

Package ‘qpcR’

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Title Modelling and analysis of real-time PCR data

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Description Model fitting, optimal model selection and calculation of various features that are essential in the analysis of quantitative real-time polymerase chain reaction (qPCR).

License GPL (>= 2)

Depends R (>= 2.13.0), MASS, minpack.lm, rgl, robustbase

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AICc

*Akaike's second-order corrected Information Criterion***Description**

Calculates the second-order corrected Akaike Information Criterion for objects of class `pcrfit`, `nls`, `lm`, `glm` or any other models from which `coefficients` and `residuals` can be extracted. This is a modified version of the original AIC which compensates for bias with small n . As qPCR data usually has $\frac{n}{k} < 40$ (see original reference), AICc was implemented to correct for this.

Usage

```
AICc(object)
```

Arguments

`object` a fitted model.

Details

Extends the AIC such that

$$AICc = AIC + \frac{2k(k+1)}{n-k-1}$$

with k = number of parameters, and n = number of observations. For large n , AICc converges to AIC.

Value

The second-order corrected AIC value.

Author(s)

Andrej-Nikolai Spiess

References

Akaike Information Criterion Statistics.
Sakamoto Y, Ishiguro M and Kitagawa G.
D. Reidel Publishing Company (1986).

Regression and Time Series Model Selection in Small Samples.
Hurvich CM & Tsai CL.
Biometrika (1989), **76**: 297-307.

See Also

[AIC](#), [logLik](#).

Examples

```
m1 <- pcrfit(reps, 1, 2, 15)
AICc(m1)
```

 akaike.weights

Calculation of Akaike weights/relative likelihoods/delta-AICs

Description

Calculates Akaike weights from a vector of AIC values.

Usage

```
akaike.weights(x)
```

Arguments

x a vector containing the AIC values.

Details

Although Akaike's Information Criterion is recognized as a major measure for selecting models, it has one major drawback: The AIC values lack intuitivity despite higher values meaning less goodness-of-fit. For this purpose, Akaike weights come to hand for calculating the weights in a regime of several models. Additional measures can be derived, such as $\Delta(AIC)$ and relative likelihoods that demonstrate the probability of one model being in favor over the other. This is done by using the following formulas:

delta AICs:

$$\Delta_i(AIC) = AIC_i - \min(AIC)$$

relative likelihood:

$$L \propto \exp\left(-\frac{1}{2}\Delta_i(AIC)\right)$$

Akaike weights:

$$w_i(AIC) = \frac{\exp(-\frac{1}{2}\Delta_i(AIC))}{\sum_{k=1}^K \exp(-\frac{1}{2}\Delta_k(AIC))}$$

Value

A list containing the following items:

| | |
|----------|---------------------------|
| deltaAIC | the $\Delta(AIC)$ values. |
| rel.LL | the relative likelihoods. |
| weights | the Akaike weights. |

Author(s)

Andrej-Nikolai Spiess

References

Classical literature:
 Akaike Information Criterion Statistics.
 Sakamoto Y, Ishiguro M and Kitagawa G.
 D. Reidel Publishing Company (1986).

Model selection and inference: a practical information-theoretic approach.
 Burnham KP & Anderson DR.
 Springer Verlag, New York, USA (2002).

A good summary:
 AIC model selection using Akaike weights. Wagenmakers EJ & Farrell S.
Psychonomic Bull Review (2004), **11**: 192-196.

See Also

[AIC, logLik.](#)

Examples

```
## apply a list of different sigmoidal models to data
## and analyze GOF statistics with Akaike weights
## on 6 different sigmoidal models
modList <- list(l5, l4, l3, b5, b4, b3)
aics <- sapply(modList, function(x) AIC(pcrfit(reps, 1, 2, x)))
akaike.weights(aics)$weights
```

 batchstat

Concatenating or calculating statistics on a 'pcrbatch'

Description

This function will either concatenate data from several pcrbatches or calculate some user-defined statistic on the runs within a pcrbatch. If the latter is chosen, a grouping vector must be supplied for defining the runs to be subjected to statistical analysis.

Usage

```
batchstat(..., group = NULL, do = c("cbind", "stat"), statfun = mean)
```

Arguments

| | |
|---------|--|
| ... | one or more pcrbatches. See 'Examples'. |
| group | in case of do = "stat", a vector defining the groups for statistical analysis. |
| do | concatenate or analyse? |
| statfun | the statistical function to be used if do = "stat". |

Details

statfun can be any internal R function, i.e. sd, median etc.

Value

Either a concatenated dataframe (do = "cbind"), or a list containing a dataframe(s) with the statistical output for each factor level defined in group, if do = "stat".

Author(s)

Andrej-Nikolai Spiess

Examples

```
## Not run:
## create 3 'pcrbatch'es
## and concatenate
dat1 <- pcrbatch(reps, fluo = 2:5, model = 14, plot = FALSE)
dat2 <- pcrbatch(reps, fluo = 6:9, model = 14, plot = FALSE)
dat3 <- pcrbatch(reps, fluo = 10:13, model = 14, plot = FALSE)
batchstat(dat1, dat2, dat3)

## one 'pcrbatch' and doing
## mean on replicates
## defined by 'group'
dat4 <- pcrbatch(reps, fluo = 2:9, model = 14, plot = FALSE)
GROUP <- c(1, 1, 1, 1, 2, 2, 2, 2)
```

```
batchstat(dat4, do = "stat", group = GROUP, statfun = mean)

## get the standard deviation
batchstat(dat4, do = "stat", group = GROUP, statfun = sd)

## do stats on many 'pcrbatch'es
## All batches must have same length!
batchstat(dat1, dat2, dat3, do = "stat",
          group = c(1, 1, 2, 2))

## End(Not run)
```

batschetal

qPCR dilution experiments from Batsch et al. (2008)

Description

High quality 4-fold dilution experiments with 5 dilution steps and 3 replicates each. See 'Details' for the different setups.

Usage

```
batsch1
batsch2
batsch3
batsch4
batsch5
```

Format

Data frames with the PCR cycles and 15 qPCR runs with 3 replicates of five 4-fold dilutions. The replicates are defined by FX.Y (X = dilution number, Y = replicate number).

Details

The real-time PCR was conducted with a Lightcycler 1.0 instrument using the following setups:

```
batsch1: Primers for rat SLC6A14, Taqman probes
batsch2: Primers for human SLC22A13, Taqman probes
batsch3: Primers for pig EMT, Taqman probes
batsch4: Primers for chicken ETT, SybrGreen
batsch5: Primers for human GAPDH, SybrGreen
```

Source

Additional File 5 to the paper.

References

Simultaneous fitting of real-time PCR data with efficiency of amplification modeled as Gaussian function of target fluorescence.

Batsch A, Noetel A, Fork C, Urban A, Lazic D, Lucas T, Pietsch J, Lazar A, Schoemig E & Gruendemann D.

BMC Bioinformatics (2008), **9**: 95.

Examples

```
m11 <- modlist(batsch1, model = 14)
plot(m11)
```

boggy

qPCR dilution experiments from Boggy et al. (2010)

Description

A dilution experiment with six 10-fold dilutions of a synthetic template, and two replicates for each dilution.

Usage

boggy

Format

A data frame with the PCR cycles and 12 qPCR runs with two replicates of six 10-fold dilutions. The replicates are defined by F1.1 - F1.2 (first dilution), F2.1 - F2.2 (second dilution) etc.

Details

The real-time PCR was conducted with primers for a synthetic template, consisting of a secondary structure-optimized random sequence (129 bp), in a Chromo4 instrument (BioRad) with Syto-13 dye.

Source

Additional File S1 to the paper.

References

A Mechanistic Model of PCR for Accurate Quantification of Quantitative PCR Data.

Boggy GJ & Woolf PJ.

PLOS One (2010), **5**: e12355.

Examples

```
m1 <- pcrfit(boggy, 1, 2, 15)
plot(m1)
```

calib

*Calculation of qPCR efficiency by dilution curve analysis***Description**

This function calculates the PCR efficiency from a classical qPCR dilution experiment. The threshold cycles are plotted against the logarithmized concentration (or dilution) values, a linear regression line is fit and the efficiency calculated by $E = 10^{\frac{-1}{\text{slope}}}$. A graph is displayed with the raw values plotted with the threshold cycle and the linear regression curve. The threshold cycles are calculated either by some arbitrary fluorescence value (i.e. as given by the qPCR software) or calculated from the second derivative maximum of the first (highest concentration) curve. If values to be predicted are given, they are calculated from the curve and also displayed within. An iterative search of the optimal threshold border can be conducted, thereby going through all slopes and intercepts and selecting the combination that minimizes the AIC of the acquired linear regression curves. See 'Details' for more information on this (maybe controversial) procedure.

Usage

```
calib(refcurve, predcurve = NULL, thresh = "cpD2", term = NULL,
      dil = NULL, plot = TRUE, plot.map = TRUE,
      conf = 0.95, opt = c("none", "inter", "slope"),
      opt.step = c(50, 50), stop.crit = c("outlier", "midpoint"),
      quan = 0.5, slope = NULL, count = 1)
```

Arguments

| | |
|-----------|---|
| refcurve | a 'modlist' containing the curves for calibration. |
| predcurve | an (optional) 'modlist' containing the curves for prediction. |
| thresh | the fluorescence value from which the threshold cycles are defined. Either "cpD2" or a numeric value. |
| term | an (optional) numeric value for the terminating intercept. See 'Details'. |
| dil | a vector with the concentration (or dilution) values corresponding to the calibration curves. |
| plot | logical. Should the optimization process be displayed? If FALSE, only values are returned. |
| plot.map | logical. Should a final heatmap display from the goodness-of-fit of all iterations be displayed? |
| conf | the confidence interval. Defaults to 95%, can be omitted with NULL. |
| opt | type of optimization. See 'Details'. |

| | |
|-----------|---|
| opt.step | a two-element vector. Number of iterations for the intercept as first item, number of slope iterations as second. |
| stop.crit | the stopping criterium for the iterations. Default is the first outlier cycle of the highest dilution curve. |
| quan | the top quantile of iterations to be shown in the heatmap. |
| slope | a slope to be defined for the threshold line. Mostly used internally by the iteration process. |
| count | internal counter for recursive purposes. Not to be altered. |

Details

The iterative function conducts a search through all combinations of slope and intercept. For each iteration, either R^2 or AIC of the resulting calibration curves are collected, and finally the combination is selected that minimized the AIC. The function goes through all combinations as to avoid local maxima that are likely to happen in this approach. The different settings for opt are:

"none" only second derivative maximum or single threshold value.

"inter" iterate along the y-axis intercept.

"slope" iterate along y-axis intercept and slope values.

The paradigm is such that the iterations will start at the second derivative of the first (lowest dilution; highest copy number) curve and terminate at the outlier cycle of the last (highest dilution; lowest copy number) curve. Alternatively a y-value can be given with term for the termination threshold. The number of iterations can be defined by opt.step but the default values usually give reasonable results. Not to forget, an iterative search only throughout all intercepts can be chosen, as well as a classical approach using the second derivative maximum of the first curve or a defined threshold value from the qPCR software, to be defined in thresh. See 'Examples'.

Value

A list with the following components:

| | |
|---------------|---|
| refcyc | the calculated threshold cycles for the calibration curves. |
| refcyc.conf | the confidence intervals for refcyc. |
| predcyc | the calculated threshold cycles for the prediction curves. |
| predconc | the predicted concentrations. |
| predconc.conf | the confidence intervals for predconc. |
| eff | the efficiency as calculated from the calibration curve. |
| aic | the AIC value of the linear fit. |
| aicc | the corrected AIC value of the linear fit. |
| rsq | The R^2 of the linear fit. |
| rsq.ad | The adjusted R_{adj}^2 of the linear fit. |
| aicMat | a matrix with the calibration curve AIC of each iteration. |

ATTENTION: If iterations were used, the values reflect the analysis of the best fit!

Author(s)

Andrej-Nikolai Spiess

Examples

```
## Define calibration curves,
## dilutions (or copy numbers)
## and curves to be predicted.
## Do background subtraction using
## average of first 8 cycles
CAL <- modlist(reps, fluo = c(2, 6, 10, 14, 18, 22), backsub = 1:8)
COPIES <- c(100000, 10000, 1000, 100, 10, 1)
PRED <- modlist(reps, fluo = c(3, 7, 11), backsub = 1:8)

## conduct normal quantification using
## the second derivative maximum of
## first curve
res1 <- calib(refcurve = CAL, predcurve = PRED, thresh = "cpD2", dil = COPIES)

## using a defined threshold value
res2 <- calib(refcurve = CAL, predcurve = PRED, thresh = 0.5, dil = COPIES)

## Not run:
## using replicates for reference curve
m11 <- modlist(reps, model = 14)
DIL <- rep(10^(6:0), each = 4)
res1 <- calib(refcurve = m11, dil = DIL)

## iterating the intercept with 50 steps
par(ask = FALSE)
res2 <- calib(refcurve = CAL, predcurve = PRED, dil = COPIES, opt = "inter",
              opt.step = c(50, 0))

## iterating the intercept/slope with 20 steps
par(ask = FALSE)
res3 <- calib(refcurve = CAL, predcurve = PRED, dil = COPIES, opt = "slope",
              opt.step = c(20, 20))

## End(Not run)
```

calib2

Calculation of qPCR efficiency by dilution curve bootstrapping

Description

This function calculates the PCR efficiency from a classical qPCR dilution experiment. The threshold cycles are plotted against the logarithmized concentration (or dilution) values, a linear regression line is fit and the efficiency calculated by $E = 10^{\frac{-1}{\text{slope}}}$. A graph is displayed with the raw values plotted with the threshold cycle and the linear regression curve. The threshold cycles are calculated

either by some arbitrary fluorescence value (i.e. as given by the qPCR software) or calculated from the second derivative maximum of the dilution curves. If values to be predicted are given, they are calculated from the curve and also displayed within. `calib2` uses a bootstrap approach if replicates for the dilutions are supplied. See 'Details'.

Usage

```
calib2(refcurve, predcurve = NULL, thresh = "cpD2", dil = NULL,
       group = NULL, plot = TRUE, conf = 0.95, B = 200)
```

Arguments

| | |
|------------------------|---|
| <code>refcurve</code> | a 'modlist' containing the curves for calibration. |
| <code>predcurve</code> | an (optional) 'modlist' containing the curves for prediction. |
| <code>thresh</code> | the fluorescence value from which the threshold cycles are defined. Either "cpD2" or a numeric value. |
| <code>dil</code> | a vector with the concentration (or dilution) values corresponding to the calibration curves. |
| <code>group</code> | a factor defining the group membership for the replicates. See 'Examples'. |
| <code>plot</code> | logical. Should the fitting (bootstrapping) be displayed? If FALSE, only values are returned. |
| <code>conf</code> | the confidence interval. Defaults to 95%, can be omitted with NULL. |
| <code>B</code> | the number of bootstraps. |

Details

`calib2` calculates confidence intervals for efficiency, AICc, adjusted R_{adj}^2 and the prediction curve concentrations. If single replicates per dilution are supplied by the user, confidence intervals for the prediction curves are calculated based on asymptotic normality. If multiple replicates are supplied, the regression curves are calculated by randomly sampling one of the replicates from each dilution group. The confidence intervals are then calculated from the bootstrapped results.

Value

A list with the following components:

| | |
|------------------------|--|
| <code>eff</code> | the efficiency. |
| <code>AICc</code> | the second-order corrected AIC. |
| <code>Rsq.ad</code> | the adjusted R_{adj}^2 . |
| <code>predconc</code> | the (log) concentration of the predicted curves. |
| <code>conf.boot</code> | a list containing the confidence intervals for the efficiency, the AICc, <code>Rsq.ad</code> and the predicted concentrations. |

A plot is also supplied for efficiency, AICc, `Rsq.ad` and predicted concentrations including confidence intervals in red.

Author(s)

Andrej-Nikolai Spiess

Examples

```
## Define calibration curves,
## dilutions (or copy numbers)
## and curves to be predicted.
## Do background subtraction using
## average of first 8 cycles
CAL <- modlist(reps, fluo = c(2, 6, 10, 14, 18, 22), backsub = 1:8)
COPIES <- c(100000, 10000, 1000, 100, 10, 1)
PRED <- modlist(reps, fluo = c(3, 7, 11), backsub = 1:8)

## conduct normal quantification using
## the second derivative maximum of
## first curve
res1 <- calib2(refcurve = CAL, predcurve = PRED, thresh = "cpD2", dil = COPIES)

## using a defined threshold value
res2 <- calib2(refcurve = CAL, predcurve = PRED, thresh = 0.5, dil = COPIES)

## using six dilutions with
## four replicates/dilution
## Not run:
CAL2 <- modlist(reps, fluo = 2:25, backsub = 1:8)
res3 <- calib2(refcurve = CAL2, predcurve = PRED, thresh = "cpD2",
              dil = COPIES, group = gl(6,4))

## End(Not run)
```

curvemean

Building a model which averages a batch of qPCR curves

Description

Starting with a batch of qPCR curves from class `modlist`, the function builds a sigmoidal model which averages the cycle numbers or expression values of the curves at every y-value (raw fluorescence). Thus, in contrast to existing qPCR averaging methods, not only the efficiencies or threshold cycles (as can be obtained from multiple reference genes) are averaged, but the complete structure of the batch at every fluorescence value. Beware: This is curve averaging, NOT model averaging!

