="class")
```

plotCtCard *Image plot of qPCR Ct values from a card format*

Description

Function for plotting high-throughput qPCR Ct values from a platform with a defined spatial layout, such as TaqMan Low Density Assay cards. The location of Ct values in the plot corresponds to the position of each well on the card.

Usage

```
plotCtCard(q, card = 1, plot = "Ct", main, nrow = 16, ncol = 24, col, col.range, na.col = "grey", na.value
```

Arguments

q	object of class qPCRset.
card	integer, the sample number to plot.
plot	character string among "Ct", "flag", "type", "class") indicating what type of plot to produce. See Details for a longer description.
main	character string, the title of the plot. Per deault this is the sample name corresponding to card.
nrow	integer, the numer of rows on the card (16 for a standard 384 well format).
ncol	integer, the numer of columns on the card (24 for a standard 384 well format).
col	vector of colors of the same length as the number of different groups for the categorical data, or the name of a colour scheme for the continuous data.
col.range	vector, the range of colours to use.
na.col	the colour used for well with NA (undetermined) Ct values.
na.value	numeric, if NA has been replaced by an (arbitrary) high Ct value in the data.
legend.cols	integer, how many columns should the legend text be split into (defaults to number of labels).
well.size	numeric, for adjusting the size of the wells on the card.
zero.center	logical, should the colours be shifted to be zero-centered.
unR	logical, should wells from the category "Unreliable" be crossed out.
unD	logical, should wells from the category "Undetermined" be crossed out.
...	any other arguments will be passed to the plot and points functions.

Details

This function may be used to plot the values of any well-specific information, such as the raw or normalized Ct values, or categorical data such as flag, gene class etc. The image follows the layout of an actual HTqPCR card.

If unR=TRUE these will wells will be crossed out using a diagonal cross (X), whereas unD=TRUE will be marked with a horisontal/vertical cross.

Value

A plot is created on the current graphics device.

Author(s)

Heidi Dvinge

See Also

image, and plotCtArray for plotting data from other high-throughput qPCR platforms (e.g. Fluidigm arrays).

Examples

```
# Load some example data
data(qPCRraw)
# Plot Ct values from first card
plotCtCard(qPCRraw)
plotCtCard(qPCRraw, card=2, col.range=c(10,35))
plotCtCard(qPCRraw, unR=TRUE, unD=TRUE)
# Other examples
plotCtCard(qPCRraw, plot="class")
plotCtCard(qPCRraw, plot="type")
plotCtCard(qPCRraw, plot="flag")
```

---

plotCtCategory *Summarising the feature categories for Ct values.*

Description

This function will provide a summary of the `featureCategory` for a qPCRset. Focus can either be on categories across samples, or across features.

Usage

```
plotCtCategory(q, cards = TRUE, by.feature = FALSE, stratify, col, xlim, main, ...)
```

Arguments

`q`	object of class qPCRset.
`cards`	integers, the number of the cards (samples) to plot.
`by.feature`	logical, should the categories be summarised for features rather than samples. See details.
`stratify`	character string, either "type" or "class" indicating if the categories should be stratified by `featureType` or `featureClass` of `q`. Ignored if `by.features` is TRUE.

col	vector with the colours to use for the categories. Default is green for "OK", yellow for "Unreliable" and red for "Undetermined". See details.
xlim	vector, the limits of the x-axis. If by.feature is FALSE, this can be used to adjust the size of the barplot to fit in the colour legend.
main	character string, the title of the plot.
...	further arguments passed to barplot or heatmap.

Details

This function is for generating two different types of plot. If by.feature=FALSE the number of each featureCategory will be counted for each card, and a barplot is made. If however by.feature=TRUE, then the categories for each feature across the selected cards will be clustered in a heatmap.

The colours given in col correspond to all the unique categories present in the entire featureCategory of q, even categories not represented for the samples selected by cards. Categories are sorted alphabetically, and colours assigned accordingly.

For by.feature=TRUE the plot can be modified extensively using calls to the underlying heatmap function, such as setting cexRow to adjust the size of row labels.

Value

A figure is produced on the current graphics device.

Author(s)

Heidi Dvinge

See Also

setCategory, and heatmap for the underlying plotting function for by.feature=TRUE.

Examples

```
# Load example preprocessed data
data(qPCRpros)
# Plot categories for samples
plotCtCategory(qPCRpros)
plotCtCategory(qPCRpros, cards=1:3, stratify="class")
# Categories for features
plotCtCategory(qPCRpros, by.feature=TRUE)
```

plotCtCor *Correlation between Ct values from qPCR data*

Description

Function for plotting the correlation based on Ct values between samples containing high-throughput qPCR data.

Usage

```
plotCtCor(q, col, col.range = c(0, 1), main, mar, ...)
```

Arguments

q	object of class qPCRset.
col	vector of colours to use, defaults to a spectrum from red to blue/purple.
col.range	vector, the range of colours to use.
main	character string, plot title.
mar	vector, the size of the borrom and right hand side margins.
...	any other arguments will be passed to the heatmap.2 function.

Details

This function may be used to cluster the samples based on Ct values and present the result in a heatmap. Per default the colours are a rainbow scale from 0 to 1.

The correlation is calculated as 1 - the 'Pearson' method. Prior to version 1.9.1 the value plotted was the correlation directly, rather than 1-correlation.

A standard heatmap is drawn, but this can be modified extensively using the arguments available in the heatmap.2 function.

Value

A plot is created on the current graphics device.

Author(s)

Heidi Dvinge

See Also

heatmap.2

Examples

```
data(qPCRraw)
plotCtCor(qPCRraw)
plotCtCor(qPCRraw, col.range=c(0,0.6))
```

plotCtDensity *Distribution plot for qPCR Ct values.*

Description

Function for plotting the density distribution of Ct values from high-throughput qPCR data.

Usage

```
plotCtDensity(q, cards = TRUE, xlab = "Ct", ylab = "Density", col, main = NULL, legend = TRUE, lwd = 2, ..
```

Arguments

q	object of class qPCRset.
cards	vector, the numbers of the cards to plot. Defaults to TRUE = all cards.
xlab	character string, label for the x-axis.
ylab	character string, label for the y-axis.
col	vector of colours to use, defaults to different colour for each card.
main	character string, plot title.
legend	logical, whether to include a colour legend or not.
lwd	numeric, the width of the lines.
...	any other arguments will be passed to the matplot function.

Details

The distribution of Ct values in the qPCRset q is calculated using density.

Value

A plot is created on the current graphics device.

Author(s)

Heidi Dvinge

See Also

matplot, density

Examples

```
# Loading the data
data(qPCRraw)
# Make plot with all samples or just a few
plotCtDensity(qPCRraw)
plotCtDensity(qPCRraw, cards=c(1,4))
```

plotCtHeatmap *Heatmap of qPCR Ct values.*

Description

Function for drawing a heatmap of Ct values from high-throughput qPCR experiments such as using TaqMan Low Density Arrays.

Usage

```
plotCtHeatmap(q, main = NULL, col, col.range, dist = "pearson", zero.center, mar, gene.names, sample.na
```

Arguments

q	object of class qPCRset.
main	character string, plot title.
col	the colours to use. See details.
col.range	vector, the range of colours to use.
dist	character string, specifying whether to use "pearson" correlation (default) or "euclidean" distance for the clustering.
zero.center	logical, should the colours be shifted to be zero-centered. See details.
mar	vector, the size of the borrom and right hand side margins.
gene.names	character vector, names to replace the genes (rows) with. See details.
sample.names	character vector, names to replace the samples (columns) with. See details.
...	any other arguments will be passed to the heatmap.2 function.

Details

This function may be used to cluster the raw or normalized Ct values, and present the result in a heatmap.

The color range is used to represent the range of values for the statistic. If col==NULL the colour will be set to a spectrum from red to blue/purple, unless there are negative values in which case it goes red-yellow-green to reflect up and down regulation of genes. If zero.center=NULL then zero.center will automatically be set to TRUE to make the colour scale symmetric around 0.

Especially gene names will often not be readable in a standard size plotting device, and might therefore be removed. If gene.names or sample.names is set to a single character (such as "" for no naming), then this character will be repeated for all rows or columns.

A standard heatmap is drawn, but this can be modified extensively using the arguments available in the heatmap.2 function.

Value

A plot is created on the current graphics device.

Author(s)

Heidi Dvinge

See Also

heatmap.2

Examples

```
# Load example data
data(qPCRraw)
# Some standard heatmaps
plotCtHeatmap(qPCRraw, gene.names="")
plotCtHeatmap(qPCRraw, gene.names="", dist="euclidean", col.range=c(10,35))
plotCtHeatmap(qPCRraw, gene.names="", dist="euclidean", col=colorRampPalette(rev(brewer.pal(9, "YlGnBu")))(20))
```

---

plotCtHistogram *Histrogram of Ct values from qPCR experiments.*

Description

The distribution of Ct values for a selected qPCR sample is shown in a histogram.

Usage

```
plotCtHistogram(q, card = 1, xlab = "Ct", col, main, n = 30, ...)
```

Arguments

q	an object of class qPCRset.
card	integer, the number of the card (sample) to plot.
xlab	character string, the label for the x-axis.
col	integer or character, the colour for the histogram.
main	character string, the plot title. Default is the name of the sample.
n	integer, number of bins to divide the Ct values into.
...	any other arguments are passed to hist.

Value

A figure is generated in the current graphics device.

Author(s)

Heidi Dvinge

See Also

plotCtDensity or plotCtBoxes for including multiple samples in the same plot.

Examples

```
# Load example data
data(qPCRraw)
# Create the plots
plotCtHistogram(qPCRraw, card=2)
plotCtHistogram(qPCRraw, card=3, n=50, col="blue")
```

---

plotCtLines *Plotting Ct values from qPCR across multiple samples.*

Description

This function is for displaying a set of features from a qPCRset across multiple samples, such as a timeseries or different treatments. Values for each feature are connected by lines, and the can be averaged across groups rather than shown for individual smaples.

Usage

```
plotCtLines(q, genes, groups, col = brewer.pal(10, "Spectral"), xlab = "Sample", ylab = "Ct", legend = T
```

Arguments

q	object of class qPCRset.
genes	numeric or character vector, selected genes to make the plot for.
groups	vector, the different groups that the samples in q belong to. See details.
col	vector, colours to use for the lines.
xlab	character string, label for the x-axis.
ylab	character string, label for the y-axis.
legend	logical, whether to include a colour legend or not.
lwd	numeric, the width of the lines.
lty	vector, line types to use. See par or lines for details.
pch	vector, if groups is set, the point types that will be used for each feature in genes.
xlim	vector of length two, the limits for the x-axis. Mainly used for adjusting the position of the legend.
...	any other arguments will be passed to the matplot function.

Details

The default plot shows the Ct values across all samples in q, with lines connecting the samples. However, if groups is set the Ct values will be averaged within groups. Lines connect these averages, but the individual values are shown with different point types, as chosen in pch.

Value

A plot is created on the current graphics device.

Author(s)

Heidi Dvinge

See Also

matplot

Examples

```
# Load some example data
data(qPCRraw)
samples <- exFiles <- read.delim(file.path(system.file("exData", package="HTqPCR"), "files.txt"))
# Draw dfferent plots
plotCtLines(qPCRraw, genes=1:10)
plotCtLines(qPCRraw, genes=1:10, groups=samples$Treatment, xlim=c(0,3))
feat <- as.numeric(as.factor(featureType(qPCRraw)[1:10]))
plotCtLines(qPCRraw, genes=1:10, col=feat)
```

---

plotCtOverview *Overview plot of qPCR Ct values across multiple conditions.*

Description

Function for high-throughput qPCR data, for showing the average Ct values for features in a barplot, either for individual samples or averaged across biological or technical groups. If Ct values are shown, error bars can be included, or the Ct values can be displayed relative to a calibrator sample.

Usage

```
plotCtOverview(q, cards = TRUE, genes, groups, calibrator, replicates = TRUE, col, conf.int = FALSE, leg
```

Arguments

q	object of class qPCRset.
cards	integer, the cards (samples) to use. Defaults to all.
genes	vector selecting the features to show. See Details.
groups	vector with groups to average the samples across. If missing all the samples are displayed individually. See Details.
calibrator	the value in groups to use as calibrator sample. See Details.
replicates	logical, if should values from replicated features in each sample be collapsed or kept separate.

col	colours to use for each sample or group. Per default a maximum of 10 colours are used, so this parameter should be set if more than 10 groups are present.
conf.int	logical, should the 95 percent confidence interval be shown. See Details.
legend	logical, should a legend be included in the plot.
...	further arguments passed to barplot.

Details

If a calibrator is chosen all values will be displayed relative to this, i.e. as Ct(sample)-Ct(calibrator). If there is no calibrator, the full Ct values are shown, including 95% confidence interval if selected. For confidence intervals when there is a calibrator, it's the variation across Ct(sample)-average(Ct(calibrator)) that is shown.

When setting replicates=TRUE it is often better to specify genes by name rather than selecting for example the first 10 features using 1:10. This literally only takes the first 10 rows of the data, although some of these features might be replicated elsewhere in the data.

The purpose of group is to tell plotCtOverview if any of the samples should be treated as biological replicates, in addition to the technical replicates that might be present on each plate. With e.g. 4 samples and groups=c("A", "B", "C", "D") they're each treated individually, and only replicates features on each plate are considered. However, groups=c("WT", "WT", "WT", "mutant") means that the first 3 are treated as biological replicates; hence for each gene in the barplot there'll be one bar for WT and one for mutant.

Value

A figure is produced in the current graphics device.

Author(s)

Heidi Dvinge

Examples