Usage

```
curvemean(ml, mean = c("cmean1", "cmean2", "amean", "gmean", "hmean"),
          which = 1, plot = TRUE)
```

Arguments

| | |
|--------------------|---|
| <code>m1</code> | a qPCR batch object of class 'modlist'. |
| <code>mean</code> | the averaging function, see 'Details'. Default is the expression mean. |
| <code>which</code> | the position of the curve in the list which is used for defining the y-values. Should be the curve with the lowest threshold cycle. |
| <code>plot</code> | should results be plotted? |

Details

Starting from the raw fluorescence values or expression values of a defined curve in the list, the cycle number of all other curves at this value are calculated and averaged with the method as under mean. After that, a new sigmoidal model is fit to the obtained data, using the same sigmoidal model as in `m1`. Note: often works better (more data points can be averaged) if `norm = TRUE` when buidling the model list with `modlist`. Five different averaging functions can be used:

Expression mean with efficiency = 2 ("cmean1"):

$$\bar{x}_{exp} = \frac{-\log\left(\frac{\sum_{i=1}^n 2^{-x_i}}{n}\right)}{\log(2)}$$

Expression mean with averaged efficiency from the runs ("cmean2"):

$$\bar{x}_{exp} = \frac{-\log\left(\frac{\sum_{i=1}^n \bar{E}^{-x_i}}{n}\right)}{\log(\bar{E})}$$

Arithmetic mean ("amean"):

$$\bar{x} = \frac{\sum_{i=1}^n x_i}{n}$$

Geometric mean ("gmean"):

$$\bar{x} = \left(\prod_{i=1}^n x_i\right)^{\frac{1}{n}}$$

Harmonic mean ("hmean"):

$$\bar{x} = \frac{n}{\sum_{i=1}^n \frac{1}{x_i}}$$

Why is `mean = "cmean1"` the default? This is because all classical versions of the 'mean' do not work in the context of real-time PCR. Consider the following example: We have a qPCR experiment with three biological replicates, yielding threshold cycles 25, 26, and 27. This means (with an efficiency = 2) that the second sample has half ($1/2^1$) and the third sample 1/forth ($1/2^2$) amount of initial copy numbers. If we plainly average (arithmetic mean) the threshold cycles, we obtain 26. But if we do this for the initial copy numbers, we obtain, for example, $(100 + 50 + 25)/3 = 58.33$. But $ct = 26$ corresponds to 50 copies, so the arithmetic mean of the threshold cycles significantly underestimates the initial copy numbers. The same accounts for the geometric and harmonic mean, as they all do not consider the exponential nature of the amplification. In contrast to this, the 'expression mean' does: `cmean1 = $-\log(\sum(2^{25-25} + 2^{26-26} + 2^{27-27})/3)/\log(2) = 25.77761$` . And

this values represents the averaged copy numbers because (calculated as ratios) $100/58.33$ gives the same as $2^{25.77761/2^{25}}$. Therefore, the expression mean calculates a cycle number which corresponds to the initial copy numbers of the replicates. If `mean = "cmean2"` is applied, the efficiency is calculated as the arithmetic mean of the efficiencies from all curves.

Value

A new model of class 'nls' and 'pcrfit' from the averaged curve.

Author(s)

Andrej-Nikolai Spiess

References

Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes.

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A & Speleman F. *Genome Biology* (2002), **3**: research0034.1-0034.11.

Examples

```
## create arithmetic mean curve of four
## serial dilutions
m11 <- modlist(reps, fluo = c(2, 6, 10, 14))
curvemmean(m11, mean = "amean")

## effect of normalizing data to [0, 1]:
## more averaged datapoints
m12 <- modlist(reps, fluo = c(2, 6, 10, 14), norm = TRUE)
curvemmean(m12, mean = "amean")

## averaging the expression value
## (i.e. copy numbers)
## using efficiency = 2
curvemmean(m12, mean = "cmean1")

## using averaged efficiencies from
## all curves at each cycle
curvemmean(m12, mean = "cmean2")
```

Description

An alternative to the classical crossing point/threshold cycle estimation as described in Guescini *et al* (2002). A tangent is fit to the first derivative maximum (point of inflection) of the modeled curve and the intersection with the x-axis is calculated.

Usage

```
Cy0(object, plot = FALSE, add = FALSE, ...)
```

Arguments

| | |
|--------|--|
| object | a fitted object of class 'pcrfit'. |
| plot | if TRUE, displays a plot of Cy0. |
| add | if TRUE, a plot is added to any other existing plot, i.e. as from <code>plot.pcrfit</code> . |
| ... | other parameters to be passed to <code>plot.pcrfit</code> or <code>points</code> . |

Details

The function calculates the first derivative maximum (cpD1) of the curve and the slope and fluorescence F_{cpD2} at that point. Cy0 is then calculated by $Cy0 = cpD1 - \frac{F_{cpD2}}{slope}$.

Value

The Cy0 value.

Author(s)

Andrej-Nikolai Spiess

References

A new real-time PCR method to overcome significant quantitative inaccuracy due to slight amplification inhibition.
 Guescini M, Sisti D, Rocchi MB, Stocchi L & Stocchi V.
BMC Bioinformatics (2008), **9**: 326.

Examples

```
## single curve with plot
m1 <- pcrfit(reps, 1, 2, 15)
Cy0(m1, plot = TRUE)

## add to 'efficiency' plot
efficiency(m1)
Cy0(m1, add = TRUE)

## compare s.d. of replicates between
## Cy0 and cpD2 method. cpD2 wins!
m11 <- modlist(reps, model = 14)
cy0 <- sapply(m11, function(x) Cy0(x))
cpd2 <- sapply(m11, function(x) efficiency(x, plot = FALSE)$cpD2)
tapply(cy0, gl(7, 4), function(x) sd(x))
tapply(cpd2, gl(7, 4), function(x) sd(x))
```

`dyemelt`*Melting curves of a 4-plex qPCR with different fluorescence dyes*

Description

A melting curve analysis of a multiplex real-time PCR on genomic DNA, producing 4 amplicons. The fluorescence dyes used were EvaGreen, SybrGreen I and Syto-13.

Usage

```
data(dyemelt)
```

Format

A data frame with the temperature values in columns 1, 2, 3 and the corresponding fluorescence data in columns 2, 4, 6.

Details

The melting curve was conducted with AZF deletion-specific primers in a Lightcycler 1.0 instrument (Roche).

Examples

```
plot(dyemelt[, 1], dyemelt[, 2], xlab = "Temperature [°C]",  
     ylab = "Raw fluorescence")
```

`eff`*The amplification efficiency curve of a fitted object*

Description

Calculates the efficiency curve from the fitted object by $E_n = \frac{F(n)}{F(n-1)}$, with E = efficiency, F = raw fluorescence, n = Cycle number. Alternatively, a cubic spline interpolation can be used on the raw data as in Shain *et al.* (2008).

Usage

```
eff(object, type = c("sigfit", "spline"), sequence = NULL, baseshift = NULL,  
     smooth = FALSE, plot = FALSE)
```

Arguments

| | |
|-----------|--|
| object | an object of class 'pcrfit'. |
| type | the efficiency curve is either calculated from the sigmoidal fit (default) or a cubic spline interpolation. |
| sequence | a 3-element vector (from, to, by) defining the sequence for the efficiency curve. Defaults to [min(Cycles), max(Cycles)] with 100 points per cycle. |
| baseshift | baseline shift value in case of type = "spline". See documentation to maxRatio . |
| smooth | logical. If TRUE and type = "spline", invokes a 5-point convolution filter (filter). See documentation to maxRatio . |
| plot | should the efficiency be plotted? |

Details

For more information about the curve smoothing, baseline shifting and cubic spline interpolation for the method as in Shain *et al.* (2008), see 'Details' in [maxRatio](#).

Value

A list with the following components:

| | |
|----------|---|
| eff.x | the cycle points. |
| eff.y | the efficiency values at eff.x. |
| effmax.x | the cycle number with the highest efficiency. |
| effmax.y | the maximum efficiency. |

Author(s)

Andrej-Nikolai Spiess

References

A new method for robust quantitative and qualitative analysis of real-time PCR.
Shain EB & Clemens JM.
Nucleic Acids Research (2008), **36**, e91.

Examples

```
## with default 100 points per cycle
m1 <- pcrfit(reps, 1, 7, 15)
eff(m1, plot = TRUE)

## not all data and only 10 points per cycle
eff(m1, sequence = c(5, 35, 0.1), plot = TRUE)

## using cubic splines
## it is preferred to use the
## smoothing option
eff(m1, type = "spline", plot = TRUE, smooth = TRUE, baseshift = 0.3)
```

Description

This function calculates the PCR efficiency of a model of class 'pcrfit', including several other important values for qPCR quantification like the first and second derivatives and the corresponding maxima thereof (i.e. threshold cycles). These values can subsequently be used for the calculation of PCR kinetics, fold induction etc. All values are included in a graphical output of the fit. Additionally, several measures of goodness-of-fit are calculated, i.e. the Akaike Information Criterion (AIC), the residual variance and the R^2 value.

Usage

```
efficiency(object, plot = TRUE, type = "cpD2", thresh = NULL,
           shift = 0, amount = NULL, ...)
```

Arguments

| | |
|--------|--|
| object | an object of class 'pcrfit'. |
| plot | logical. If TRUE, a graph is displayed. If FALSE, values are printed out. |
| type | the method of efficiency estimation. See 'Details'. |
| thresh | an (optional) numeric value for a fluorescence threshold border. Overrides type. |
| shift | a user defined shift in cycles from the values defined by type. See 'Examples'. |
| amount | the template amount or molecule number for quantitative calibration. |
| ... | additional parameters to be passed to eff or plot.pcrfit . |

Details

The efficiency is always (with the exception of `type = "maxRatio"`) calculated from the efficiency curve (in blue), which is calculated according to $E_n = \frac{F_n}{F_{n-1}}$ from the fitted curve, but taken from different points at the curve, as to be defined in `type`:

"cpD2" taken from the maximum of the second derivative curve,

"cpD1" taken from the maximum of the first derivative curve,

"maxE" taken from the maximum of the efficiency curve,

"expR" taken from the exponential region by $expR = cpD2 - (cpD1 - cpD2)$,

"CQ" taken from the 20% value of the fluorescence at "cpD2" as developed by Corbett Research (comparative quantification),

"Cy0" the intersection of a tangent on the first derivative maximum with the abscissa as calculated according to Guescini et al. or

a numeric value taken from the threshold cycle output of the PCR software, i.e. 15.24 as defined in `type` or

a numeric value taken from the fluorescence threshold output of the PCR software as defined in `thresh`.

The initial fluorescence F_0 for relative or absolute quantification is either calculated by setting $x = 0$ in the sigmoidal model of object giving `init1` or by calculating an exponential model down (`init2`) with $F_0 = \frac{F_n}{E^n}$, with F_n = raw fluorescence, E = PCR efficiency and n = the cycle number defined by type. If a template amount is defined, a conversion factor $cf = \frac{amount}{F_0}$ is given. The different measures for goodness-of-fit give an overview for the validity of the efficiency estimation. First and second derivatives are calculated from the fitted function and the maxima of the derivatives curve and the efficiency curve are obtained.

If `type = "maxRatio"`, the maximum efficiency is calculated from the cubic spline interpolated raw fluorescence values and therefore NOT from the sigmoidal fit. This is a different paradigm and will usually result in fairly the same threshold cycles as with `type = "cpD2"`, but the efficiencies are generally lower. See documentation to `maxRatio`. This method is usually not applied for calculating efficiencies that are to be used for relative quantification, but one might try...

Value

A list with the following components:

| | |
|---------------------|--|
| <code>eff</code> | the PCR efficiency. |
| <code>resVar</code> | the residual variance. |
| <code>AICc</code> | the bias-corrected Akaike Information Criterion. |
| <code>AIC</code> | the Akaike Information Criterion. |
| <code>Rsq</code> | the R^2 value. |
| <code>Rsq.ad</code> | the adjusted R_{adj}^2 value. |
| <code>cpD1</code> | the first derivative maximum (point of inflection in 'l4' or 'b4' models, can be used for defining the threshold cycle). |
| <code>cpD2</code> | the second derivative maximum (turning point of <code>cpD1</code> , more often used for defining the threshold cycle). |
| <code>cpE</code> | the PCR cycle with the highest efficiency. |
| <code>cpR</code> | the PCR cycle within the exponential region calculated as under 'Details'. |
| <code>cpT</code> | the PCR cycle corresponding to the fluorescence threshold as defined in <code>thresh</code> . |
| <code>Cy0</code> | the PCR threshold cycle 'Cy0' according to Guescini et al. See 'Details'. |
| <code>cpCQ</code> | the PCR cycle corresponding to the 20% fluorescence value at 'cpD2'. |
| <code>cpMR</code> | the PCR cycle corresponding to the 'maxRatio', if this was selected. |
| <code>fluo</code> | the raw fluorescence value at the point defined by <code>type</code> or <code>thresh</code> . |
| <code>init1</code> | the initial template fluorescence from the sigmoidal model, calculated as under 'Details'. |
| <code>init2</code> | the initial template fluorescence from an exponential model, calculated as under 'Details'. |
| <code>cf</code> | the conversion factor between raw fluorescence and template amount, if the latter is defined. |

If object was of type 'modlist', the results are given as a matrix, with samples in columns.

Note

Three parameter models ('b3' or 'l3') do not work very well in calculating the PCR efficiency. It is advisable not to take too many cycles of the plateau phase prior to fitting the model as this has a strong effect on the validity of the efficiency estimates.

Author(s)

Andrej-Nikolai Spiess

References

Validation of a quantitative method for real time PCR kinetics.
Liu W & Saint DA.
BBRC (2002), **294**: 347-353.

A new real-time PCR method to overcome significant quantitative inaccuracy due to slight amplification inhibition.
Guescini M, Sisti D, Rocchi MB, Stocchi L & Stocchi V.
BMC Bioinformatics (2008), **9**: 326.

Examples

```
## Fitting initial model
m1 <- pcrfit(reps, 1, 2, 14)
efficiency(m1)

## Using one cycle 'downstream'
## of second derivative max
efficiency(m1, type = "cpD2", shift = -1)

## using "maxE" method, with calculation of PCR efficiency
## 2 cycles 'upstream' from the cycle of max efficiency
efficiency(m1, type = "maxE", shift = 2)

## using the exponential region
efficiency(m1, type = "expR")

## using threshold cycle (i.e. 15.32)
## from PCR software
efficiency(m1, type = 15.32)

## using Cy0 method from
## Guescini et al. (2008),
## add Cy0 tangent
efficiency(m1, type = "Cy0")
Cy0(m1, add = TRUE)

## using a defined fluorescence
## threshold value from PCR software
efficiency(m1, thresh = 1)
```

```

## using the first 30 cycles and a template amount
## (optical calibration)
m2 <- pcrfit(reps[1:30, ], 1, 2, 15)
efficiency(m2, amount = 1E3)

## using 'maxRatio' method from Shain et al. (2008)
## baseshifting essential!
efficiency(m1, type = "maxRatio", baseshift = 0.2)

## Not run:
## on a modlist with plotting
## of the efficiencies
m11 <- modlist(reps, model = 15)
res <- sapply(m11, function(x) efficiency(x)$eff)
barplot(as.numeric(res))

## End(Not run)

```

evidence

Evidence ratio for model comparisons with AIC, AICc or BIC

Description

The evidence ratio

$$\frac{1}{\exp(-0.5 \cdot (IC2 - IC1))}$$

is calculated for one of the information criteria $IC = AIC, AICc, BIC$ either from two fitted models or two numerical values. Models can be compared that are not nested and where the f-test on residual-sum-of-squares is not applicable.

Usage

```
evidence(x, y, type = c("AIC", "AICc", "BIC"))
```

Arguments

| | |
|------|---|
| x | a fitted object or numerical value. |
| y | a fitted object or numerical value. |
| type | any of the three Information Criteria AIC, AICc or BIC. |

Details

Small differences in values can mean substantial more 'likelihood' of one model over the other. For example, a model with $AIC = -130$ is nearly 150 times more likely than a model with $AIC = -120$.

Value

A value of the first model x being more likely than the second model y. If large, first model is better. If small, second model is better.

Author(s)

Andrej-Nikolai Spiess

Examples

```
## compare two four-parameter and five-parameter
## log-logistic models
m1 <- pcrfit(reps, 1, 2, 14)
m2 <- pcrfit(reps, 1, 2, 15)
evidence(m2, m1)

## ratio of two AIC's
evidence(-120, -123)
```

expcomp*Comparison of all sigmoidal models within the exponential region*

Description

The exponential region of the qPCR data is identified by the studentized outlier method, as in [expfit](#). The root-mean-squared-error (RMSE) of all available sigmoidal models within this region is then calculated. The result of the fits are plotted and models returned in order of ascending RMSE.

Usage

```
expcomp(object, ...)
```

Arguments

| | |
|--------|--|
| object | an object of class 'pcrfit'. |
| ... | other parameters to be passed to <code>expfit</code> . |

Details

The following sigmoidal models are fitted: b3, b4, b5, b6, b7, l3, l4, l5, l6, l7

Value

A dataframe with names of the models, in ascending order of RMSE.