```
# Load example data
data(qPCRraw)
exPath <- system.file("exData", package="HTqPCR")
samples <- read.delim(file.path(exPath, "files.txt"))
# Show all samples for the first 10 genes
g <- featureNames(qPCRraw)[1:10]
plotCtOverview(qPCRraw, genes=g, xlim=c(0,90))
plotCtOverview(qPCRraw, genes=g, xlim=c(0,50), groups=samples$Treatment)
plotCtOverview(qPCRraw, genes=g, xlim=c(0,60), groups=samples$Treatment, conf.int=TRUE, ylim=c(0,55))
# Relative to a calibrator sample
plotCtOverview(qPCRraw, genes=g, groups=samples$Treatment, calibrator="Control")
plotCtOverview(qPCRraw, genes=g, groups=samples$Treatment, calibrator="Control", conf.int=TRUE, ylim=c(-0.5,0.5)
plotCtOverview(qPCRraw, genes=g, groups=samples$Treatment, calibrator="LongStarve")
```

plotCtPairs *Pairwise scatterplot of multiple sets of Ct values from qPCR data.*

Description

Produces a plot of high-throughput qPCR Ct values from N number of samples plotted pairwise against each other in an N by N plot. The Ct values will be in the upper triangle, and the correlation between samples in the lower. Features can be marked based on for example feature class or type.

Usage

```
plotCtPairs(q, cards = TRUE, lower.panel = panel.Ct.cor, upper.panel = panel.Ct.scatter, Ct.max = 35, co
```

Arguments

q	object of class qPCRset.
cards	vector, the cards to plot against each other.
lower.panel	function, to use for plotting the lower triangle.
upper.panel	function, to use for plotting the upper triangle.
Ct.max	numeric, Ct values above this limit will be excluded when calculating the correlation.
col	vector with the colour(s) to use for the points, or a character string ("type" or "class") indicating whether points should be coloured according to featureType or featureClass of q.
pch	integer or single character, which plotting symbol to use for the points.
cex.cor	numeric, the expansion factor for the text in panel.Ct.cor.
cex.pch	numeric, the expansion factor for the points in panel.Ct.scatter.
diag	logical, should the diagonal line y=x be plotted.
...	any other arguments are passed to the panel function or pairs.

Details

Per default, the lower panels contain the correlations between data sets. For each correlation all complete pairs are used, i.e. NAs are ignored. If there are no complete observations between two samples the correlation will be set to NA.

Value

A figure is generated in the current graphics device.

Author(s)

Heidi Dvinge

See Also

pairs or plotCtScatter for plotting just two samples.

Examples

```
# Load example data
data(qPCRraw)
# Various types of plot
plotCtPairs(qPCRraw, cards=1:4)
plotCtPairs(qPCRraw, col="black")
plotCtPairs(qPCRraw, Ct.max=40)
```

---

plotCtPCA *PCA for qPCR Ct values.*

Description

Perform and plot a principal component analysis for high-throughput qPCR data from any platform, for doing clustering.

Usage

```
plotCtPCA(q, s.names, f.names, scale = TRUE, features = TRUE, col, cex = c(1, 1))
```

Arguments

q	a matrix or an object of class qPCRset containing Ct values.
s.names	character vector, names of samples. See details.
f.names	character vector, names of features. See details.
scale	logical, should the variables be scaled to have unit variance. Passed on to prcomp.
features	logical, should the features be plotted. See details.
col	vector, the colours to use for the samples if features=FALSE.
cex	vector of length 2, the expansion to use for features and samples respectively if features=FALSE.

Details

Per default the sample names from the qPCRset are used, however the feature names are replaced by "*" to avoid cluttering the plot.

If features=TRUE then a biplot including all features is produced, with samples represented by vectors. I.e. both observations and variables are plotted, which can potentially be used to identify outliers among the features. For features=FALSE only the samples will be included in the plot. This might be more useful for clustering.

In case of high-throughput arrays, some samples may be all NAs. These are ignored during the PCA calculation.

Value

A plot is created on the current graphics device.

Note

This is still a work in progress, and the function is not particularly sophisticated. Suggestions/wishes are welcome though.

Author(s)

Heidi Dvinge

See Also

prcomp, biplot

Examples

```
# Load example data
data(qPCRraw)
# Plot
plotCtPCA(qPCRraw)
# Include feature names; make them smaller
plotCtPCA(qPCRraw, f.names=featureNames(qPCRraw), cex=c(0.5,1))
# Plot only the samples
plotCtPCA(qPCRraw, features=FALSE)
```

---

plotCtReps *Scatter plot of features analysed twice during each qPCR experiment.*

Description

In high-throughput qPCR data some features may be present twice on each card (sample). This function will make a scatter plot of one replicate versus the other for each sample individually, as well as mark genes with very deviating replicate values.

Usage

```
plotCtReps(q, card = 1, percent = 20, verbose = TRUE, col = 1, ...)
```

Arguments

q	object of class qPCRset.
card	integer, the sample number to plot.
percent	numeric, features with replicate values differ more than this percentage from their average will be marked on the plot.
verbose	logical, should the deviating genes and their Ct values be printed to the terminal.
col	integer or character; the colour of the points in the scatter plot.
...	any other arguments are passed to plot.

Details

This function will look through the data in the qPCRset, find all genes with are presented twice on the array, and plot the Ct values of these replicated genes against each other. Whether a genes goes to the x or y-axis depends on the first occurrence of the gene names.

All genes where abs(rep1-rep2) > percent/100*replicate mean will be marked by an open circle, and the gene names written in red letters.

Value

An plot is created on the current graphics device. Also, a data.frame with the names and values of deviating genes is returned invisibly.

Author(s)

Heidi Dvinge

See Also

plot, and par for the plotting parameters.

Examples

```
# Load example data
data(qPCRraw)
# Plot replicates
plotCtReps(qPCRraw, card=1, percent=30)
plotCtReps(qPCRraw, card=2, percent=10)
reps <- plotCtReps(qPCRraw, card=2, percent=20)
reps
```

---

plotCtRQ *Plot the relative quantification of Ct values from qPCR experiments.*

Description

Function for plotting the relative quantification (RQ) between two groups of data, whose Ct values have been tested for significant differential expression.

Usage

```
plotCtRQ(qDE, comparison = 1, genes, transform = "log2", p.val = 0.1, mark.sig = TRUE, p.sig = 0.05, p.ve
```

Arguments

qDE	list or data.frame, the result from ttestCtData or limmaCtData.
comparison	integer or character string, indicating which component to use if qDE is a list.
genes	numeric or character vector, selected genes to make the plot for.
transform	character string, how should the data be displayed. Options are "none", "log2" or "log10". See details
p.val	numeric between 0 and 1, if genes is not supplied all given with (adjusted) p-value below this threshold will be included.
mark.sig	logical, should significant features be marked.
p.sig	numeric, the cut-off for significant p-values that will be marked by *.
p.very.sig	numeric, the cut-off for very significant p-values that will be marked by ".
mark.un	logical, should data with unreliable target or calibrator samples be marked. See details.
un.tar	colour to use for the undetermined targets. See details.
un.cal	colour to use for the undetermined calibrators. See details.
col	vector, colours to use for the bars.
legend	logical, should a legend be included in the barplot.
xlim	vector of length 2, the limits on the x-axis. Mainly used for moving the legend to the left of bars.
mar	vector with 4 values, the size of the margins. See par for more info.
main	character string, the image title. Default to the name of the chosen comparison.
...	any other arguments will be passed to the barplot function.

Details

The relative quantification is calculated as RQ=2^-ddCT, where ddCT is the deltadeltaCt value.

If mark.un=TRUE, those bars where either the calibrator or target sample measurements were undetermined are marked using diagonal lines. Whether either of these are called undetermined (includes unreliable values) or not depends on all the input Ct values in ttestCtData or limmaCtData, and whether stringent=TRUE was used in these functions.

Value

A plot is created on the current graphics device.

Author(s)

Heidi Dvinge

See Also

ttestCtData and limmaCtData for testing the Ct data for differential expression.

`plotCtScatter` *Scatterplot of two sets of Ct values from qPCR data.*

Description

Produces a plot of Ct values from two samples plotted against each other. Features can be marked based on for example feature class or type.

Usage

```
plotCtScatter(q, cards = c(1, 2), col = "class", pch = 20, diag = FALSE, cor = TRUE, Ct.max = 35, legend =
```

Arguments

`q`	object of class qPCRset.
`cards`	vector, the two cards to plot against each other.
`col`	vector with the colour(s) to use for the points, or a character string ("type" or "class") indicating whether points should be coloured according to `featureType` or `featureClass` of `q`.
`pch`	integer, the point type to use for the plot.
`diag`	logical, should the diagonal line y=x be plotted.
`cor`	logical, should information about the correlation between the two samples be included in the plot. The correlation is calculated both with and without removing Ct values above Ct.max.
`Ct.max`	numeric, all Ct values above this will be removed for calculating one of the correlations.
`legend`	logical, if `col` is either "type" or "class", should a colour legend for these be included.
`...`	any other arguments are passed to `plot`.

Value

A figure is generated in the current graphics device.

Author(s)

Heidi Dvinge

Examples

```
# Load example data
data(qPCRraw)
# Various types of plot
plotCtScatter(qPCRraw, cards=c(1,2))
plotCtScatter(qPCRraw, cards=c(1,4), col="type")
plotCtScatter(qPCRraw, cards=c(1,4), col="black", cor=FALSE, diag=TRUE)
```

plotCtSignificance *Barplot with Ct values between genes from qPCR.*

Description

Function for producing a barplot of the Ct values from high-throughput qPCR samples. A comparison is made between two groups which have been tested for differential expression, and all individual Ct values are shown, to identify potential outliers.

Usage

```
plotCtSignificance(qDE, q, comparison = 1, genes, p.val = 0.1, groups, calibrator, target, p.sig = 0.05,
```

Arguments

qDE	list or data.frame, the result from ttestCtData or limmaCtData.
q	the qPCRset data that was used for testing for differential expression.
comparison	integer or character string, indicating which component to use if x is a list.
genes	numeric or character vector, selected genes to make the plot for.
p.val	numeric between 0 and 1, if genes is not supplied all given with (adjusted) p-value below this threshold will be included.
groups	vector, the groups of all the samples in q.
calibrator	character string, which of the groups is the calibrator.
target	character string, which of the groups is the target.
p.sig	numeric, the cut-off for significant p-values that will be marked by *.
p.very.sig	numeric, the cut-off for very significant p-values that will be marked by ".
mark.sig	logical, should significant features be marked.
col	vector, colours to use for the two sets of bars, one per sample type.
un.col	integer or character string, the colour to use for all Ct values that are "Unreliable" or "Undetermined".
point.col	integer or character string, the colour to use for all other Ct values.
legend	logical, should a legend be included int eh barplot.
mar	vector with 4 values, the size of the margins. See par for more info.
main	character string, the image title. Default to the name of the chosen comparison.
jitter	numeric, between 0 and 1. If Ct values are very similar, the individual points might lie on top of each other in the bars. This adds a jittering factor along the x-axis. If 0 the points will all be aligned.
...	any other arguments will be passed to the barplot function.

Details

This function will make a barplot with the average Ct values for the test and reference samples for the selected genes. All the individual Ct values are plotted on top of the bars though, and the "Unreliable" or "Undetermined" ones are marked, to do a visual assessment of the impact of non-valid measurements on the average.

It's up to the user to specify the correct `calibrator` and `target` for the given comparison; no checking is done.

Value

A plot is created on the current graphics device.

Author(s)

Heidi Dvinge

See Also

`barplot` and `plotCtRQ` or `plotCtOverview` for a plot of the relative quantification between samples.

---

plotCtVariation *Plot variation in Ct values across replicates*

Description

Examine the variation in Ct values, either across features present multiple times on each card, or for within different groups of samples. The function supports both a summarised and a more detailed output.

Usage