Author(s)

Andrej-Nikolai Spiess

Examples

```
m1 <- pcrfit(reps, 1, 2, 14)
expcomp(m1)
```

 expfit

Calculation of PCR efficiency by fitting an exponential model

Description

An exponential model is fit to a window of defined size on the qPCR raw data. The window is identified either by the 'studentized outlier' method as described in Tichopad *et al.* (2003), the 'midpoint' method (Peirson *et al.*, 2003) or by subtracting the difference of cpD1 and cpD2 from cpD2 ('ERBCP', unpublished).

Usage

```
expfit(object, method = c("outlier", "midpoint", "ERBCP"), pval = 0.05,
       n.outl = 3, n.ground = 1:5, corfact = 1,
       fix = c("top", "bottom", "middle"), nfit = 5, plot = TRUE, ...)
```

Arguments

| | |
|----------|---|
| object | an object of class 'pcrfit'. |
| method | one of the three possible methods to be used for defining the position of the fitting window. |
| pval | for method = "outlier", the p-value for the outlier test. |
| n.outl | for method = "outlier", the number of successive outlier cycles. |
| n.ground | for method = "midpoint", the number of cycles in the noisy ground phase to calculate the standard deviation from. |
| corfact | for method = "ERBCP", the correction factor for finding the exponential region. See 'Details'. |
| fix | for methods "midpoint" and "ERBCP", the orientation of the fitting window based on the identified point. See 'Details'. |
| nfit | the size of the fitting window. |
| plot | logical. If TRUE, a graphical display of the curve and the fitted region is shown. |
| ... | other parameters to be passed to the plotting function. |

Details

The exponential growth function $f(x) = b \cdot \exp(k \cdot x) + e$ is fit to the data. Calls `takeoff` for the calculation of the studentized residuals and 'outlier' cycle, and `midpoint` for calculation of the exponential phase 'midpoint'. For method 'ERBCP' (Exponential Region By Crossing Points), the exponential region is calculated by $\text{expR} = \text{cpD2} - \text{corfact} \cdot (\text{cpD1} - \text{cpD2})$. The efficiency is calculated a) from the exponential fit with $E = \exp(k)$ and b) for each cycle within the exponential region from the raw fluorescence values by $E_n = \frac{F_n}{F_{n-1}}$. The initial template fluorescence F_0 is derived from $F_0 = b \cdot \exp(k)$.

Value

A list with the following components:

| | |
|------------|--|
| point | the point within the exponential region as identified by one of the three methods. |
| cycles | the cycles of the identified region as defined by method, fix and nfit. |
| eff | the efficiency calculated from the exponential fit. |
| eff.cycles | the efficiencies of all points within the identified region. |
| AIC | the Akaike Information Criterion of the fit. |
| resVar | the residual variance of the fit. |
| RMSE | the root-mean-squared-error of the fit. |
| init | the initial template fluorescence. |
| mod | the exponential model of class 'nls'. |

Author(s)

Andrej-Nikolai Spiess

References

Standardized determination of real-time PCR efficiency from a single reaction set-up.
Tichopad A, Dilger M, Schwarz G & Pfaffl MW.
Nucleic Acids Research (2003), **31**:e122.

Comprehensive algorithm for quantitative real-time polymerase chain reaction.
Zhao S & Fernald RD.
J Comput Biol (2005), **12**:1047-64.

Examples

```
## using 'outlier' method
m1 <- pcrfit(reps, 1, 2, 15)
expfit(m1)

## 'midpoint' method and 7 cycle window
expfit(m1, method = "midpoint", nfit = 7)

## 'ERBCP' method with window centered around
## fixpoint
expfit(m1, method = "ERBCP", fix = "middle")
```

fitchisq

*The chi-square goodness-of-fit***Description**

Calculates χ^2 , reduced χ^2_ν and the χ^2 fit probability for objects of class `pcrfit`, `lm`, `glm`, `nls` or any other object with a `call` component that includes formula and data. The function checks for replicated data (i.e. multiple same predictor values). If replicates are not given, the function needs error values, otherwise NA's are returned.

Usage

```
fitchisq(object, error = NULL)
```

Arguments

`object` a single model of class 'pcrfit', a 'replisq' or any fitted model of the above.
`error` in case of a model without replicates, a single error for all response values or a vector of errors for each response value.

Details

The variance of a fit s^2 is also characterized by the statistic χ^2 defined as followed:

$$\chi^2 \equiv \sum_{i=1}^n \frac{(y_i - f(x_i))^2}{\sigma_i^2}$$

The relationship between s^2 and χ^2 can be seen most easily by comparison with the reduced χ^2 :

$$\chi^2_\nu = \frac{\chi^2}{\nu} = \frac{s^2}{\langle \sigma_i^2 \rangle}$$

whereas ν = degrees of freedom (N - p), and $\langle \sigma_i^2 \rangle$ is the weighted average of the individual variances. If the fitting function is a good approximation to the parent function, the value of the reduced chi-square should be approximately unity, $\chi^2_\nu = 1$. If the fitting function is not appropriate for describing the data, the deviations will be larger and the estimated variance will be too large, yielding a value greater than 1. A value less than 1 can be a consequence of the fact that there exists an uncertainty in the determination of s^2 , and the observed values of χ^2_ν will fluctuate from experiment to experiment. To assign significance to the χ^2 value, we can use the integral probability

$$P_\chi(\chi^2; \nu) = \int_{\chi^2}^{\infty} P_\chi(x^2, \nu) dx^2$$

which describes the probability that a random set of n data points sampled from the parent distribution would yield a value of χ^2 equal to or greater than the calculated one. This is calculated by $1 - pchisq(\chi^2, \nu)$.

Value

A list with the following items:

| | |
|----------|---|
| chi2 | the χ^2 value. |
| chi2.red | the reduced χ^2 . |
| p.value | the fit probability as described above. |

Author(s)

Andrej-Nikolai Spiess

References

Data Reduction and Error Analysis for the Physical Sciences.
Bevington PR & Robinson DK.
McGraw-Hill, New York (2003).

Applied Regression Analysis.
Draper NR & Smith H.
Wiley, New York, 1998.

Examples

```
## using replicates by making
## a 'replist'
m11 <- modlist(reps, fluo = 2:5)
r11 <- replist(m11, group = c(1, 1, 1, 1))
fitchisq(r11[[1]])

## using single model with
## added error
m1 <- pcrfit(reps, 1, 2, 15)
fitchisq(m1, 0.1)
```

getPar

*Batch calculation of qPCR fit parameters/efficiencies/threshold cycles
with simple output, especially tailored to high-throughput data*

Description

This is a cut-down version of [pcrbatch](#), starting with data of class 'modlist', which delivers a simple dataframe output, with either the parameters of the fit or calculated threshold cycles/efficiencies. The column names are deduced from the run names. All calculations have been error-protected through [tryCatch](#), so whenever there is any kind of error (parameter extraction, efficiency estimation etc), NA is returned. This function can be used with high throughput data quite conveniently. All methods as in [pcrbatch](#) are available. The results are automatically copied to the clipboard.

Usage

```
getPar(x, type = c("fit", "curve"), cp = "cpD2", eff = "sigfit", ...)
```

Arguments

| | |
|------|---|
| x | an object of class 'pcrfit' or 'modlist'. |
| type | fit will extract the fit parameters, curve will invoke efficiency and return threshold cycles/efficiencies. |
| cp | which method for threshold cycle estimation. Any of the methods in efficiency , i.e. "cpD2" (default), "cpD1", "maxE", "expR", "Cy0", "CQ", "maxRatio". |
| eff | which method for efficiency estimation. Either "sigfit" (default), "sliwin" or "expfit". |
| ... | other parameters to be passed to efficiency , sliwin or expfit . |

Details

Takes about 4 sec for 100 runs on a Pentium 4 Quad-Core (3 Ghz) when using type = "curve". When using type = "fit", the fitted model parameters are returned. If type = "curve", threshold cycles and efficiencies are calculated by [efficiency](#) based on the parameters supplied in ... (default cpD2).

Value

A dataframe, which is automatically copied to the clipboard.

Author(s)

Andrej-Nikolai Spiess.

Examples

```
## Not run:
## simple example with
## fit parameters
m11 <- modlist(rutledge, model = 15)
getPar(m11, type = "fit")

## simple example with
## plotting of threshold cycles
res1 <- getPar(m11, type = "curve", cp = "cpD2", eff = "sliwin")
barplot(res1[1, ], las = 2)

## using a mechanistic model such as
## 'mak3' and extracting D0 values
## => initial template fluorescence
m12 <- modlist(rutledge, 1, 2:41, model = mak3)
res <- getPar(m12, type = "fit")
barplot(log10(res[1, ]), las = 2)

## End(Not run)
```

`guescinietal`*qPCR dilution experiments from Guescini et al. (2008)*

Description

`guescini1`: A high quality 10-fold dilution experiment with 7 dilution steps and 12 replicates each.
`guescini2`: A high quality experiment in which a decreasing amount of PCR mix mimics PCR inhibition.

Usage

```
guescini1  
guescini2
```

Format

`guescini1`: A data frame with the PCR cycles and 84 qPCR runs with 12 replicates of seven 10-fold dilutions. The replicates are defined by FX.Y (X = dilution number, Y = replicate number).
`guescini2`: A data frame with the PCR cycles and 60 qPCR runs from 12 replicates with 5 decreasing steps of PCR mix. The replicates are defined by FX.Y (X = PCR mix dilution number, Y = replicate number).

Details

The real-time PCR was conducted with primers for the NADH dehydrogenase 1 in a Lightcycler 480 (Roche). The data is background subtracted.

Source

Supplemental data 1 to the paper.

References

A new real-time PCR method to overcome significant quantitative inaccuracy due to slight amplification inhibition.
Guescini M, Sisti D, Rocchi MB, Stocchi L & Stocchi V.
BMC Bioinformatics (2008), **9**: 326.

Examples

```
## Not run:  
## dilution set with replicates  
m11 <- modlist(guescini1, model = 14)  
plot(m11)  
  
## effect of decreasing mix  
## on PCR efficiency,
```

```
## mean for the replicates
m12 <- modlist(guescini2, model = 14)
effs <- sapply(m12, function(x) efficiency(x, plot = FALSE)$eff)
tapply(effs, gl(5, 12), function(x) mean(x, na.rm = TRUE))

## End(Not run)
```

 HQIC

Hannan-Quinn Information Criterion

Description

Calculates the Hannan-Quinn Information Criterion for objects of class `pcrfit`, `nls`, `lm`, `glm` or any other models from which `logLik`, `coef` and `residuals` can be extracted. It is somewhat similar to `BIC`, but penalizes n even more by double logarithmation.

Usage

```
HQIC(object)
```

Arguments

`object` a fitted model.

Details

$$HQIC = -2 \cdot \log(\mathcal{L}_{max}) + 2 \cdot k \cdot \log(\log(n))$$

with \mathcal{L}_{max} = maximum likelihood, k = number of parameters and n = number of observations.

Value

The Hannan-Quinn Information Criterion.

Note

For $n > \sim 20$ or so `BIC` is the strictest in penalizing loss of degree of freedom by having more parameters in the fitted model. For $n > \sim 40$ `AIC` is the least strict of the three and `HQIC` holds the middle ground, or is the least penalizing for $n < \sim 20$.

Author(s)

Andrej-Nikolai Spiess

References

The Determination of the Order of an Autoregression.
 Hannan EJ & Quinn BG.
J Roy Stat Soc B (1979), **41**: 190-195.

See Also

[AIC, BIC.](#)

Examples

```
m1 <- pcrfit(reps, 1, 2, 15)
HQIC(m1)
```

htPCR

High throughput qPCR experiment with 8858 runs

Description

A high throughput experiment containing 8858 runs from a 95 x 96 PCR grid. Some wells have been removed, probably due to failed amplification.

Usage

```
data(htPCR)
```

Format

A data frame with 8858 PCR runs.

Details

The real-time PCR was conducted with proprietary primers in a Fluidigm instrument (Biomark) using EvaGreen and ROX normalization.

Source

Kindly supplied by Roman Bruno.

Examples

```
## Not run:
data(htPCR)
m1 <- modlist(htPCR, 1, 2:200, model = 15)
getPar(m1, type = "curve")

## End(Not run)
```

`is.outlier`*Outlier summary for objects of class 'modlist' or 'replist'*

Description

For model lists of class 'modlist' or 'replist', `is.outlier` returns a vector of logicals for each run if they are outliers (i.e. sigmoidal or kinetic) or not.

Usage

```
is.outlier(object)
```

Arguments

`object` an object of class 'modlist' or 'replist'.

Value

A vector of logicals with run names.

Author(s)

Andrej-Nikolai Spiess

See Also

[KOD](#).

Examples

```
## analyze in respect to amplification
## efficiency outliers
m11 <- modlist(reps)
res1 <- KOD(m11, check = "uni2")

## which runs are outliers?
outl <- is.outlier(res1)
outl
which(outl)

## test for sigmoidal outliers
## with the 'testdat' dataset
m12 <- modlist(testdat, model = 15, check = "uni2")
is.outlier(m12)
```

KOD

*(K)inetic (O)utlier (D)etection using several methods***Description**

Identifies and/or removes qPCR runs according to several published methods or own ideas. The univariate measures are based on efficiency or difference in first/second derivative maxima. Multivariate methods are implemented that describe the structure of the curves according to several fixpoints such as first/second derivative maximum, slope at first derivative maximum or plateau fluorescence. These measures are compared with a set of curves using the [mahalanobis](#) distance with a robust covariance matrix and calculation of statistics by a χ^2 distribution. See 'Details'.

Usage

```
KOD(object, method = c("uni1", "uni2", "multi1", "multi2", "multi3"),
     par = parKOD(), remove = FALSE, verbose = TRUE, plot = TRUE, ...)
```

Arguments

| | |
|---------|--|
| object | an object of class 'modlist' or 'replist'. |
| method | which method to use for kinetic outlier identification. Method "uni1" is default. See 'Details' for all methods. |
| par | parameters for the different methods. See parKOD . |
| remove | logical. If TRUE, outlier runs are removed and the object is updated. If FALSE, the individual qPCR runs are tagged as 'outliers' or not. See 'Details'. |
| verbose | logical. If TRUE, all calculation steps and results are displayed on the console. |
| plot | logical. If TRUE, a multivariate plot is displayed. |
| ... | any other parameters to be passed to sliwin , efficiency or expfit . |

Details**The following methods for the detection of kinetic outliers are implemented**

uni1: KOD method according to Bar et al. (2003). Outliers are defined by removing the sample efficiency from the replicate group and testing it against the remaining samples' efficiencies using a Z-test:

$$P = 2 * \left[1 - \Phi \left(\frac{e_i - \mu_{train}}{\sigma_{train}} \right) \right] < 0.05$$

uni2: This method from the package author is more or less a test on sigmoidal structure for the individual curves. It is different in that there is no comparison against other curves from a replicate set. The test is simple: The difference between first and second derivative maxima should be less than 10 cycles:

$$\left(\frac{\partial^3 F(x; a, b, \dots)}{\partial x^3} = 0 \right) - \left(\frac{\partial^2 F(x; a, b, \dots)}{\partial x^2} = 0 \right) < 10$$

Sounds astonishingly simple, but works: Runs are defines as 'outliers' that really failed to amplify, i.e. have no sigmoidal structure or are very shallow. It is the default setting in `modlist`.

`multi1`: KOD method according to Tichopad et al. (2010). Assuming two vectors with first and second derivative maxima t_1 and t_2 from a 4-parameter sigmoidal fit within a window of points around the first derivative maximum, a linear model $t_2 = t_1 \cdot b + a + \tau$ is made. Both t_1 and the residuals from the fit $\tau = t_2 - \hat{t}_2$ are Z-transformed:

$$t_{1norm} = \frac{t_1 - \bar{t}_1}{\sigma_{t_1}}, \tau_{1norm} = \frac{\tau_1 - \bar{\tau}_1}{\sigma_{\tau_1}}$$

Both t_1 and τ are used for making a robust covariance matrix. The outcome is plugged into a `mahalanobis` distance analysis using the 'adaptive reweighted estimator' from package 'mvoutlier' and p-values for significance of being an 'outlier' are deduced from a χ^2 distribution. If more than two parameters are supplied, `princomp` is used instead.

`multi2`: Second KOD method according to Tichopad et al. (2010), mentioned in the paper. Uses the same pipeline as `multi1`, but with the slope at the first derivative maximum and maximum fluorescence as parameters:

$$\frac{\partial F(x; a, b, \dots)}{\partial x}, F_{max}$$

`multi3`: KOD method according to Sisti et al. (2010). Similar to `multi2`, but uses maximum fluorescence, slope at first derivative maximum and y-value at first derivative maximum as fixpoints:

$$\frac{\partial F(x; a, b, \dots)}{\partial x}, F \left(\frac{\partial^2 F(x; a, b, \dots)}{\partial x^2} = 0 \right), F_{max}$$

All essential parameters for the methods can be tweaked by `parKOD`. See there and in 'Examples'.