```
plotCtVariation(q, cards = TRUE, variation = "var", type = "summary", sample.reps, feature.reps, log = F
```

Arguments

`q`	object of class qPCRset.
`cards`	vector, the numbers of the cards to plot. Defaults to TRUE = all cards.
`variation`	character string indication whether to calculate the variation, "var", or standard deviation, "sd".
`type`	character string indicating whether to output the results in a summarised boxplot, "summary" or as a more detailed scatter plot, "detail". See Details and the examples.
`sample.reps`	a vector grouping the samples (see Details). Overrides `feature.reps`.
`feature.reps`	a vector grouping the features according to which are replicates. Per default `featureNames(q)` are used.

log	logical, should the results be converted into log10 values.
add.featurenames	logical, if type="detail" should the names of each feature be added to the scatter plot.
ylab	character, the label of the y-axis.
n.col	integer, if type="detail" how many columns should the scatterplots be presented in. Defaults to 3, or n.samples(q) if <3.
...	further arguments passed to boxplot or plot.

Details

It is often useful to examine the data to determine if some samples are inherently more variable than other, or if the concordance between replicates on each qPCR card is acceptable. Using type="summary" generates a boxplot with all the variation values, either across genes (if sample.reps is set) or with each samples (default, or if feature.reps is set). That way the general distribution of variation or standard deviation values can be compared quickly.

If it looks like there's an unacceptable (or interesting) difference in the variation, this can be further investigated using type="detail". This will generate multiple sub-plots, containing a single scatterplot of variation versus mean for each gene (if sample.reps is set) or each sample (default, or if feature.reps is set). Including the mean in the plot can be used to assess heteroskedasticity in the data.

Value

A plot is created on the current graphics device. The variation and mean across each type of replicate is returned invisibly in a list with "Var" and "Mean" slots.

Author(s)

Heidi Dvinge

See Also

plotCtReps for cases where the qPCR card only contains two replicates of each feature. plotCVBoxes for other ways of plotting variation within different groups.

Examples

```
# Load some example data
data(qPCRraw)


# Detailed summary of variation versus mean Ct value for replicated features within each sample
plotCtVariation(qPCRraw, type="detail", log=TRUE)
plotCtVariation(qPCRraw, type="detail")
# Add feature names to see which the highly varying replicates are.
plotCtVariation(qPCRraw, type="detail", add.featurenames=TRUE, pch=" ", cex=0.8)
# Use different information to indicate which features are replicates
plotCtVariation(qPCRraw, type="detail", feature.reps=paste("test", rep(1:96, each=4)))
```

```
# Examine variation across samples for the first 9 features
plotCtVariation(qPCRraw[1:9,], type="detail", sample.reps=paste("mutant", rep(1:3,2)), add.featurenames=TRUE)

# Examine the output
test <- plotCtVariation(qPCRraw, variation="sd")
names(test)
head(test[["Var"]])
```

---

plotCVBoxes *Boxplots of CV for qPCR Ct values.*

Description

Function that will calculate the coefficients of variation across selected qPCR data, and plot the results in a boxplot.

Usage

```
plotCVBoxes(q, cards = TRUE, xlab = "", ylab = "CV", col = brewer.pal(5, "Spectral"), main = NULL, strati
```

Arguments

q	object of class qPCRset.
cards	vector, the numbers of the cards to plot. Defaults to TRUE = all cards.
xlab	character string, label for the x-axis.
ylab	character string, label for the y-axis.
col	vector of colours to use.
main	character string, plot title.
stratify	character, specifying what to stratify the Ct values by. NULL, the default means no stratification, "type" is the feature types of the qPCRset, and "class" the feature class.
...	any other arguments will be passed to the boxplot function.

Details

The CV is calculated across all the selected cards based on each well position, without taking possibly replicated genes on the cards into consideration. "type" and "class" are automatically extracted from the qPCRset using featureType and featureClass.

Value

A plot is created on the current graphics device. The CV values are returned invisibly.

Author(s)

Heidi Dvinge

See Also

boxplot

Examples

```
# Load example data
data(qPCRraw)
# Make plot with all samples or just a few
plotCVBoxes(qPCRraw)
plotCVBoxes(qPCRraw, cards=c(1,4))
plotCVBoxes(qPCRraw, stratify="class")
x <- plotCVBoxes(qPCRraw, stratify="type")
x[1:10]
```

---

qPCRpros *Example processed qPCR data*

Description

Processed version of the raw data in qPCRraw, to be used as example data in the HTqPCR package. The data has been processed with `setCategory` to mark the feature categories, and with `normalizeCtData` using rank invariant normalisation.

Usage

```
data(qPCRpros)
```

Format

The format is: Formal class 'qPCRset' [package ".GlobalEnv"] with 8 slots ..@ CtHistory :'data.frame': 3 obs. of 1 variable: .. ..$ history: chr [1:3] "readCtData(files = exFiles$File, path = exPath)" "setCategory(q = qPCRraw, groups = exFiles$Treatment)" "normalizeCtData(q = qPCRpros, norm = \"norm.rankinvariant\")" ..@ assayData :<environment: 0x1180c9400> ..@ phenoData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots .. .. ..@ varMetadata :'data.frame': 1 obs. of 1 variable: .. ..$ labelDescription: chr "Sample numbering" .. .. ..@ data :'data.frame': 6 obs. of 1 variable: .. ..$ sample: int [1:6] 1 2 3 4 5 6 .. .. ..@ dimLabels : chr [1:2] "sampleNames" "sampleColumns" .. .. ..@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots .. ..@ .Data:List of 1 .. ..$: int [1:3] 1 1 0 ..@ featureData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots .. .. ..@ varMetadata :'data.frame': 4 obs. of 1 variable: .. ..$ labelDescription: chr [1:4] NA NA NA NA .. .. ..@ data :'data.frame': 384 obs. of 4 variables: .. ..$ featureNames: Factor w/ 191 levels "Gene1","Gene10",..: 1 104 115 126 137 148 159 170 181 2 ... .$ featureType : Factor w/ 2 levels "Endogenous Control",..: 1 2 2 2 2 2 2 2 2 2 ... .$ featurePos : Factor w/ 384 levels "A1","A10","A11",..: 1 12 18 19 20 21 22 23 24 2 ... .$ featureClass: Factor w/ 3 levels "Kinase","Marker",..: 1 2 1 3 2 2 2 3 1 2 ... .@ dimLabels : chr [1:2] "featureNames" "featureColumns" .. .. ..@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots .. ..@ .Data:List of 1 .. ..$: int [1:3] 1 1 0 ..@ experimentData :Formal class 'MIAME' [package "Biobase"]

with 13 slots .. .. ..@ name : chr "" .. .. ..@ lab : chr "" .. .. ..@ contact : chr "" .. .. ..@ title : chr "" .. .. ..@ abstract : chr "" .. .. ..@ url : chr "" .. .. ..@ pubMedIds : chr "" .. .. ..@ samples : list() .. .. ..@ hybridizations : list() .. .. ..@ normControls : list() .. .. ..@ preprocessing : list() .. .. ..@ other : list() .. .. ..@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots .. ..@ .Data:List of 2 .. ..$: int [1:3] 1 0 0 .. ..$: int [1:3] 1 1 0 ..@ annotation : chr(0) ..@ protocolData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots .. .. ..@ varMetadata :'data.frame': 0 obs. of 1 variable: .. ..$ labelDescription: chr(0) .. .. ..@ data :'data.frame': 6 obs. of 0 variables .. .. ..@ dimLabels : chr [1:2] "sampleNames" "sampleColumns" .. .. ..@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots .. ..@ .Data:List of 1 .. ..$: int [1:3] 1 1 0 ..@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots .. .. ..@ .Data:List of 3 .. ..$: int [1:3] 2 14 0 .. ..$: int [1:3] 2 14 0 .. ..$: int [1:3] 1 3 0

Examples

```
data(qPCRpros)
```

---

qPCRraw *Example raw qPCR data.*

Description

Six qPCR samples, performed on the TaqMan Low Density Arrays from Applied Biosystem. Each sample contains 384 PCR reactions, and there are 3 different samples with 2 replicates each. To be used as example data in the HTqPCR package.

Usage

```
data(qPCRraw)
```

Format

The format is: Formal class 'qPCRset' [package ".GlobalEnv"] with 8 slots ..@ CtHistory :'data.frame': 1 obs. of 1 variable: .. ..$ history: chr "readCtData(files = exFiles$File, path = exPath)" ..@ assayData :<environment: 0x118094e30> ..@ phenoData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots .. .. ..@ varMetadata :'data.frame': 1 obs. of 1 variable: .. ..$ labelDescription: chr "Sample numbering" .. .. ..@ data :'data.frame': 6 obs. of 1 variable: .. ..$ sample: int [1:6] 1 2 3 4 5 6 .. .. ..@ dimLabels : chr [1:2] "sampleNames" "sampleColumns" .. .. ..@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots .. ..@ .Data:List of 1 .. ..$: int [1:3] 1 1 0 ..@ featureData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots .. .. ..@ varMetadata :'data.frame': 4 obs. of 1 variable: .. ..$ labelDescription: chr [1:4] NA NA NA NA .. .. ..@ data :'data.frame': 384 obs. of 4 variables: .. ..$ featureNames: Factor w/ 191 levels "Gene1","Gene10",..: 1 104 115 126 137 148 159 170 181 2 ... .$ featureType : Factor w/ 2 levels "Endogenous Control",..: 1 2 2 2 2 2 2 2 2 2 ... .$ featurePos : Factor w/ 384 levels "A1","A10","A11",..: 1 12 18 19 20 21 22 23 24 2 ... .$ featureClass: Factor w/ 3 levels "Kinase","Marker",..: 1 2 1 3 2 2 2 3 1 2 ... .@ dimLabels : chr [1:2] "featureNames" "featureColumns" .. .. ..@ .__classVersion__:Formal

class 'Versions' [package "Biobase"] with 1 slots .. ..@ .Data:List of 1 .. ..$: int [1:3] 1 1 0 ..@ experimentData :Formal class 'MIAME' [package "Biobase"] with 13 slots .. .. ..@ name : chr "" .. .. ..@ lab : chr "" .. .. ..@ contact : chr "" .. .. ..@ title : chr "" .. .. ..@ abstract : chr "" .. .. ..@ url : chr "" .. .. ..@ pubMedIds : chr "" .. .. ..@ samples : list() .. .. ..@ hybridizations : list() .. .. ..@ normControls : list() .. .. ..@ preprocessing : list() .. .. ..@ other : list() .. .. ..@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots .. ..@ .Data:List of 2 .. ..$: int [1:3] 1 0 0 .. ..$: int [1:3] 1 1 0 ..@ annotation : chr(0) ..@ protocolData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots .. .. ..@ varMetadata :'data.frame': 0 obs. of 1 variable: .. ..$ labelDescription: chr(0) .. .. ..@ data :'data.frame': 6 obs. of 0 variables .. .. ..@ dimLabels : chr [1:2] "sampleNames" "sampleColumns" .. .. ..@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots .. ..@ .Data:List of 1 .. ..$: int [1:3] 1 1 0 ..@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots .. .. ..@ .Data:List of 3 .. ..$: int [1:3] 2 14 0 .. ..$: int [1:3] 2 14 0 .. ..$: int [1:3] 1 3 0

Examples

```
data(qPCRraw)
```

`qPCRset-class` *Class "qPCRset"*

Description

This is a class for containing the raw or normalized cycle threshold (Ct) values and some related quality information. It is suitable for TaqMan Low Density Arrays or any other type of (high-throughput) qPCR data, where gene expression is measured for any number of genes, across several samples/conditions. It inherits from `eSet` for microarray data.

Objects from the Class

Objects can be created by calls of the form `new("qPCRset", assayData, phenoData, featureData, experimentData, annotation, protocolData, ...)` or using `readCtData`.

Slots

`CtHistory`**:** Object of class `"data.frame"` indicating how the data has been read in, normalized, filtered etc. Gives the exact commands used during these operations.

`assayData`**:** Object of class `"AssayData"`, containing the Ct values.

`phenoData`**:** Object of class `"AnnotatedDataFrame"`, where information about samples can be added.

`featureData`**:** Object of class `"AnnotatedDataFrame"`, where information about features can be added. If the object is from `readCtData`, the featureData will contain the columns 'featureName', 'featurePos' and 'featureType'.

`experimentData`**:** Object of class `"MIAxE"`, where details about the experiment can be stored.

`annotation`**:** Object of class `"character"`, where the name of the qPCR assay can be stored.

`protocolData`**:** Object of class `"AnnotatedDataFrame"`, where details of the protocol can be stored.

`.__classVersion__`**:** Object of class `"Versions"`.

Furthermore, the following information is contained within the object.

`flag`**:** Object of class `"data.frame"` containing the flag for each Ct value, as supplied by the input files.

`featureCategory`**:** Object of class `"data.frame"` representing the quality of the measurement for each Ct value, such as "OK", "Undetermined" or "Unreliable" if the Ct value is considered too high.

Extends

Class `"eSet"`, directly. Class `"VersionedBiobase"`, by class "eSet", distance 2. Class `"Versioned"`, by class "eSet", distance 3.

Methods

[`signature(x = "qPCRset")`: Subsets by genes or samples.

exprs `signature(object = "qPCRset")`: Extracts the Ct matrix. Is identical to `getCt`

exprs<- `signature(object = "qPCRset", value = "matrix")`: Replaces the Ct matrix. Is identical to `setCt<-`

getCt `signature(object = "qPCRset")`: Extracts the Ct matrix. Is identical to `exprs`.

setCt<- `signature(object = "qPCRset", value = "matrix")`: Replaces the Ct matrix. Is identical to `exprs<-`.

featureNames `signature(object = "qPCRset")`: Extracts the features (gene names) on the card.

featureNames<- `signature(object = "qPCRset", value = "character")`: Replaces the features (gene names) on the card.

sampleNames `signature(object = "qPCRset")`: Extracts the sample names.

sampleNames<- `signature(object = "qPCRset", value = "character")`: Replaces the sample names.

featureType `signature(object = "qPCRset")`: Extracts the different types of features on the card, such as controls and target genes.

featureType<- `signature(object = "qPCRset", value = "factor")`: Replaces the feature type for each gene.

featurePos `signature(object = "qPCRset")`: Extracts the position of each feature (gene) on the assay, representing the location "well" (such as well A1, A2, ...). If data does not come from a card format, the positions will be given consecutive names.

featurePos<- `signature(object = "qPCRset", value = "character")`: Replaces the position of each feature (gene) on the card.

featureClass `signature(object = "qPCRset")`: Extracts the feature class for each gene.

featureClass<- `signature(object = "qPCRset", value = "factor")`: Replaces the feature class for each gene, for example if it is a marker, transcription factor or similar.

featureCategory `signature(object = "qPCRset")`: Extracts the category of each Ct value.

featureCategory<- `signature(object = "qPCRset", value = "data.frame")`: Replaces the category of each Ct value.

flag `signature(object = "qPCRset")`: Extracts the flag of each Ct value.

flag<- `signature(object = "qPCRset")`: Replaces the flag of each Ct value.

n.wells `signature(object = "qPCRset")`: Extracts information about the number of wells on the card.

n.samples `signature(object = "qPCRset")`: Extracts information about the number of samples in the set.

getCtHistory `signature(object = "qPCRset")`: Extracts data frame containing information about the history of the object (which operations have been performed on it).

setCtHistory<- `signature(object = "qPCRset")`: Add information about the history of the object.

show `signature(object = "qPCRset")`: Displays some abbreviated information about the data object.

summary `signature(object = "qPCRset")`: Displays a summary of the Ct values from each sample.

Author(s)

Heidi Dvinge

Examples