Value

An object of the same class as in object that is 'tagged' in its name (**name**) if it is an outlier and also with an item `$isOutlier` with outlier information (see `is.outlier`). If `remove = TRUE`, the outlier runs are removed (and the fitting updated in case of a 'replis').

Author(s)

Andrej-Nikolai Spiess

References

- Kinetic Outlier Detection (KOD) in real-time PCR.
Bar T, Stahlberg A, Muszta A & Kubista M.
Nucl Acid Res (2003), **31**: e105.
- Quality control for quantitative PCR based on amplification compatibility test.
Tichopad A, Bar T, Pecan L, Kitchen RR, Kubista M &, Pfaffl MW.
Methods (2010), **50**: 308-312.
- Shape based kinetic outlier detection in real-time PCR.
Sisti D, Guescini M, Rocchi MBL, Tibollo P, D'Atri M & Stocchi V.
BMC Bioinformatics (2010), **11**: 186.

See Also

Function `is.outlier` to get an outlier summary.

Examples

```
## kinetic outliers:
## on a 'modlist', using efficiency from sigmoidal fit
## and alpha = 0.01.
## F7.3 detected as outlier (shallower => low efficiency)
m11 <- modlist(reps, 1, c(2:5, 28), model = 15)
res1 <- KOD(m11, method = "uni1", par = parKOD(eff = "sliwin", alpha = 0.01))
plot(res1)

## sigmoidal outliers:
## remove runs without sigmoidal structure
m12 <- modlist(testdat, model = 15)
res2 <- KOD(m12, method = "uni2", remove = TRUE)
plot(res2, which = "single")

## multivariate outliers:
## a few runs are identified
m13 <- modlist(reps, model = 15)
res3 <- KOD(m13, method = "multi1")

## on a 'replist',
## several outliers identified
r13 <- replist(m13, group = gl(7, 4))
res4 <- KOD(r13, method = "uni1")
```

lievensetal

qPCR dilution and inhibition data from Lievens et al. (2012)

Description

lievens1: High quality 5-fold dilution experiments with 5 dilution steps and 18 replicates.
lievens2: Inhibition data with five different concentrations of isopropanol and 18 replicates.
lievens3: Inhibition data with five different amounts of tannic acid per reaction and 18 replicates.

Usage

```
lievens1
lievens2
lievens3
```

Format

lievens1: 90 qPCR runs with five 5-fold dilutions and 18 replicates each. Named by SX.Y, with X = dilution step and Y = replicate.

lievens2: 90 qPCR runs with five different concentrations of isopropanol (2.5%, 0.5%, 0.1%, 0.02% and 0.004% (v/v)) and 18 replicates each. Named by SX.Y, with X = concentration step and Y = replicate.

lievens3: 90 qPCR runs with five different amounts of tannic acid per reaction (5 ng, 1 ng, 0.2 ng, 0.04 ng and 0.008 ng) and 18 replicates each. Named by SX.Y, with X = concentration step and Y = replicate.

Details

The real-time PCR was conducted with an ABI7300 (ABI) or Biorad IQ5 (Biorad) instrument using SybrGreen I chemistry and primers for the soybean lectin endogene Le1.

Source

Supplementary Data to the paper.

References

Enhanced analysis of real-time PCR data by using a variable efficiency model: FPK-PCR.
Lievens A, Van Aelst S, Van den Bulcke M & Goetghebeur E.
Nucleic Acids Res (2012), **40**:e10.

Examples

```
## Not run:  
## lievens1  
m11 <- modlist(lievens1, model = 14)  
plot(m11)  
  
## lievens2  
COL <- rep(1:4, each = 18)  
m12 <- modlist(lievens2, model = 14)  
plot(m12, col = COL)  
  
## End(Not run)
```

llratio

Calculation of likelihood ratios for nested models

Description

Calculates the likelihood ratio and p-value from a chi-square distribution for two nested models.

Usage

```
llratio(objX, objY)
```

Arguments

| | |
|------|--|
| objX | Either a value of class <code>logLik</code> or a model for which <code>logLik</code> can be applied. |
| objY | Either a value of class <code>logLik</code> or a model for which <code>logLik</code> can be applied. |

Details

The likelihood ratio statistic is

$$LR = \frac{f(X, \hat{\phi}, \hat{\psi})}{f(X, \phi, \psi_0)}$$

The usual test statistic is

$$\Lambda = 2 \cdot (l(\hat{\phi}, \hat{\psi}) - l(\phi, \psi_0))$$

Following the large sample theory, if H_0 is true, then

$$\Lambda \sim \chi_p^2$$

Value

A list containing the following items:

| | |
|---------|--|
| ratio | the likelihood ratio statistic. |
| df | the change in parameters. |
| p.value | the p-value from a χ^2 distribution. See Details. |

Author(s)

Andrej-Nikolai Spiess

See Also

[AIC](#), [logLik](#).

Examples

```
## compare l5 and l4 model
m1 <- pcrfit(reps, 1, 2, 15)
m2 <- pcrfit(reps, 1, 2, 14)
llratio(m1, m2)
```

| | |
|----------|---|
| LOF.test | <i>Formal lack-Of-Fit test of a nonlinear model against a one-way ANOVA model</i> |
|----------|---|

Description

Tests the nonlinear model against a more general one-way ANOVA model and from a likelihood ratio test. P-values are derived from the F- and χ^2 distribution, respectively.

Usage

LOF.test(object)

Arguments

object an object of class 'replst', 'pcrfit' or 'nls', which was fit with replicate response values.

Details

The one-way ANOVA model is constructed from the data component of the nonlinear model by factorizing each of the predictor values. Hence, the nonlinear model becomes a submodel of the one-way ANOVA model and we test both models with the null hypothesis that the ANOVA model can be simplified to the nonlinear model (Lack-of-fit test). This is done by two approaches:

- 1) an F-test (Bates & Watts, 1988).
- 2) a likelihood ratio test (Huet *et al*, 2004).

P-values are derived from an F-distribution (1) and a χ^2 distribution (2).

Value

A list with the following components:

| | |
|-----|---|
| pF | the p-value from the F-test against the one-way ANOVA model. |
| pLR | the p-value from the likelihood ratio test against the one-way ANOVA model. |

Author(s)

Andrej-Nikolai Spiess

References

Nonlinear Regression Analysis and its Applications.
 Bates DM & Watts DG.
 John Wiley & Sons (1988), New York.

Statistical Tools for Nonlinear Regression: A Practical Guide with S-PLUS and R Examples.
 Huet S, Bouvier A, Poursat MA & Jolivet E.
 Springer Verlag (2004), New York, 2nd Ed.

Examples

```
## Example with a 'replis'
## no lack-of-fit
m11 <- modlist(reps, fluo = 2:5, model = 15)
r11 <- replis(m11, group = c(1, 1, 1, 1))
LOF.test(r11)

## Example with a 'nls' fit
## => there is a lack-of-fit
DNase1 <- subset(DNase, Run == 1)
fm1DNase1 <- nls(density ~ SSlogis(log(conc), Asym, xmid, scal), DNase1)
LOF.test(fm1DNase1)
```

| | |
|-----|---|
| LRE | <i>Calculation of qPCR efficiency by the 'linear regression of efficiency' method</i> |
|-----|---|

Description

The LRE method is based on a linear regression of raw fluorescence versus efficiency, with the final aim to obtain cycle dependent individual efficiencies E_n . A linear model is then fit to a sliding window of defined size(s) and within a defined border. Regression coefficients are calculated for each window, and from the window of maximum regression, parameters such as PCR efficiency and initial template fluorescence are calculated. See 'Details' for more information. This approach is quite similar to the one in [sliwin](#), but while [sliwin](#) regresses cycle number versus log(fluorescence), LRE regresses raw fluorescence versus efficiency. Hence, the former is based on assuming a constant efficiency for all cycles while the latter is based on a per-cycle individual efficiency.

Usage

```
LRE(object, wsize = 6, basecyc = 1:6, base = 0, border = NULL,
     plot = TRUE, verbose = TRUE, ...)
```

Arguments

| | |
|---------|---|
| object | an object of class 'pcrfit'. |
| wsize | the size(s) of the sliding window(s), default is 6. A sequence such as 4:6 can be used to optimize the window size. |
| basecyc | if base != 0, which cycles to use for an initial baseline estimation based on the averaged fluorescence values. |
| base | either 0 for no baseline optimization, or a scalar defining multiples of the standard deviation of all baseline points obtained from basecyc. These are iteratively subtracted from the raw data. See 'Details' and 'Examples'. |
| border | either NULL (default) or a two-element vector which defines the border from the take-off point to points nearby the upper asymptote (saturation phase). See 'Details'. |

| | |
|---------|--|
| plot | if TRUE, the result is plotted with the fluorescence/efficiency curve, sliding window, regression line and baseline. |
| verbose | logical. If TRUE, more information is displayed in the console window. |
| ... | only used internally for passing the parameter matrix. |

Details

To avoid fits with a high R^2 in the baseline region, some border in the data must be defined. In LRE, this is by default (`base = NULL`) the region in the curve starting at the take-off cycle (*top*) as calculated from `takeoff` and ending at the transition region to the upper asymptote (saturation region). The latter is calculated from the first and second derivative maxima: $asympt = cpD1 + (cpD1 - cpD2)$. If the border is to be set by the user, border values such as `c(-2, 4)` extend these values by $top + border[1]$ and $asympt + border[2]$. The efficiency is calculated by $E_n = \frac{F_n}{F_{n-1}}$ and regressed against the raw fluorescence values F : $E = F\beta + \epsilon$. For the baseline optimization, 100 baseline values Fb_i are interpolated in the range of the data:

$$F_{min} \leq Fb_i \leq base \cdot \sigma(F_{basecyc[1]} \dots F_{basecyc[2]})$$

and subtracted from F_n . For all iterations, the best regression window in terms of R^2 is found and its parameters returned. Two different initial template fluorescence values F_0 are calculated in LRE:

`init1`: Using the single maximum efficiency E_{max} (the intercept of the best fit) and the fluorescence at second derivative maximum F_{cpD2} , by

$$F_0 = \frac{F_{cpD2}}{E_{max}}$$

`init2`: Using the cycle dependent efficiencies E_n from $n = 1$ to the near-lowest integer (floor) cycle of the second derivative maximum $n = \lfloor cpD2 \rfloor$, and the fluorescence at the floor of the second derivative maximum $F_{\lfloor cpD2 \rfloor}$, by

$$F_0 = \frac{F_{\lfloor cpD2 \rfloor}}{\prod E_n}$$

This approach corresponds to the paradigm described in Rutledge & Stewart (2008), by using cycle-dependent and decreasing efficiencies Δ_E to calculate F_0 .

Value

A list with the following components:

| | |
|--------|---|
| eff | the maximum PCR efficiency E_{max} calculated from the best window. |
| rsq | the maximum R^2 . |
| base | the optimized baseline value. |
| window | the best window found within the borders. |
| parMat | a matrix containing the parameters as above for each iteration. |
| init1 | the initial template fluorescence F_0 assuming constant efficiency E_{max} as described under 'Details'. |
| init2 | the initial template fluorescence F_0 , assuming cycle-dependent efficiency E_n as described under 'Details'. |

Author(s)

Andrej-Nikolai Spiess

References

A kinetic-based sigmoidal model for the polymerase chain reaction and its application to high-capacity absolute quantitative real-time PCR.
Rutledge RG & Stewart D.
BMC Biotech (2008), **8**: 47.

Examples

```
## sliding window of size 5
## between take-off point and 3 cycles
## upstream of the upper asymptote turning point,
## no baseline optimization
m1 <- pcrfit(reps, 1, 2, 14)
LRE(m1, wsize = 5, border = c(0, 3), base = 0)

## Not run:
## optimizing with window sizes of 4 to 6,
## between 0/+2 from lower/upper border,
## and baseline up to 2 standard deviations
LRE(m1, wsize = 4:6, border = c(0, 2), base = 2)

## End(Not run)
```

maxRatio

The maxRatio method as in Shain et al. (2008)

Description

The maximum ratio (MR) is determined along the cubic spline interpolated curve of $\frac{F_n}{F_{n-1}}$ and the corresponding cycle numbers FCN and its adjusted version FCNA are then calculated for MR.

Usage

```
maxRatio(x, type = c("spline", "sigfit"), baseshift = NULL,
         smooth = TRUE, plot = TRUE, ...)
```

Arguments

x an object of class 'pcrfit' (single run) or 'modlist' (multiple runs).
type the parameters are either calculated from the cubic spline interpolation (default) or a sigmoidal fit.
baseshift numerical. Shift value in case of type = "spline". See 'Details'.

| | |
|--------|---|
| smooth | logical. If TRUE and type = "spline", invokes a 5-point convolution filter (filter). See 'Details'. |
| plot | Should diagnostic plots be displayed? |
| ... | other parameters to be passed to eff or plot . |

Details

In a first step, the raw fluorescence data can be smoothed by a 5-point convolution filter. This is optional but feasible for many qPCR setups with significant noise in the baseline region, and therefore set to TRUE as default. If `baseshift` is a numeric value, this is added to each response value $F_n = F_n + \text{baseshift}$ (baseline shifting). Finally, a cubic spline is fit with a resolution of 0.01 cycles and the maximum ratio (efficiency) is calculated by $MR = \max(\frac{F_n}{F_{n-1}} - 1)$. FCN is then calculated as the cycle number at MR and from this additionally an adjusted $FCNA = FCN - \log_2(MR)$. Sometimes problems are encountered in which, due to high noise in the background region, randomly high efficiency ratios are calculated. This must be resolved by tweaking the `baseshift` value.

Value

A list with the following components:

| | |
|-------|---|
| eff | the maximum efficiency. Equals to $mr + 1$. |
| mr | the maximum ratio. |
| fcn | the cycle number at mr . |
| fcna | an adjusted <code>fcn</code> , as described in Shain et al. |
| names | the names of the runs as taken from the original dataframe. |

Note

This function has been approved by the original author (Eric Shain).

Author(s)

Andrej-Nikolai Spiess

References

A new method for robust quantitative and qualitative analysis of real-time PCR.
Shain EB & Clemens JM.
Nucleic Acids Research (2008), **36**: e91.

Examples

```
## on single curve
## using baseline shifting
m1 <- pcrfit(reps, 1, 2, 15)
maxRatio(m1, baseshift = 0.3)

## on a 'modlist'
```

```
## using 'baseline shifting'  
## Not run:  
m11 <- modlist(reps, model = 15)  
maxRatio(m11, baseshift = 0.5)  
  
## End(Not run)
```

meanlist

Amalgamation of single data models into an averaged model

Description

Starting from a 'modlist' containing qPCR models from single data, meanlist amalgamates the models according to the grouping structure as defined in group. The result is a 'modlist' with models obtained from averaging the replicates by [pcrfit](#).

Usage

```
meanlist(object, group, type = c("mean", "median"))
```

Arguments

| | |
|--------|--|
| object | an object of class 'modlist'. |
| group | a vector defining the replicates for each group. |
| type | how to average the data. |

Details

As being defined by group, the average data of the curves is subjected to [pcrfit](#) and a new modlist with the averaged models is created. Similar to [replist](#) but does not contain the replicates within the 'nls' model but the averaged model with only ONE curve.

Value

An object of class 'modlist' containing the averaged models of class 'nls'/'pcrfit'.

Author(s)

Andrej-Nikolai Spiess

See Also

[modlist](#), [replist](#).

Examples

```
m11 <- modlist(reps, model = 14)  
res1 <- meanlist(m11, group = gl(7, 4))  
plot(res1)  
efficiency(res1[[1]])
```

| | |
|-----------|---|
| meltcurve | <i>Melting curve analysis with (iterative) T_m identification and peak area calculation/cutoff</i> |
|-----------|---|

Description

This function conducts a melting curve analysis from the melting curve data of a real-time qPCR instrument. The data has to be preformatted in a way that for each column of temperature values there exists a corresponding fluorescence value column. See `edit(dyemelt)` for a proper format. The output is a graph displaying the raw fluorescence curve (black), the first derivative curve (red) and the identified melting peaks. The original data together with the results ($-\frac{\partial F}{\partial T}$ values, T_m values) are returned as a list. An automatic optimization procedure is also implemented which iterates over `span.smooth` and `span.peaks` values and finds the optimal parameter combination that delivers minimum residual sum-of-squares of the identified T_m values to known T_m values. For all peaks, the areas can be calculated and only those included which have areas higher than a given cutoff (`cut.Area`). If no peak was identified meeting the cutoff values, the melting curves are flagged with a 'bad' attribute. See 'Details'.