```
# The data format
data(qPCRraw)
show(qPCRraw)
getCtHistory(qPCRraw)
showClass("qPCRset")
str(qPCRraw)
# Information about samples
phenoData(qPCRraw)
pData(qPCRraw)
pData(qPCRraw)[,"Rep"] <- c(1,1,2,2,3,3)
# Information about features
featureData(qPCRraw)
head(fData(qPCRraw))
```

`readCtData` *Reading Ct values from qPCR experiments data into a qPCRset*

Description

This function will read tab separated text files with Ct values and feature meta-data from high-throughput qPCR experiments into a qPCRset containing all the relevant information.

Usage

```
readCtData(files, path = NULL, n.features = 384, format="plain", column.info, flag, feature, type, posi
```

Arguments

files	character vector with the names of the files to be read.
path	character string with the path to the folder containing the data files.
n.features	integer, number of features present on each array (e.g. 384). See details.
format	character, the format of the input file. Options are "plain", "SDS", "LightCycler", "CFX", "OpenArray" and "BioMark". See Details.
column.info	list, indicating which column number or name the information of interest is in. It is set automatically by format, but this can be overridden manually. The names list slots can be 'flag', 'feature', 'position', 'type and 'Ct'. See Details. Note than when indicating column names, these are sometimes changed by R to be syntactically valid, so replacing e.g. brackets by dots.
flag, feature, Ct, type, position	deprecated, use column.info instead.
header	logical, does the file contain a header row or not. Only used for format="plain".
SDS	deprecated, use format="SDS" instead.
n.data	integer vector, same length as files. Indicates the number of samples that are present in each file. For each file in files, n.data*n.features lines will be read.
samples	character vector with names for each sample. Per default the file names are used.
na.value	integer, a Ct value that will be assigned to all undetermined/NA wells.
sample.info	object of class AnnotatedDataFrame, given the phenoData of the object. Can be added later.
...	any other arguments are passed to read.table or read.csv.

Details

This is the main data input function for the HTqPCR package for analysing qPCR data. It extracts the threshold cycle, Ct value, of each well on the card, as well as information about the quality (e.g.~passed/failed) of the wells. The function is tuned for data from TaqMan Low Density Array cards, but can be used for any kind of qPCR data.

The information to be extracted is:

- flag integer indicating the number of column containing information about the flags.
- feature integer indicating the number of column containing information about the individual features (typically gene names).
- type integer indicating the number of column containing information about the type of each feature.
- position integer indicating the number of column containing information about the position of features on the card.
- Ct integer indicating the number of column containing information about the Ct values. Per default, this information is assumed to be in certain columns depending on the input format.

featureNames, featureType and featurePos will be extracted from the first file. If flag, type or position are not included into column.info, this means that this information is not available in the file. flag will then be set to "Passed", type to "Target" and position to "feature1", "feature2", ... etc until the end of the file. Especially position might not be available in case the data does not come from a card format, but it is required in subsequent functions in order to disambiguate in case some features are present multiple times.

format indicates the format of the input file. The options currently implemented are:

- plain A tab-separated text file, containing no header unless header=TRUE. The information extracted defaults to column.info=list(flag=4, feature=6, type=7, position=3, Ct=8).
- SDS An output file from the SDS Software program. This is often used for the TaqMan Low Density Arrays from Applied Biosystems, but can also be used for assays from other vendors, such as Exiqon. column.info is the same as for "plain".
- OpenArray The TaqMan OpenArray Real-Time PCR Plates. The information extracted defaults to column.info=list(flag="ThroughHole.Outlier", feature="Assay.Assay.ID", type="Assay.Assay.Type", position="ThroughHole.Address", Ct="ThroughHole.Ct").
- BioMark The BioMark HD System from Fluidigm, currently including the 48.48 and 96.96 assays. The information extracted defaults to column.info=list(flag="Call", feature="Name.1", position="ID", Ct="Value").
- CFX The CFX Automation System from Bio-Rad. The information extracted defaults to column.info=list(feature="Content", position="Well", Ct="Cq.Mean").
- LightCycler The LightCycler System from Roche Applied Science. The information extracted defaults to column.info=list(feature="Name", position="Pos", Ct="Cp").

The BioMark and OpenArray assays always contain information multiple samples on each assay, such as 48 features for 48 samples for the BioMark 48.48. The results across these samples are always present in a single file, e.g. with 48x48=2304 rows. Setting n.features=2304 will read in all the information and create a qPCRset object with dimensions 2304x1. Setting n.data=48 and n.features=48 will however automatically convert this into a 48x48 qPCRset. See openVignette(package="HTqPCR") for examples. The samples are being read in the order in which they're present in the file, i.e. from row 1 onwards, regardless of how they're loaded onto the particular platform.

If the data was analysed using for example SDS Software it may contain a variable length header specifying parameters for files that were analysed at the same time. If format="SDS" then readCtData will scan through the first 100 lines of each file, and skip all lines until (and including) the line beginning with "#", which is the header. The end of the file might also contain some plate ID information, but only the number of lines specified in n.features will be read.

n.features indicates the number of features present on each array. For example, for a 384 well plate with just 1 sample, the number would be 382. For a plate with 2 individual samples loaded onto it, n.features=196 and n.data=2. For 1 file with 5 plates and 2 samples per plate, the numbers are n.features=196 and n.data=10. n.features*n.data must correspond to the total number of lines to be read from each file.

Value

A "qPCRset" object.

Warnings

The files are all assumed to belong to the same design, i.e.~have the same features (genes) in them and in identical order.

Author(s)

Heidi Dvinge

See Also

`read.delim` for further information about reading in data, and `"qPCRset"` for a definition of the resulting object.

Examples