Usage

```
meltcurve(data, temps = NULL, fluos = NULL, window = NULL,
           norm = FALSE, span.smooth = 0.05, span.peaks = 51,
           is.deriv = FALSE, Tm.opt = NULL, Tm.border = c(1, 1),
           plot = TRUE, peaklines = TRUE, calc.Area = TRUE,
           plot.Area = TRUE, cut.Area = 0, ...)
```

Arguments

| | |
|--------------------------|---|
| <code>data</code> | a dataframe containing the temperature and fluorescence data. |
| <code>temps</code> | a vector of column numbers reflecting the temperature values. If NULL, they are assumed to be 1, 3, 5, |
| <code>fluos</code> | a vector of column numbers reflecting the fluorescence values. If NULL, they are assumed to be 2, 4, 6, |
| <code>window</code> | a user-defined window for the temperature region to be analyzed. See 'Details'. |
| <code>norm</code> | logical. If TRUE, the fluorescence values are scaled between [0, 1]. |
| <code>span.smooth</code> | the window span for curve smoothing. Can be tweaked to optimize T_m identification. |
| <code>span.peaks</code> | the window span for peak identification. Can be tweaked to optimize T_m identification. Must be an odd number. |
| <code>is.deriv</code> | logical. Use TRUE, if data is already in first derivative transformed format. |
| <code>Tm.opt</code> | a possible vector of known T_m values to optimize <code>span.smooth</code> and <code>span.peaks</code> against. See 'Details' and 'Examples'. |
| <code>Tm.border</code> | for peak area calculation, a vector containing left and right border temperature values from the T_m values. Default is -1/+1 °C. |

| | |
|-----------|---|
| plot | logical. If TRUE, a plot with the raw melting curve, derivative curve and identified T_m values is displayed for each sample. |
| peaklines | logical. If TRUE, lines that show the identified peaks are plotted. |
| calc.Area | logical. If TRUE, all peak areas are calculated. |
| plot.Area | logical. If TRUE, the baselined area identified for the peaks is plotted by filling the peaks in red. |
| cut.Area | a peak area value to identify only those peaks with a higher area. |
| ... | other parameters to be passed to plot . |

Details

The melting curve analysis is conducted with the following steps:

- 1a) Temperature and fluorescence values are selected in a region according to window.
- 1b) If `norm = TRUE`, the fluorescence data is scaled into [0, 1] by `qpcR:::rescale`. Then, the function `qpcR:::TmFind` conducts the following steps:
 - 2a) A cubic spline function ([splinefun](#)) is fit to the raw fluorescence melt values.
 - 2b) The first derivative values are calculated from the spline function for each of the temperature values.
 - 2c) Friedman's supersmoother ([supsmu](#)) is applied to the first derivative values.
 - 2d) Melting peaks (T_m) values are identified by `qpcR:::peaks`.
 - 2e) Raw melt data, first derivative data, best parameters, residual sum-of-squares and identified T_m values are returned.
- Peak areas are then calculated by `qpcR:::peakArea`:
 - 3a) A linear regression curve is fit from the leftmost temperature value ($T_m - Tm.border[1]$) to the rightmost temperature value ($T_m + Tm.border[2]$) by [lm](#).
 - 3b) A baseline curve is calculated from the regression coefficients by [predict.lm](#).
 - 3c) The baseline data is subtracted from the first derivative melt data (baselining).
 - 3d) A [splinefun](#) is fit to the baselined data.
 - 3e) The area of this spline function is [integrated](#) from the leftmost to rightmost temperature value.
- 4) If calculated peak areas were below `cut.Area`, the corresponding T_m values are removed.
- Finally,
- 5) A matrix of xyy-plots is displayed using `qpcR:::xyy.plot`.

`is.deriv` must be set to TRUE if the exported data was already transformed to $-\frac{\partial F}{\partial T}$ by the PCR system (i.e. Stratagene MX3000P).

If values are given to `Tm.opt` (see 'Examples'), then `meltcurve` is iterated over all combinations of `span.smooth = seq(0, 0.2, by = 0.01)` and `span.peaks = seq(11, 201, by = 10)`. For each iteration, T_m values are calculated and compared to those given by measuring the residual sum-of-squares between the given values `Tm.opt` and the T_m values obtained during the iteration:

$$RSS = \sum_{i=1}^n (Tm_i - Tm.opt_i)^2$$

The returned list items containing the resulting data frame each has an attribute "quality" which is set to "bad" if none of the peaks met the `cut.Area` criterion (or "good" otherwise).

Value

A list with as many items as melting curves, named as in data, each containing a data.frame with the temperature (*Temp*), fluorescence values (*Fluo*), first derivative (*dF.dT*) values, (optimized) parameters of *span.smooth/span.peaks*, residual sum-of-squares (if *Tm.opt* != NULL), identified melting points (*Tm*), calculated peak areas (*Area*) and peak baseline values (*baseline*).

Note

The peaks function is derived from a R-Help mailing list entry in Nov 2005 by Martin Maechler.

Author(s)

Andrej-Nikolai Spiess

Examples

```
## default columns
data(dyemelt)
res1 <- meltcurve(dyemelt, window = c(75, 86))
res1

## selected columns and normalized fluo values
res2 <- meltcurve(dyemelt, temps = c(1, 3), fluos = c(2, 4),
                  window = c(75, 86), norm = TRUE)

## removing peaks based on peak area
## => two peaks have smaller areas and are not
## included
res3 <- meltcurve(dyemelt, temps = 1, fluos = 2, window = c(75, 86),
                  cut.Area = 0.2)
attr(res3[[1]], "quality")

## if all peak areas do not meet the cutoff value, meltcurve is
## flagged as 'bad'
res4 <- meltcurve(dyemelt, temps = 1, fluos = 2, window = c(75, 86),
                  cut.Area = 0.5)
attr(res4[[1]], "quality")

## optimizing span and peaks values
## Not run:
res5 <- meltcurve(dyemelt[, 1:6], window = c(74, 88),
                  Tm.opt = c(77.2, 80.1, 82.4, 84.8))

## End(Not run)
```

midpoint

Calculation of the 'midpoint' region

Description

Calculates the exponential region midpoint using the algorithm described in Peirson *et al.* (2003).

Usage

```
midpoint(object, noise.cyc = 1:5)
```

Arguments

object a fitted object of class 'pcrfit'.
noise.cyc the cycles defining the background noise.

Details

The 'midpoint' region is calculated by

$$F_{noise} \cdot \sqrt{\frac{F_{max}}{F_{noise}}}$$

with F_{noise} = the standard deviation of the background cycles and F_{max} = the maximal fluorescence.

Value

A list with the following components:

f.mp the 'midpoint' fluorescence.
cyc.mp the 'midpoint' cycle, as predicted from f.mp.

Author(s)

Andrej-Nikolai Spiess

References

Experimental validation of novel and conventional approaches to quantitative real-time PCR data analysis.
Peirson SN, Butler JN & Foster RG.
Nucleic Acids Research (2003), **31**: e73.

Examples

```
m1 <- pcrfit(reps, 1, 2, 15)
mp <- midpoint(m1)
plot(m1)
abline(h = mp$f.mp, col = 2)
abline(v = mp$mp, col = 2)
```

modlist

Create nonlinear models from a dataframe and coerce them into a list

Description

Essential function to create a list of nonlinear models from the columns (runs) of a qPCR dataframe. This function houses different methods for curve transformation prior to fitting, such as normalization in [0, 1], smoothing, background subtraction etc. Runs that failed to fit or that have been identified as kinetic outliers (by default: lack of sigmoidal structure) can be removed automatically as well as their entries in an optionally supplied label vector.

Usage

```
modlist(x, cyc = 1, fluo = NULL, model = 14, check = "uni2",
        checkPAR = parKOD(), remove = c("none", "fit", "KOD"),
        exclude = NULL, labels = NULL, norm = FALSE, backsub = NULL,
        smooth = c("none", "smooth", "lowess", "supsmu", "spline"),
        smoothPAR = list(span = 0.1), factor = 1, opt = FALSE,
        optPAR = list(sig.level = 0.05, crit = "ftest"), verbose = TRUE, ...)
```

Arguments

| | |
|----------|--|
| x | a dataframe containing the qPCR data or a single qPCR run of class 'pcrfit'. |
| cyc | the column containing the cycle data. Defaults to first column. |
| fluo | the column(s) (runs) to be analyzed. If NULL, all runs will be considered. |
| model | the model to be used for all runs. |
| check | the method for kinetic outlier detection. Default is check for sigmoidal structure, see KOD . To turn off, use NULL. |
| checkPAR | parameters to be supplied to the check method, see KOD . |
| remove | which runs to remove. Either "none", those which failed to "fit" or from the "KOD" outlier method. |
| exclude | either "" for samples with missing column names or a regular expression defining columns (samples) to be excluded from modlist. See 'Details'. |
| labels | a vector containing labels, i.e. for defining replicate groups prior to ratiobatch . |
| norm | logical. Should the raw data be normalized within [0, 1] before model fitting? |
| backsub | background subtraction. An optional numeric sequence defining the cycle numbers for background averaging and subtraction, such as 1:8. |

| | |
|-----------|---|
| smooth | which curve smoothing method to use. See 'Details'. |
| smoothPAR | parameters to be supplied to the smoothing functions. See 'Details'. |
| factor | a multiplication factor for the fluorescence response values (barely useful, but who knows...). |
| opt | logical. Should model selection be applied to each model? |
| optPAR | parameters to be supplied to mselect . |
| verbose | logical. If TRUE, fitting and tagging results will be displayed in the console. |
| ... | other parameters to be passed to pcrfit . |

Details

The following smoothing methods are available for the fluorescence values: [smooth](#), [lowess](#), [supsmu](#) and [spline](#). See documentation there. The author of this package favors "supsmu" with span = 0.1. In case of unsuccessful model fitting and if remove = "none" (default), the original data is included in the output, albeit with no fitting information. This is useful since using `plot.pcrfit` on the 'modlist' shows the non-fitted runs. If remove = "fit", the non-fitted runs are automatically removed and will thus not be displayed. If remove = "KOD", by default all runs without sigmoidal structure are removed likewise. If a labels vector lab is supplied, the labels from the failed fits are removed and a new label vector lab_mod is written to the global environment. This way, an initial labeling vector for all samples can be supplied, bad runs and their labels automatically removed and these transferred to downstream analysis (i.e. to [ratiobatch](#)) without giving errors. `exclude` offers an option to exclude samples from the modlist by some regular expression or by using "" for samples with empty column names. See 'Examples'.

Value

A list with each item containing the model from each column. A names item (which is tagged by *NAME*, if fitting failed) containing the column name is attached to each model as well as an item `isFitted` with either TRUE (fitting converged) or FALSE (a fitting error occurred). This information is useful when [ratiocalc](#) is to be applied and unsuccessful fits should automatically removed from the given group definition. If kinetic outlier detection is selected, an item `isOutlier` is attached, defining the run as an outlier (TRUE) or not (FALSE).

Author(s)

Andrej-Nikolai Spiess

See Also

[pcrbatch](#) for batch analysis using different methods.

Examples

```
## calculate efficiencies for each run in
## the 'reps' data
## subtract background using the first 8 cycles
m11 <- modlist(reps, model = 15, backsub = 1:8)
sapply(m11, function(x) efficiency(x, plot = FALSE)$eff)
```

```

## 'crossing points' for the first 3 runs (normalized)
## and using best model from Akaike weights
m12 <- modlist(reps, 1, 2:5, model = 15, norm = TRUE,
              opt = TRUE, optPAR = list(crit = "weights"))
sapply(m12, function(x) efficiency(x, plot = FALSE)$cpD2)

## convert a single run to a 'modlist'
m <- pcrfit(reps, 1, 2, 15)
m13 <- modlist(m)

## using the 'testdat' set
## include failed fits
m14 <- modlist(testdat, 1, 2:9, model = 15)
plot(m14, which = "single")

## remove failed fits and update a label vector
GROUP <- c("g1s1", "g1s2", "g1s3", "g1s4", "g1c1", "g1c2", "g1c3", "g1c4")
m15 <- modlist(testdat, 1, 2:9, model = 15, labels = GROUP, remove = "KOD")
plot(m15, which = "single")
GROUP_mod

## Not run:
## use one of the mechanistic models
## get D0 values
m16 <- modlist(reps, model = mak3)
sapply(m16, function(x) coef(x)[1])

## exclude first sample in each
## replicate group of dataset 'reps'
m17 <- modlist(reps, exclude = ".1")
plot(m17, which = "single")

## End(Not run)

```

mselect

Sigmoidal model selection by different criteria

Description

Model selection by comparison of different models using

- 1) the maximum log likelihood value,
- 2) Akaike's Information Criterion (AIC),
- 3) bias-corrected Akaike's Information Criterion (AICc),
- 4) the estimated residual variance,
- 5) the p-value from a nested F-test on the residual variance,
- 6) the p-value from the likelihood ratio,
- 7) the Akaike weights based on AIC,

- 8) the Akaike weights based on AICc, and
- 9) the reduced chi-square, χ^2_ν , if replicates exist.

The best model is chosen by 5), 6), 8) or 9) and returned as a new model.

Usage

```
mselect(object, fctList = NULL, sig.level = 0.05, verbose = TRUE,
        crit = c("ftest", "ratio", "weights", "chisq"), do.all = FALSE, ...)
```

Arguments

| | |
|-----------|--|
| object | an object of class 'pcrfit' or 'replst'. |
| fctList | a list of functions to be analyzed, i.e. for a non-nested regime. Should also contain the original model. |
| sig.level | the significance level for the nested F-test. |
| verbose | logical. If TRUE, the result matrix is displayed in the console. |
| crit | the criterium for model selection. Either "ftest"/"ratio" for nested models or "weights"/"fitprob" for nested and non-nested models. |
| do.all | if TRUE, all available sigmoidal models are tested and the best one is selected based on AICc weights. |
| ... | other parameters to be passed to fitchisq . |

Details

Criteria 5) and 6) cannot be used for comparison unless the models are nested. Criterion 8), Akaike weights, can be used for nested and non-nested regimes, which also accounts for the reduced χ^2_ν . For criterion 1) the larger the better. For criteria 2), 3) and 4): the smaller the better. The best model is chosen either from the nested F-test ([anova](#)), likelihood ratio ([llratio](#)), corrected Akaike weights ([akaike.weights](#)) or reduced χ^2_ν ([fitchisq](#)) and returned as a new model. When using "ftest"/"ratio" the corresponding nested functions are analyzed automatically, i.e. b3/b4/b5/b6/b7; l3/l4/l5/l6/l7. If supplying nested models, please do this with ascending number of parameters.

Value

A model of the best fit selected by one of the criteria above. The new model has an additional list item 'retMat' with a result matrix of the criterion tests.

Author(s)

Andrej-Nikolai Spiess

See Also

[llratio](#), [akaike.weights](#) and [fitchisq](#).

Examples

```
## choose best model based on F-tests
## on the corresponding nested models
m1 <- pcrfit(reps, 1, 2, 13)
m2 <- mselect(m1)
summary(m2) ## Converted to 17 model!

## use Akaike weights on non-nested models
## compare to original model
m2 <- mselect(m1, fctList = list(13, 15, b3), crit = "weights")
summary(m2) ## Converted to 15 model!

## try all sigmoidal models
m3 <- pcrfit(reps, 1, 20, 14)
mselect(m3, do.all = TRUE) ## 17 wins by far!

## on replicated data
## using reduced chi-square
m11 <- modlist(reps, fluo = 2:5, model = 14)
r11 <- replist(m11, group = c(1, 1, 1, 1))
mselect(r11, crit = "chisq") ## converted to 16!
```

neill.test

Neill's lack-of-fit test when replicates are lacking

Description

This is a test for nonlinear models in the absence of replicates. A grouping of the predictor values has to be provided. If missing, it is calculated from cutting the dendrogram into groups of at least 2 predictor values. See 'References' for details. Works on any model WITHOUT replicates of class 'pcrfit' or 'nls'.

Usage

```
neill.test(object, grouping)
```

Arguments

object an object of class 'pcrfit'.
grouping vector that provides the grouping of the predictor ('Cycles') values.

Details

Let the total number of observations be denoted by $\sum n_i$ and let $\bar{G}(\theta)$ be defined by

$$\bar{G}(\theta) = (G(x_1, \theta)j'_{n_1}, \dots, G(x_M, \theta)j'_{n_M})'$$

, where j_{n_i} is an $n_i \times 1$ vector of ones, $i = 1, 2, \dots, M$. Also, $Y = (Y_{11}, \dots, Y_{1n_1}, \dots, Y_{Mn_m})'$ and $\bar{Y} = (\bar{Y}_{1 \cdot j'_{n_1}}, \dots, \bar{Y}_{M \cdot j'_{n_M}})'$. To test

$$H_0 : E(Y) = \bar{G}(\theta)$$

vs.

$$H_\alpha : E(Y) \neq \bar{G}(\theta)$$

let the statistic F be defined by

$$F = \frac{N - M}{M - p} \cdot \frac{\left(\|\bar{Y} - \bar{G}(\hat{\theta})\| \right)^2}{\left(\|Y - \bar{Y}\| \right)^2}$$

, where $\hat{\theta}$ is the least-squares parameter estimator of θ . Reject H_0 if observed $F > F_{M-p, N-M}^\alpha$. This is a nonlinear analogue to the Lack-of-fit test in linear models with replication.

Value

The p-value from the test.

Author(s)

Andrej-Nikolai Spiess, taken in part from function `neill.test` of the 'drc' package.

References

Testing for lack-of-fit in nonlinear regression.
Neill JW.
Ann Statist (1988), **16**: 733-740.

Lack-of-fit tests for assessing mean structures for continuous dose-response data.
Ritz C & Martinussen T.
Environ Ecol Stat (2011), **18**: 349-366.

Examples

```
## compare two models
m1 <- pcrfit(reps, 1, 2, 14)
m2 <- pcrfit(reps, 1, 2, 15)
neill.test(m1)
neill.test(m2)

## Not run:
## using example from 'nls'
## Fails when replicates are given
DNase1 <- subset(DNase, Run == 1)
fm1DNase1 <- nls(density ~ SSlogis(log(conc), Asym, xmid, scal), DNase1)
neill.test(fm1DNase1)
## But works if replicates are removed
DNase2 <- DNase1[-c(2, 4, 6, 8, 10, 12, 14, 16),]
```

```
fm1DNase2 <- nls(density ~ SSlogis(log(conc), Asym, xmid, scal), DNase2)
neill.test(fm1DNase2)

## End(Not run)
```

parKOD *Parameters that can be changed to tweak the kinetic outlier methods*

Description

A control function with different list items that change the performance of the different (kinetic) outlier functions as defined in [KOD](#).

Usage

```
parKOD(eff = c("sliwin", "sigfit", "expfit"), train = TRUE,
       alpha = 0.05, cp.crit = 10, cut = c(-6, 2))
```

Arguments

| | |
|---------|---|
| eff | uni1. The efficiency method to be used. Either sliwin, sigfit or expfit. |
| train | uni1. If TRUE, the sample's efficiency is NOT included in the calculation of the average efficiency (default), if FALSE it is. |
| cp.crit | uni2. The cycle difference between first and second derivative maxima, default is 10. |
| cut | multi1. A 2-element vector defining the lower and upper border from the first derivative maximum from where to cut the complete curve. |
| alpha | the p-value cutoff value for all implemented statistical tests. |

Details

For more details on the function of the parameters within the different kinetic and sigmoidal outlier methods, see [KOD](#).

Value

If called, returns a list with the parameters as items.

Author(s)

Andrej-Nikolai Spiess

Examples

```
## multivariate outliers,
## adjusting the 'cut' parameter
m11 <- modlist(reps, 1, 2:5, model = 15)
res1 <- KOD(m11, method = "multi1", par = parKOD(cut = c(-5, 2)))
```

 parMAK

Parameters that can be changed to tweak the mak2/mak3 methods

Description

A function to set (at the moment) three parameters that change the selfStart performance of mak2/mak3 models. This is feasible if the fitting fails to converge, or if there are other problems such as a high background noise level.

Usage

```
parMAK(SS.offset = 0, SS.method = "LM", SS.deriv = c("sigfit", "spline"))
```

Arguments

| | |
|-----------|--|
| SS.offset | number of cycles to add to the second derivative maximum cut-off point. |
| SS.method | fitting algorithm to be used for selfStart parameter estimation. Either "LM" or any of the methods in optim . |
| SS.deriv | method to calculate the second derivative maximum cut-off point. Either "sigfit" based on 4-par sigmoidal fit or "spline" based on smoothing and estimation from the smoothed data by two-times diff . |

Details

Calling the function writes a list named parMAKs with the three parameters to the global environment. In the **mak2** and **mak3** methods, these parameters are passed to \$ssFct and \$fct_ssFct. Selecting SS.deriv = "spline" usually leads to 1-2 cycles less for cutoff compared to "sigfit". The user should try which is the best setting by using some calibration data and choosing the method delivering the highest R^2 in the linear regression plot.

Value

A list with the three items is written to the global environment.

Author(s)

Andrej-Nikolai Spiess

Examples

```
## Not run:
## fitting a mak2 model (no slope parameter)
m1 <- pcrfit(reps, 1, 2, mak2)
plot(m1)
BIC(m1)

## fitting a mak3 model (with slope parameter)
m2 <- pcrfit(reps, 1, 2, mak3)
```

```

plot(m2, add = TRUE, col = 2)
BIC(m2)

## using a spline fit
parMAK(SS.deriv = "spline")
m3 <- pcrfit(reps, 1, 2, mak3)

## End(Not run)

```

pcrbatch

Batch calculation of qPCR efficiency and other qPCR parameters

Description

This function batch calculates the results obtained from [efficiency](#), [sliwin](#), [expfit](#), [LRE](#) or the coefficients from any of the mak models on a dataframe containing many qPCR runs. The input can also be a list obtained from [modlist](#), which simplifies things in many cases. The output is a dataframe with the estimated parameters and model descriptions. Very easy to use on datasheets containing many qPCR runs, i.e. as can be imported from Excel. The result is automatically copied to the clipboard.

Usage

```

pcrbatch(x, cyc = 1, fluo = NULL,
         methods = c("sigfit", "sliwin", "expfit", "LRE"),
         model = l4, check = "uni2", checkPAR = parKOD(),
         remove = c("none", "fit", "KOD"), exclude = NULL,
         type = "cpD2", labels = NULL, norm = FALSE, backsub = NULL,
         smooth = c("none", "smooth", "lowess", "supsmu", "spline"),
         smoothPAR = list(span = 0.1), factor = 1, opt = FALSE,
         optPAR = list(sig.level = 0.05, crit = "ftest"),
         group = NULL, names = c("group", "first"), plot = TRUE,
         verbose = TRUE, ...)

```

Arguments

| | |
|----------|--|
| x | a dataframe containing the qPCR raw data from the different runs or a list obtained from modlist . |
| cyc | the column containing the cycle data. Defaults to first column. |
| fluo | the column(s) (runs) to be analyzed. If NULL, all runs will be considered. |
| methods | a character vector defining the methods to use. See 'Details'. |
| model | the model to be used for all runs. |
| check | the method for outlier detection in KOD . Default is check for sigmoidal structure. |
| checkPAR | parameters to be supplied to the check method. |
| remove | which runs to remove. Either none, those which failed to fit or from the outlier methods. |

| | |
|-----------|---|
| exclude | either "" for samples with missing column names or a regular expression defining columns (samples) to be excluded from pcrbatch. See 'Details' and 'Examples' in <code>modlist</code> . |
| type | the point on the amplification curve from which the efficiency is estimated. See <code>efficiency</code> . |
| labels | a vector containing labels, i.e. for defining replicate groups prior to <code>radiobatch</code> . |
| norm | logical. Should the raw data be normalized within [0, 1] before model fitting? |
| backsub | background subtraction. An optional numeric sequence defining the cycle numbers for background averaging and subtraction, such as 1:8. |
| smooth | which curve smoothing method to use. See 'Details'. |
| smoothPAR | parameters to be supplied to the smoothing functions. See 'Details'. |
| factor | a multiplication factor for the fluorescence response values (barely useful, but who knows...). |
| opt | logical. Should model selection be applied to each model? |
| optPAR | parameters to be supplied to <code>mselect</code> . |
| group | a vector containing the grouping for possible replicates. |
| names | how to name the grouped fit. Either 'group_1, ...' or the first name of the replicates. |
| plot | logical. If TRUE, the single runs are plotted from the internal 'modlist' for diagnostics. |
| verbose | logical. If TRUE, fitting and tagging results will be displayed in the console. |
| ... | other parameters to be passed to downstream methods. |

Details

The methods vector is used for defining the different methods from which pcrbatch will concatenate the results. The mak models are omitted by default, because fitting is time-expensive. If they should be included, just add "mak3" to methods. See 'Examples'. The qPCR raw data should be arranged with the cycle numbers in the first column with the name "Cycles". All subsequent columns must be plain raw data with sensible column descriptions. If replicates are defined by group, the output will contain a numbering of groups (i.e. "group_1" for the first replicate group). The model selection process is optional, but we advocate using this for obtaining better parameter estimates. Normalization has been described to improve certain qPCR analyses, but this has still to be independently evaluated. Background subtraction is done by averaging the backsub cycles of the run and subtracting this from all data points. In case of unsuccessful model fitting or lack of sigmoidal structure, the names are tagged by *NAME* or **NAME**, respectively (if `remove = FALSE`). However, if `remove = TRUE`, the failed runs are excluded from the output. Likewise to `modlist`, if a labels vector lab is supplied, the labels from the failed fits are removed and a new label vector lab_mod is written to the global environment.

Value

A dataframe with the results in columns containing the calculated values, fit parameters and (tagged) model name together with the different methods used as the name prefix. A plot shows a plot matrix of all amplification curves/sigmoidal fits and failed amplifications marked with asterisks.

Note

IMPORTANT: When subsequent use of [ratiocalc](#) is desired, use pcrbatch on the single run level with `group = NULL` and `remove = FALSE`, so that [ratiocalc](#) can automatically delete the failed runs from its group definition. Otherwise error propagation will fail.

Author(s)

Andrej-Nikolai Spiess

See Also

The function [modlist](#) for creating a list of models, which is used internally by pcrbatch.

Examples

```
## first 4 runs and return parameters of fit
## do background subtraction using the first 8 cycles
res1 <- pcrbatch(reps, fluo = 2:5, backsub = 1:8)

## first 8 runs, with 4 replicates each, 15 model
res2 <- pcrbatch(reps, fluo = 2:9, model = 15, group = c(1,1,1,1,2,2,2,2))

## using model selection (Akaike weights)
## on the first 4 runs, runs 1 and 2 are replicates
res3 <- pcrbatch(reps, fluo = 2:5, group = c(1,1,2,3),
                opt = TRUE, optPAR = list(crit = "weights"))

## Not run:
## fitting a sigmoidal and 'mak3' mechanistic model
res4 <- pcrbatch(reps, methods = c("sigfit", "mak3"))
View(res4)

## converting a 'modlist' to 'pcrbatch'
m11 <- modlist(reps, 1, 2:5, b3)
res5 <- pcrbatch(m11)

## End(Not run)
```

pcrboot

Bootstrapping and jackknifing qPCR data

Description

Confidence intervals for the estimated parameters and goodness-of-fit measures are calculated for a nonlinear qPCR data fit by either

- a) bootstrapping the residuals of the fit or
- b) jackknifing and refitting the data.

Confidence intervals can also be calculated for all parameters obtained from the [efficiency](#) analysis.

Usage

```
pcrboot(object, type = c("boot", "jack"), B = 100, njack = 1,
        plot = TRUE, do.eff = TRUE, conf = 0.95, verbose = TRUE, ...)
```

Arguments

| | |
|---------|---|
| object | an object of class 'pcrfit'. |
| type | either bootstrapping or jackknifing. |
| B | numeric. The number of iterations. |
| njack | numeric. In case of type = "jack", how many datapoints to exclude. Defaults to leave-one-out. |
| plot | should the fitting and final results be displayed as a plot? |
| do.eff | logical. If TRUE, efficiency analysis will be performed. |
| conf | the confidence level. |
| verbose | logical. If TRUE, the iterations will be printed on the console. |
| ... | other parameters to be passed on to the plotting functions. |

Details

Non-parametric bootstrapping is applied using the centered residuals.

1) Obtain the residuals from the fit:

$$\hat{\epsilon}_t = y_t - f(x_t, \hat{\theta})$$

2) Draw bootstrap pseudodata:

$$y_t^* = f(x_t, \hat{\theta}) + \epsilon_t^*$$

where ϵ_t^* are i.i.d. from distribution \hat{F} , where the residuals from the original fit are centered at zero.

3) Fit $\hat{\theta}^*$ by nonlinear least-squares.

4) Repeat B times, yielding bootstrap replications

$$\hat{\theta}^{*1}, \hat{\theta}^{*2}, \dots, \hat{\theta}^{*B}$$

One can then characterize the EDF and calculate confidence intervals for each parameter:

$$\theta \in [EDF^{-1}(\alpha/2), EDF^{-1}(1 - \alpha/2)]$$

The jackknife alternative is to perform the bootstrap on the data-predictor vector, i.e. eliminating a certain number of datapoints.

If the residuals are correlated or have non-constant variance the latter is recommended. This may be the case in qPCR data, as the variance in the low fluorescence region (ground phase) is usually much higher than in the rest of the curve.

Value

A list containing the following items:

ITER a list containing each of the results from the iterations.
CONF a list containing the confidence intervals for each item in ITER.

Each item contains subitems for the coefficients (coef), root-mean-squared error (rmse), residual sum-of-squares (rss), goodness-of-fit measures (gof) and the efficiency analysis (eff). If plot = TRUE, all data is plotted as boxplots including confidence intervals.

Author(s)

Andrej-Nikolai Spiess

References

Nonlinear regression analysis and its applications.
Bates DM & Watts DG.
Wiley, Chichester, UK, 1988.

Nonlinear regression.
Seber GAF & Wild CJ.
Wiley, New York, 1989.

Bootstrap accuracy for non-linear regression models.
Roy T.
J Chemometrics (1994), **8**: 37-44.

Examples

```
## simple bootstrapping with  
## too less iterations...  
par(ask = FALSE)  
m1 <- pcrfit(reps, 1, 2, 14)  
pcrboot(m1, B = 20)  
  
## jackknifing with leaving  
## 5 datapoints out  
m2 <- pcrfit(reps, 1, 2, 14)  
pcrboot(m2, type = "jack", njack = 5, B = 20)
```

pcrfit

*Workhorse function for qPCR model fitting***Description**

This is the main workhorse function of the qpcR package that fits one of the available models to qPCR data using nonlinear least-squares fitting from `nls`, with sensible starting parameters obtained from either `nls.lm` (default) or `optim`.

Usage

```
pcrfit(data, cyc = 1, fluo, model = 14, do.optim = TRUE,
       opt.method = "all", nls.method = "all",
       start = NULL, robust = FALSE, weights = NULL,
       verbose = TRUE, ...)
```

Arguments

| | |
|-------------------------|--|
| <code>data</code> | the name of the dataframe containing the qPCR runs. |
| <code>cyc</code> | the column containing the cycle data. Defaults to 1. |
| <code>fluo</code> | the column(s) containing the raw fluorescence data of the run(s). If more than one column is given, the model will be built with the replicates. See 'Details' and 'Examples'. |
| <code>model</code> | the model to be used for the analysis. Defaults to '14'. |
| <code>do.optim</code> | if FALSE, refinement of starting values by any of the optimization methods (see 'Details') will be skipped. |
| <code>opt.method</code> | all or one of the available optimization methods. See 'Details'. |
| <code>nls.method</code> | all or one of the available methods in <code>nls</code> . See 'Details'. |
| <code>start</code> | a vector of starting values that can be supplied externally. |
| <code>robust</code> | logical. If TRUE, robust nonlinear regression is used. See 'Details'. |
| <code>weights</code> | a vector with same length as <code>data</code> containing possible weights for the nonlinear fit. |
| <code>verbose</code> | logical. If TRUE, fitting and convergence results will be displayed in the console. |
| <code>...</code> | other parameters to be passed to <code>nls.lm</code> , <code>optim</code> or <code>nls</code> . |

Details

The fitting procedure works as follows (ensuring maximum safeness against convergence errors):

- 1) Approximate starting values are acquired from `model$ssfct`.
- 2) Starting values are refined by any of the methods available in `optim` or the Levenberg-Marquardt algorithm (`nls.lm`). The default setting is `all`, meaning that the optimization is done in the order "LM", "BFGS", "Nelder-Mead", and "SANN". If any of the methods successfully converged, the remaining are skipped and the refined starting values transferred to `nls`. The `opt.methods` can be

combined to tweak the robustness, whereby the starting parameters are passed to each succeeding method, i.e. `rep("Nelder", 5)` will do 5 successive Nelder-Mead optimisations or `c("LM", "Nelder")` will pass the starting values from "LM" to "Nelder". Levenberg-Marquardt ("LM") is very fast and reliable in many scenarios and is thus the default first method.

3) The refined starting values from 2) are fitted with `nls` using the order "port", "default" (Gauss-Newton) and "plinear". Again, if any of the methods converged, the remaining are skipped.

This function is to be used at the single run level or on replicates (by giving several columns). The latter will build a single model based on the replicate values. If many models should be built on a cohort of replicates, use `modlist` and `replist`.

The output from the optim methods is checked by ensuring all eigenvalues from the hessian are positive, otherwise a notice will occur and the next method in queue is used.

If `robust = TRUE`, robust nonlinear fitting will be used. To do this, the internal function `qpcR:::rnls` is called which is a modification of the `nls` function of the 'robustbase' package. Modifications were done such that all available generic functions for objects of class 'nls' can be used on the output of `qpcR:::rnls`, such as `predict`, `confint` etc.

Value

A model of class 'nls' and 'pcrfit' with the following items attached:

| | |
|-------------------------|--|
| <code>DATA</code> | the initial data used for fitting. |
| <code>MODEL</code> | the model used for fitting. |
| <code>call12</code> | the call to <code>pcrfit</code> . |
| <code>parMat</code> | the trace of the starting values for each applied method. Can be used to track problems. |
| <code>opt.method</code> | the successful (convergence) optimization methods. |

Author(s)

Andrej-Nikolai Spiess

References

Bioassay analysis using R.
Ritz C & Streibig JC.
J Stat Soft (2005), **12**: 1-22.

A Method for the Solution of Certain Problems in Least Squares.
K. Levenberg.
Quart Appl Math (1944), **2**: 164-168.

An Algorithm for Least-Squares Estimation of Nonlinear Parameters.
D. Marquardt.
SIAM J Appl Math (1963), **11**: 431-441.