```
# Locate example data and create qPCRset object
exPath <- system.file("exData", package="HTqPCR")
exFiles <- read.delim(file.path(exPath, "files.txt"))
raw <- readCtData(files=exFiles$File, path=exPath)
# Example of adding missing information (random data in this case)
featureClass(raw) <- factor(rep(c("A", "B", "C"), each=384/3))
pData(raw)[,"rep"]  <- c(1,1,2,2,3,3)

## See the package vignette for more examples, including different input formats.
```

---

setCategory *Assign categories to Ct values from qPCR data.*

Description

Data in qPCRset objects will have feature categories ("Unreliable", "Undetermined") assigned to them based on different Ct criteria.

Usage

```
setCategory(q, Ct.max = 35, Ct.min = 10, replicates = TRUE, quantile = 0.9, groups, flag = TRUE, flag.out
```

Arguments

`q`	qPCRset object.
`Ct.max`	numeric, the maximum tolerated Ct value. Everything above this will be "Undetermined".
`Ct.min`	numeric, the minimum tolerated Ct value. Everything below this will be "Unreliable".
`replicates`	logical, should Ct values from genes replicated within each sample be collapsed for the standard deviation.

quantile	numeric from 0 to 1, the quantile interval accepted for standard deviations. See details. NULL means that variation between replicates is not used for setting the categories.
groups	vector, grouping of cards, for example biological or technical replicates. NULL means that variation between groups os samples is not assessed, same as for setting quantile=NULL.
flag	logical, should categories also be set to "Unreliable" according to the content of flag(q).
flag.out	character vector, if flag=TRUE, what are the flag(s) to be set as "Unreliable".
verbose	logical, should a summary about category counts per sample be printed to the prompt.
plot	logical, should some plots of the standard deviations be created.
...	any other arguments are passed to plot.

Details

Categories can be assigned to the featureCategory of the qPCRset using either just simple criteria (max/min of Ct values or flag of q) or by looking at the standard deviation of Ct values across biological and technical replicates for each gene.

When looking at replicates, the standard deviation and mean are calculated and a normal distribution following these parameters is generated. Individual Ct values that are outside the interval set by quantile are set as "Unreliable". So if e.g. quantile=90 the values outside the top 5% and lower 5% of the normal distribution with the given mean and standard deviation are removed.

"Undetermined" has priority over "Unreliable", so if a value is outside quantile but also above Ct.max it will be "Undetermined".

NB: When setting categories based on replicates, the Ct values are assumed to follow a normal distribution. This might not be the case if the number of samples within each group is small, and there are no replicates on the genes within each sample.

If the number of replicates vary significantly between biological groups, this will influence the thresholds used for determining the range of "OK" Ct values.

Value

If plot=TRUE one figure per sample group is returned to the current graphics device. A qPCRset with the new feature categories is returned invisibly.

Note

It's adviced to try several different values for quantile, depending on the input data set. Using the function PlotCtCategory(..., by.feature=FALSE) or plotCtCategory(..., by.feature=TRUE) might help assess the result of different quantile choices.

Author(s)

Heidi Dvinge

See Also

filterCategory, plotCtCategory

Examples

```
# Load example data
data(qPCRraw)
exFiles <- read.delim(file.path(system.file("exData", package="HTqPCR"), "files.txt"))
 # Set categories in various ways
setCategory(qPCRraw, flag=FALSE, quantile=NULL)
```

---

ttestCtData *Differentially expressed features with qPCR: t-test*

Description

Function for calculating t-test and p-values across two groups for the features present in high-throughput qPCR data, such as from TaqMan Low Density Arrays.

Usage

```
ttestCtData(q, groups = NULL, calibrator, alternative = "two.sided", paired = FALSE, replicates = TRUE,
```

Arguments

q	qPCRset object.
groups	factor, assigning each sample to one of two groups.
calibrator	which of the two groups is to be considered as the reference and not the test? Defaults to the first group in groups.
alternative	character string (first letter is enough), specifying the alternative hypothesis, "two.sided" (default), "greater" or "less".
paired	logical, should a paired t-test be used.
replicates	logical, if replicated genes are present on the array, the statistics will be calculated for all the replicates combined, rather than the individual wells.
sort	boolean, should the output be sorted by p-values.
stringent	boolean, for flagging results as "Undetermined". See details.
p.adjust	character string, which method to use for p-value adjustment for multiple testing. See details.
...	any other arguments will be passed to the t.test function.

Details

Once the Ct values have been normalised, differential expression can be calculated. This function deals with just the simple case, where there are two types of samples to compare. For more complex studies, see `limmaCtData`.

All results are assigned to a category, either "OK" or "Undetermined" depending on the input Ct values. If `stringent=TRUE` any unreliable or undetermined measurements among technical and biological replicates will result in the final result being "Undetermined". For `stringent=FALSE` the result will be "OK" unless at least half of the Ct values for a given gene are unreliable/undetermined.

The argument `p.adjust` is passed on to the `p.adjust` function. Options include e.g. "BH" (Benjamini & Hochberg, the default), "fdr" and "bonferroni". See `p.adjust` for more information on the individual methods.

Value

A data.frame containing the following information:

`genes`	The names of the features on the card.
`feature.pos`	The `featurePos` of the genes. If replicated genes are used, the feature positions will be concatenated together.
`t.test`	The value of the t-test.
`p.value`	The corresponding p-value.
`ddCt`	The delta delta Ct values.
`FC`	The fold change; 2^(-ddCt).
`meanCalibrator`	The average expression level of each gene in the calibrator sample(s).
`meanTarget`	The average expression level of each gene in the target sample(s).
`categoryCalibrator`	The category of the Ct values ("OK", "Undetermined") across the calibrator.
`categoryTarget`	Ditto for the target.

Author(s)

Heidi Dvinge

See Also

`t.test`, `limmaCtData`, `mannwhitneyCtData`. `plotCtRQ` and `plotCtSignificance` can be used for visualising the results.

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