Examples

```

## simple l4 fit of F1.1 of the 'reps' dataset
m1 <- pcrfit(reps, 1, 2, l4)
plot(m1)

## using BFGS and Nelder from 'optim'
pcrfit(reps, 1, 2, l5, opt.method = c("BFGS", "Nelder-Mead"))

## skip 'optim' method and supply
## own starting values
pcrfit(reps, 1, 2, l4, do.optim = FALSE, start = c(-5, -0.05, 11, 16))

## make a robust model
pcrfit(reps, 1, 2, l4, robust = TRUE)

## make a replicate model
m2 <- pcrfit(reps, 1, 2:5, l5)
plot(m2)

## Not run:
## fit a mechanistic mak3 model
m3 <- pcrfit(reps, 1, 2, mak3)
plot(m3)

## End(Not run)

```

pcrGOF

Summarize measures for the goodness-of-fit

Description

Calculates all implemented measures for the goodness-of-fit and returns them as a list. Works for objects of class `pcrfit`, `lm`, `glm`, `nls`, `drc` and many others...

Usage

```
pcrGOF(object, PRESS = FALSE)
```

Arguments

| | |
|---------------------|--|
| <code>object</code> | a fitted object. |
| <code>PRESS</code> | logical. If TRUE, the more calculation intensive P^2 is also returned. |

Value

A list with all implemented Information criteria (AIC, AICc, BIC, HQIC), residual variance, root-mean-squared-error, the p-value from `neill.test` (if no replicates), the reduced χ^2_ν from `fitchisq` (if replicates) and the `PRESS` P^2 value (if `PRESS = TRUE`).

Author(s)

Andrej-Nikolai Spiess

Examples

```
## single fit without replicates
## including PRESS statistic
m1 <- pcrfit(reps, 1, 2, 15)
pcrGOF(m1, PRESS = TRUE)

## fit containing replicates:
## calculation of reduced
## chi-square included!
m2 <- pcrfit(reps, 1, 2:5, 15)
pcrGOF(m2)
```

pcrimport

Advanced qPCR data import function

Description

Advanced function to easily import/preformat qPCR data from delimited text files, the clipboard or the workspace. The data files can be located in a directory which is automatically browsed for all files. In a series of steps, the data can be imported and transformed to the appropriate format of the 'qpcR' package (such as in dataset `reps`, with 'Cycles' in the first column and named runs with raw fluorescence data in remaining columns). A dataset can function as a transformation template, and the remaining files in the directory are then formatted according to the established parameters. See 'Details' and tutorial video in <http://www.dr-spiess.de/qpcR/tutorials.html>.

Usage

```
pcrimport(file = NA, sep = NA, dec = NA, delCol = NA, delRow = NA,
          format = c(NA, "col", "row"), sampleDat = NA, refDat = NA,
          names = NA, sampleLen = NA, refLen = NA, check = TRUE,
          usePars = TRUE, dirPars = NULL, needFirst = TRUE, ...)
```

Arguments

| | |
|---------------------|---|
| <code>file</code> | either a directory such as "c:/temp" containing the data file(s), the Windows "clipboard" or an object in the workspace such as "reps". |
| <code>sep</code> | the field separator character, i.e. "\t" for tabs. |
| <code>dec</code> | the decimal separator, i.e. ".". |
| <code>delCol</code> | unnneeded columns to delete after successful import, i.e. 2, 1:3, seq(1, 5, by = 2), etc.... |
| <code>delRow</code> | unnneeded rows to delete after successful import, i.e. 2, 1:3, seq(1, 5, by = 2), etc.... |

| | |
|-----------|---|
| format | how the data is organized, i.e. in columns or rows. |
| sampleDat | the columns with the raw fluorescence reporter dye data. |
| refDat | optional columns with the raw fluorescence reference dye data. |
| names | the row(s) that should be used for naming the runs. |
| sampleLen | the rows with the reporter dye cycles. |
| refLen | the rows with the reference dye cycles. |
| check | logical. If TRUE, a window displaying the transformed data after each step is displayed. This assists in choosing the right parameters. |
| usePars | logical. If TRUE, then all files in the directory are batch analysed using the stored parameters. See 'Details'. |
| dirPars | an optional directory such as "c:/pars" where the formatting parameters can be stored. If NULL, the 'qpcR' directory is used. |
| needFirst | logical. If TRUE, then the (alphabetically) first file in the directory is used for an initial definition of transformation parameters. |
| ... | other parameters to be passed to read.delim . |

Details

This function has been designed to offer maximal flexibility in importing qPCR data from all kinds of systems. This is accomplished by asking the user for many formatting options in single steps, with the final goal of obtaining a dataset that is transformed in a way suitable for [pccrfit](#), as in all datasets in this package (i.e. 'reps'): it must be a dataframe with the first column containing the cycle numbers ("Cycles") and all subsequent columns with sensible sample names, such as "S1_1". In detail, the following steps are queried:

- 1) Location of the file. Either a directory containing the file(s), the (Windows) clipboard or a dataframe in the workspace.
- 2) How are the fields separated, i.e. by tabs?
- 3) What is the decimal separator?
- 4) Which columns can be deleted? For analysis, we only need the raw fluorescence values and sample names. Everything else should be deleted.
- 5) Which rows can be deleted? Same as above.
- 6) Are the runs organized in rows or in columns?
After these steps, the unwanted rows/columns are deleted and the data transformed into vertical format (if it was in rows).
- 7) In which columns are the runs with reporter dye data (i.e. SybrGreen)?
- 8) If a reference dye (i.e. ROX) was used, in which columns are the runs?
- 9) How should the runs be named (automatically or from a row/rows containing names)? If more than one row is supplied, the names in the rows are pasted together, i.e. "A4.GAPDH".
- 10) Which are the rows containing the raw fluorescence data from cycle to cycle for the reporter dye?
- 11) If a reference dye was used, which are the rows with the cycle to cycle data?
After these steps, a 'Cycles' column is prepended to the data which should then be in the right format for downstream analysis.

ATTENTION: Because of this step, if the imported data also initially had a column containing

cycle numbers, these should be removed in steps 2) or 3)!

One major advantage of this function is that the formatting parameters are stored in a file and can be reused with new data, most conveniently when doing a batch analysis of several files in a directory. When `needFirst = TRUE`, the alphabetically first run in the directory is used for defining the formatting parameters, and if `usePars = TRUE` these are applied on all remaining datasets. If the initial definition of formatting parameters is not needed, then setting `needFirst = FALSE` will apply the last stored parameters on all datasets. By using different `dirPars`, one can establish different formatting options for different qPCR systems.

The function will query (if `needFirst = TRUE`) all parameters that are defined as NA. For example, using `res <- pcrimport(file = "c:/temp", sep = "\t", dec = ".", delCol = c(1, 3), delRow = 1:2, ...)` will result in these parameters not being queried.

If reference dye data was supplied, the function checks if the data is of same dimensions than the reporter dye data. The output is then the normalized fuorescence data $\frac{F_{rep}}{F_{ref}}$.

The 'Examples' feature internal datasets, but this function is best understood by the tutorial under <http://www.dr-spiess.de/qpcR/tutorials.html>.

Value

A list with the transformed data as `data.frame` list items, suitable for downstream analysis.

Author(s)

Andrej-Nikolai Spiess

Examples

```
## Not run:
## EXAMPLE 1:
## Internal dataset format01.txt (in 'add01' directory)
## with 384 runs.
## Tab delimited, 30 cycles, only reporter dye,
## data in rows, and some unneeded columns and rows.
## This is the example data path, but could be any path
## with data such as c:/temp.
PATH <- .path.package("qpcR")
PATHall <- paste(PATH, "/add01/", sep = "")
res <- pcrimport(PATHall)

## Answer queries with the following parameters and
## verify the effects in the 'View' windows:
## 1 => data is tab delimited
## 1 => decimal separator is "."
## c(1, 3) => remove columns 1 + 3
## 1:2 => remove rows 1 + 2
```

```

## 2 => data is in rows
## 0 => all data is from reporter dye
## 1 => sample names are in row #1
## 0 => reporter data goes until end of table
## Data is stored as dataframe list items and can
## then be analyzed:
ml <- modlist(res[[1]], model = 15)
plot(ml, which = "single")

## Alternative without query:
res <- pccrimport(PATHall, sep = "\t", dec = ".",
  delCol = c(1, 3), delRow = 1:2,
  format = "row", sampleDat = 0,
  names = 1, sampleLen = 0,
  check = FALSE)

## Do something useful with the data:
ml <- modlist(res[[1]], model = 15)
plot(ml, which = "single")

## EXAMPLE 2:
## Internal datasets format02a.txt - format02d.txt
## (in 'add02' directory) with 96 runs.
## Tab delimited, 40 cycles, reporter dye + reference dye,
## data in columns, and some unneeded columns and rows.
PATH <- .path.package("qpcR")
PATHall <- paste(PATH, "/add02/", sep = "")
res <- pccrimport(PATHall)

## Answer queries with the following parameters and
## verify the effects in the 'View' windows:
## 1 => data is tab delimited
## 1 => decimal separator is "."
## 1 => remove column 1 with cycle data
## c(1, 43, 44) => remove rows 1, 43, 44
## 1 => data is in columns
## 1:96 => data columns for reporter dye
## -2 => reference dye stacked under reporter dye
## 1 => sample names are in row #1
## 1:40 => reporter data is in rows 1-40
## -1 => reference data is stacked under samples
## Data is stored as dataframe list items and can
## then be analyzed.

## Alternative without query:
res2 <- pccrimport(PATHall, sep = "\t", dec = ".",
  delCol = 1, delRow = c(1, 43, 44),
  format = "col", sampleDat = 1:96,
  refDat = -2, names = 1,
  sampleLen = 1:40, refLen = -1,
  check = FALSE)

## Do something useful with the data:

```

```
m12 <- modlist(res2[[1]], model = 15)
plot(m12)

## End(Not run)
```

pcrimport2

Simple qPCR data import function (i.e. from text files or clipboard)

Description

Simple wrapper function to easily import qPCR data from the clipboard (default) or tab-delimited text files. In contrast to [pcrimport](#), this function has no enhanced formatting features, but is quick and easy to use on data that has been pre-formatted, i.e. as in dataset [reps](#) ('Cycles' in the first column, all remaining columns with sensible names).

Usage

```
pcrimport2(file = "clipboard", sep = "\t", header = TRUE, quote = "",
           dec = ".", colClasses = "numeric", ...)
```

Arguments

| | |
|------------|--|
| file | the name of the file which the data are to be read from (full path). |
| sep | the field separator character. |
| header | a logical value indicating whether the file contains the names of the variables as its first line. |
| quote | the set of quoting characters. |
| dec | the character used in the file to denote decimal points. |
| colClasses | character. A vector of classes to be assumed for the columns. |
| ... | further arguments to be passed on to read.table . |

Details

For a more detailed description of the arguments see [read.table](#).

Value

A data frame containing a representation of the data in the file.

Note

This function is the former [pcrimport](#) from packages 1.3-3 downward. See [pcrimport](#) for an enhanced version offering formatting in the presence of reference dyes, columns/rows deletion, transforming from wide to long format, and automatic batch analysis.

Author(s)

Andrej-Nikolai Spiess

Examples

```
## paste some Excel data into the clipboard ###
## Not run:
temp <- pcrimport2()

## End(Not run)
## from a tab-delimited text file ###
## Not run:
temp <- pcrimport2("c:\temp\foo.txt")

## End(Not run)
```

pcropt1

Combinatorial elimination of plateau and ground phase cycles

Description

The estimation of PCR efficiency and calculation of initial fluorescence F_0 is analyzed by refitting the (optimized) model on subsets of the data, thereby using all possible combinations of datapoints. The estimated parameters are then collated in a dataframe. This is intended to be the prerequisite for finding the optimal datapoints that minimize the fit or exhibit the best correlation to a calibration curve. This approach is an extension to the method described in Rutledge *et al.* (2004). The result of any collected parameter can then be displayed by a rank-colored bubbleplot. See 'Examples'.

Usage

```
pcropt1(object, fact = 3, opt = FALSE, plot = TRUE, bubble = NULL, ...)
```

Arguments

| | |
|--------|--|
| object | an object of class 'pcrfit'. |
| fact | numeric. The multiplier for the scan border. See 'Details'. |
| opt | logical. If true, model selection is applied for each combination of cycles. Beware: Slow!! |
| plot | logical. If TRUE, the iterative plotting is displayed, which makes the method a bit slower. |
| bubble | either NULL for no bubble plot or any parameter (given as a character vector) in the result matrix to be displayed as a bubble plot. See 'Examples'. |
| ... | other parameters to be passed on to efficiency , mselect or qpcR:::bubbleplot . |

Details

It has been shown by Rutledge (2004) that the estimation of PCR efficiency gives more realistic values when the number of plateau cycles are decreased. This paradigm is the basis for this function, but we also consider the cycles in the ground phase and all combinations between ground/plateau cycles. All datapoints between the lower border $cpD1 - fact * (cpD1 - cpD2)$ and upper border $cpD1 + fact * (cpD1 - cpD2)$ are cycled through.

Value

A matrix with the selected border values, goodness-of-fit measures as obtained from `pcrGOF` and efficiency and F_0 values from `efficiency`.

Author(s)

Andrej-Nikolai Spiess

References

Sigmoidal curve fitting redefines quantitative real-time PCR with the prospective of developing automated high-throughput applications.
Rutledge RG.
Nucleic Acids Research (2004), **32**: e178.

Examples

```
## Not run:
## optimize fit and display
## bubbleplot of R-square
m1 <- pcrfit(reps, 1, 2, 14)
res1 <- pcropt1(m1, plot = FALSE, bubble = "Rsq")

## End(Not run)
```

pcropt2

Elimination of qPCR cycles with low/high impact on fitted parameters

Description

The qPCR curve containing n cycles is refitted $n-1$ times, each time leaving out one cycle. The difference of the new coefficients of the fit in comparison to the original coefficients is calculated and those cycles are eliminated that have a weak (strong) influence on the change of coefficients. A new model is returned with the selected cycles left out.

Usage

```
pcropt2(object, plot = TRUE, which.par = "all", quan = 0.1,
        delete = c("low", "high"), ...)
```

Arguments

| | |
|-----------|--|
| object | an object of class 'pcrfit'. |
| plot | logical. If TRUE, the refitting and the final result are plotted. |
| which.par | The coefficient(s) to be analysed. Either "all" for all coefficients, or the coefficient name, i.e. "b". |
| quan | the quantile for selecting the cycles exhibiting weak (strong) influence on the coefficient estimation. |
| delete | which cycles to delete. Those with low influence on the coefficients or those with a high one. |
| ... | other parameters to be passed on to the plotting functions. |

Details

For each deletion of cycle $i = 1, \dots, n$, the qPCR data is refitted yielding new parameter estimates

$$\hat{\theta}^{*1}, \dots, \hat{\theta}^{*i}$$

The difference to the original coefficients $\hat{\theta}$ is calculated by

$$crit = \frac{|\hat{\theta} - \hat{\theta}^{*i}|}{s.e.(\hat{\theta})}$$

with s.e. = standard error. The user then chooses the cycles with $F^{-1}(p) = \inf\{crit \in \mathbf{R} : F(crit) \geq p\}$ with p = the selected quantile.

Value

A new model of class 'pcrfit' with the corresponding cycles removed.

Author(s)

Andrej-Nikolai Spiess

References

Nonlinear regression analysis and its applications.
Bates DM & Watts DG.
Wiley, Chichester, UK, 1988.

See Also

The function [pcropt1](#) which removes cycles sequentially from both sides of the curve.

Examples

```
## which cycles have low influence
## on parameter 'c' (the lower
## asymptote)?
m1 <- pcrfit(reps, 1, 2, 14)
pcropt2(m1, which.par = "c", quan = 0.3, delete = "low")

## Not run:
## and on 'b' and 'e'?
pcropt2(m1, which.par = c("b", "e"), quan = 0.3, delete = "low")

## very high influence on 'd'
## (upper asymptote)?
pcropt2(m1, which.par = c("d"), quan = 0.1, delete = "high")

## End(Not run)
```

pcrsim

Simulation of sigmoidal qPCR data with goodness-of-fit analysis

Description

Simulated sigmoidal qPCR curves are generated from an initial model to which some user-defined homoscedastic or heteroscedastic noise is added. One or more models can then be fit to this random data and goodness-of-fit (GOF) measures are calculated for each of the models. This is essentially a Monte-Carlo approach testing for the best model in dependence to some noise structure in sigmoidal models.

Usage

```
pcrsim(object, nsim = 100, error = 0.02,
       errfun = function(y) 1, plot = TRUE,
       fitmodel = NULL, select = FALSE,
       statfun = function(y) mean(y, na.rm = TRUE),
       PRESS = FALSE, ...)
```

Arguments

| | |
|----------|---|
| object | an object of class 'pcrfit'. |
| nsim | the number of simulated curves. |
| error | the gaussian error used for the simulation. See 'Details'. |
| errfun | an optional function for the error distribution. See 'Details'. |
| plot | should the simulated and fitted curves be displayed? |
| fitmodel | a model or model list to test against the initial model. |
| select | if TRUE, a matrix is returned with the best model in respect to each of the GOF measures. |

| | |
|---------|--|
| statfun | a function to be finally applied to all collected GOF measures, default is the average. |
| PRESS | logical. If set to TRUE, the computationally expensive PRESS statistic will be calculated. |
| ... | other parameters to be passed on to plot or pcrfit . |

Details

The value defined under `error` is just the standard deviation added plainly to each `y` value from the initial model, thus generating a dataset with homoscedastic error. With aid of `errfun`, the distribution of the error along the `y` values can be altered and be used to generate heteroscedastic error along the curve, i.e. as a function of the magnitude.

Example:

```
errfun = function(y) 1
same variance for all y, as is.
```

```
errfun = function(y) y
variance as a function of the y-magnitude.
```

```
errfun = function(y) 1/y
variance as an inverse function of the y-magnitude.
```

For the effect, see 'Examples'.

Value

A list containing the following items:

| | |
|----------|--|
| cyc | same as in 'arguments'. |
| fluoMat | a matrix with the simulated qPCR data in columns. |
| coefList | a list with the coefficients from the fits for each model, as subitems. |
| gofList | a list with the GOF measures for each model, as subitems. |
| statList | a list with the GOF measures summarized by <code>statfun</code> for each model, as subitems. |
| modelMat | if <code>select = TRUE</code> , a matrix with the best model for each GOF measure and each simulation. |

Author(s)

Andrej-Nikolai Spiess

Examples

```
## generate initial model
m1 <- pcrfit(reps, 1, 2, 14)

## simulate homoscedastic error
## and test 14 and 15 on data
res1 <- pcrsim(m1, error = 0.2, nsim = 20,
```

```

fitmodel = list(14, 15))

## Not run:
## use heteroscedastic noise typical for
## qPCR: more noise at lower fluorescence
res2 <- pcrsim(m1, error = 0.01, errfun = function(y) 1/y,
              nsim = 20, fitmodel = list(14, 15, 16))

## get 95% confidence interval for
## the models GOF in question (14, 15, 16)
res3 <- pcrsim(m1, error = 0.2, nsim = 20, fitmodel = list(14, 15, 16),
              statfun = function(y) quantile(y, c(0.025, 0.975)))
res3$statList

## count the selection of the 'true' model (14)
## for each of the GOF measures,
## use PRESS statistic => SLOW!
## BIC wins!!
res4 <- pcrsim(m1, error = 0.05, nsim = 20, fitmodel = list(13, 14, 15),
              select = TRUE, PRESS = TRUE)
apply(res4$modelMat, 2, function(x) sum(x == 2))

## End(Not run)

```

plot.pcrfit

Plotting qPCR data with fitted curves/confidence bands/error bars

Description

A plotting function for data of class 'pcrfit' (single curves), 'modlist' (batch curves) or 'replist' (replicate curves) displaying the data points, the fitted curve and (if desired) confidence/prediction bands/error bars on replicates. Four different plot types are available, namely plotting all curves in a 2D graph, a 2D plot matrix, a 3D graph or a heatmap-like image plot.

Usage

```

## S3 method for class 'pcrfit'
plot(x, which = c("all", "single", "3D", "image"),
     fitted = TRUE, add = FALSE, col = NULL,
     confband = c("none", "confidence", "prediction"),
     errbar = c("none", "sd", "se", "conf"), par3D = list(),
     par2D = list(), parCI = list(), parSD = list(), ...)

```

Arguments

x an object of class 'pcrfit', 'modlist' or 'replist'.

which plots all curves in 2D ("all"), a plot matrix with many curves ("single"), a 3D plot ("3D") or a heatmap-like image plot (image).

| | |
|----------|---|
| fitted | should the fitted lines be displayed? |
| add | should the curve be added to an existing plot? |
| col | an optional color vector for the individual curves. Is recycled to the number of runs in x. |
| confband | should confidence/prediction bands be displayed? See confint . |
| errbar | the type of error bar on the plot if replicates exist. See 'Examples'. |
| par3D | a list containing graphical parameters to change the 3D-plot: plot3d , points3d , lines3d , axis3d or mtext3d . |
| par2D | a list containing graphical parameters to change the 2D-plots: plot , points or lines . |
| parCI | a list containing graphical parameters to change the confidence band: lines . |
| parSD | a list containing graphical parameters to change the error bars: arrows . |
| ... | other parameters to be passed to predict . |

Details

Uses the 'rgl' package for 3D plots. If the 'modlist' contains runs that failed to fit, these are displayed with RED asterisked names. Additionally, if an outlier method from [KOD](#) was applied on the 'modlist' with option `remove = FALSE`, outlier runs will be displayed in BLUE with double asterisked names. This approach makes the identification of failed runs easy and works only with `which = "single"`. See 'Examples'.

For high-throughput data, the user of this function is encouraged to use the "image" kind of plot, as one can see quite nicely the differences in the amplification profiles of several hundred runs. Of course, this plot type does not display the fitted curve. See 'Examples'.

Value

A 2D, multiple 2D, 3D or heatmap-like qPCR plot. If object was of class 'replot', colour coding of the curves is done automatically (but can be overridden).

Author(s)

Andrej-Nikolai Spiess

Examples

```
## single plot
m1 <- pcrfit(reps, 1, 2, 15)
plot(m1)

## add another plot in blue
## with 99% confidence interval
m2 <- pcrfit(reps, 1, 12, 15)
plot(m2, add = TRUE, col = 4, confband = "confidence", level = 0.99)

## plot a 'modlist' batch with coloring of replicates
m11 <- modlist(reps, 1, 2:13, model = 14)
plot(m11, col = gl(3,4))
```

```

## subset of data
plot(m11, type = "n", col = rep(1:3, each = 4),
      par2D = list(xlim = c(10, 30)))

## plot a 'replist'
r11 <- replist(m11, group = gl(3, 4))
plot(r11)

## standard deviation instead of
## replicate points; suppress plotting
## of point symbols
plot(r11, type = "l", errbar = "sd",
      par2D = list(pch = ""))

## 95% confidence values
plot(r11, errbar = "conf",
      par2D = list(pch = ""))

## plot single curves.
## good for diagnostics...
plot(m11, which = "single", col = rep(1:3, each = 4))

## 3D plots of 'modlist's or 'replist's
plot(m11, which = "3D", col = rep(1:3, each = 4))
rgl.close()
plot(r11, which = "3D")
rgl.close()

## Not run:
## example for "image" type when
## using large data
m12 <- modlist(vermeulen2)
plot(m12, which = "image")

## example for outlier identification:
## RED/*name* indicates failed fitting,
## BLUE/**name** indicates sigmoidal outlier
## using 'testdat' set
m13 <- modlist(testdat, model = 15)
plot(m13, which = "single")

## End(Not run)

```

predict.pcrfit

Value prediction from a fitted qPCR model

Description

After fitting the appropriate model, either the raw fluorescence values can be predicted from the cycle number or *vice versa*.

Usage

```
## S3 method for class 'pcrfit'
predict(object, newdata, which = c("y", "x"),
        interval = c("none", "confidence", "prediction"),
        level = 0.95, ...)
```

Arguments

| | |
|----------|--|
| object | an object of class 'pcrfit'. |
| newdata | a dataframe containing the values to estimate from, using the same variable naming as in the fitted model. |
| which | either "y" (default) for prediction of the raw fluorescence or "x" for prediction of the cycle number. |
| interval | if not "none", confidence or prediction intervals are calculated. |
| level | the confidence level. |
| ... | some methods for this generic require additional arguments. None are used in this method. |

Details

y-values (Fluorescence) are estimated from `object$MODEL$expr`, x-values (Cycles) are estimated from `object$MODEL$inv`. Confidence intervals are calculated from the gradient of the function and the variance-covariance matrix of object by $\nabla f(x) \cdot cov(y) \cdot \nabla f(x)$ and are based on asymptotic normality (t-distribution).

Value

A dataframe containing the estimated values and (if chosen) standard error/upper confidence limit/lower confidence limit. The gradient is attached to the dataframe and can be accessed with `attr(..., "gradient")`.

Note

The estimation of x (cycles) from fluorescence data if `which = "x"` is problematic in the asymptotic regions of the sigmoidal curves (often gives NaN, due to logarithmation of negative values) and works fairly well in the ascending part.

Author(s)

Andrej-Nikolai Spiess

Examples

```
m1 <- pcrfit(reps, 1, 2, 15)

## which raw fluorescence value at cycle number = 17?
predict(m1, newdata = data.frame(Cycles = 17))
```

```
## cycle numbers 20:25, with 95% confidence?
predict(m1, newdata = data.frame(Cycles = 20:25), interval = "confidence")

## which cycle at Fluo = 4, with 95% prediction?
predict(m1, newdata = data.frame(Fluo = 4), which = "x", interval = "prediction")
```

 PRESS

Allen's PRESS (Prediction Sum-Of-Squares) statistic, aka P-square

Description

Calculates the PRESS statistic, a leave-one-out refitting and prediction method, as described in Allen (1971). Works for any regression model with a `call` slot, an `update` and a `predict` function, hence all models of class `lm`, `glm`, `nls` and `drc` (and maybe more...). The function also returns the PRESS analog to R-square, the P-square.

Usage

```
PRESS(object, verbose = TRUE)
```

Arguments

| | |
|----------------------|--|
| <code>object</code> | a fitted model. |
| <code>verbose</code> | logical. If TRUE, iterations are displayed on the console. |

Details

From a fitted model, each of the predictors $x_i, i = 1 \dots n$ is removed and the model is refitted to the $n - 1$ points. The predicted value $\hat{y}_{i,-i}$ is calculated at the excluded point x_i and the PRESS statistic is given by:

$$\sum_{i=1}^n (y_i - \hat{y}_{i,-i})^2$$

The PRESS statistic is a surrogate measure of crossvalidation of small sample sizes and a measure for internal validity. Small values indicate that the model is not overly sensitive to any single data point. The P-square value, the PRESS equivalent to R-square, is given by

$$P^2 = \frac{\sum_{i=1}^n \hat{\epsilon}_{-i}^2}{\sum_{i=1}^n (y_i - \bar{y})^2}$$

with $\hat{\epsilon}_{-i} = y_i - \hat{y}_{-i}$.

Value

A list with the following components:

| | |
|------------------------|--|
| <code>stat</code> | The PRESS statistic. |
| <code>residuals</code> | a vector containing the PRESS residuals for each x_i . |
| <code>P.square</code> | the P-square value. See 'Details'. |

Note

There is also a PRESS function in library 'MPV' that works solely for lm models using the hat matrix.

Author(s)

Andrej-Nikolai Spiess

References

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Second Edition (1990), Duxbury Press (PWS-KENT Publishing Company), 299-304.

Examples

```
## example for PCR analysis
m1 <- pcrfit(reps, 1, 2, 15)
PRESS(m1)

## compare PRESS statistic in models
## with fewer parameters
m2 <- pcrfit(reps, 1, 2, 14)
PRESS(m2)
m3 <- pcrfit(reps, 1, 2, 13)
PRESS(m3)

## example for linear regression
x <- 1:10
y <- rnorm(10, x, 0.1)
mod <- lm(y ~ x)
PRESS(mod)

## example for NLS fitting
DNase1 <- subset(DNase, Run == 1)
fm1DNase1 <- nls(density ~ SSlogis(log(conc), Asym, xmid, scal), DNase1)
res <- PRESS(fm1DNase1)

## PRESS residuals plot
barplot(res$residuals)
```

propagate

General error analysis function using different methods

Description

A general function for the calculation of errors. Can be used for qPCR data, but any data that should be subjected to error analysis will do. The different error types can be calculated for any given expression from either replicates or from statistical summary data (mean & standard deviation). These are:

1) Monte-Carlo simulation:

For each variable in the dataset, simulated data with `nsim` samples is generated from a multivariate normal distribution using the mean μ and standard deviation σ of each variable. All data is coerced into a new dataset that has the same covariance structure as the initial dataset. Each row of the simulated dataset is evaluated and statistics are calculated from the `nsim` evaluations.

2) Permutation approach:

The data of the original dataset is permuted `nperm` times by binding observations together according to `ties`. The `ties` bind observations that can be independent measurements from the same sample. In qPCR terms, this would be a real-time PCR for two different genes on the same sample. If `ties` is omitted, the observations are shuffled independently. In detail, two datasets are created for each permutation: Dataset1 samples the rows (replicates) of the data according to `ties`. Dataset2 is obtained by sampling the columns (samples), also binding columns as in `ties`. For both datasets, the permutations are evaluated and statistics are collected. The confidence interval is calculated from all evaluations of Dataset1. A p-value is calculated from all permutations that follow `perm.crit`, whereby `init` reflects the permutations of the initial data and `perm` the permutations of the randomly reallocated samples. Thus, the p-value gives a measure against the null hypothesis that the result in the initial group is just by chance. See 'Details' and 'Examples'.

3) Error propagation:

For all variables in the original dataset, μ and σ are calculated. Through gaussian error propagation (first-order Taylor expansion), the propagated error is calculated using a matrix approach (see 'Details') by either omitting or including the dataset covariance structure.

Usage

```
propagate(expr, data, type = c("raw", "stat"), do.sim = FALSE,
          use.cov = FALSE, nsim = 10000, do.perm = FALSE,
          perm.crit = NULL, ties = NULL, nperm = 2000,
          alpha = 0.05, plot = TRUE, logx = FALSE, verbose = FALSE, ...)
```

Arguments

| | |
|-------------------|--|
| <code>expr</code> | an expression, such as <code>expression(x/y)</code> . |
| <code>data</code> | a dataframe or matrix containing either a) the replicates in columns or b) the means in the first row and the standard deviations in the second row. The variable names must be defined in the column headers. |

| | |
|-----------|---|
| type | either raw if replicates are given, or stat if means and standard deviations are supplied. |
| do.sim | logical. Should Monte Carlo error analysis be applied? |
| use.cov | logical or variance-covariance matrix with the same column descriptions and column order as data. If TRUE together with replicates, the covariances are calculated from these and used within the Monte-Carlo simulation and error propagation. If type = "stat", a square variance-covariance matrix can be supplied in the right dimensions (n x n, n = number of variables). If FALSE, the Monte-Carlo simulation and error propagation use only the diagonal (variances). |
| nsim | the number of simulations to be performed, minimum is 5000. |
| do.perm | logical. Should permutation error analysis be applied? |
| perm.crit | a character string of one or more criteria defining the null hypothesis for the permutation p-value. See 'Details'. |
| ties | a vector defining the columns that should be tied together for the permutations. See 'Details'. |
| nperm | the number of permutations to be performed. |
| alpha | the confidence level. |
| plot | logical. Should histograms with confidence intervals (in blue) be plotted for all analyses? |
| logx | logical. Should the x-axis of the graphs have logarithmic scale? |
| verbose | logical. If TRUE, a longer output is given including the simulated data, derivatives, covariance matrix etc. |
| ... | other parameters to be supplied to hist, boxplot or abline. |

Details

The propagated error is calculated by gaussian error propagation. Often omitted, but important in models where the variables are dependent (i.e. linear regression), is the second covariance term.

$$\sigma_Y^2 = \sum_i \left(\frac{\partial f}{\partial X_i} \right)^2 \sigma_i^2 + \sum_{i \neq j} \sum_{j \neq i} \left(\frac{\partial f}{\partial X_i} \right) \left(\frac{\partial f}{\partial X_j} \right) \sigma_{ij}$$

propagate calculates the propagated error either with or without covariances by using the matrix representation

$$C_Y = F_X C_X F_X^T$$

with C_Y = the propagated error, F_X = the p x n matrix with the results from the partial derivatives, C_X = the p x p variance-covariance matrix and F_X^T = the n x p transposed matrix of F_X . Depending on the input formula, the error propagation may result in an error that is not normally distributed. The Monte Carlo simulation, starting with normal distributions of the variables, can clarify this. The plots obtained from this function will also clarify this potential caveat. A high tendency from deviation of normality is encountered in formulas where the error of the denominator is relatively high or in exponential models where the exponent has a high error. This is one of the problems that is inherent in real-time PCR analysis, as the classical ratio calculation with efficiencies (i.e. by the delta-ct method) is usually of the exponential type.

The criterium for the permutation p-value (`perm.crit`) has to be defined by the user. For example, let's say we calculate some value 0.2 which is a ratio between two groups. We would hypothesize that by randomly reallocating the values between the groups the mean values are not equal or smaller than in the initial data. We would thus define `perm.crit` as "`perm < init`" meaning that we want to test if the mean of the initial data (`init`) is frequently smaller than by the randomly allocated data (`perm`). The default (NULL) is to test all three variants `perm.crit = c("perm > init", "perm == init", "perm < init")`.

Value

A plot containing the histograms of the Monte-Carlo simulation, the permutation values and histogram of the error propagation. Additionally inserted in the plots are a boxplot, the median values in red and the confidence intervals as blue borders.

A list with the following components (if `verbose = TRUE`):

| | |
|------------------------|---|
| <code>data.Sim</code> | the Monte Carlo simulated data with the evaluations in the last column. |
| <code>data.Perm</code> | the data of the permuted observations and samples with the corresponding evaluations and the decision according to <code>perm.crit</code> . |
| <code>data.Prop</code> | <code>nsim</code> values taken from a normal distribution with mean and s.d. calculated from the propagated error. |
| <code>derivs</code> | a list containing the partial derivatives expressions for each variable. |
| <code>covMat</code> | the covariance matrix used for the Monte-Carlo simulation and error propagation. |
| <code>summary</code> | a summary of the collected statistics, given as a dataframe. These are: mean, s.d. median, mad, lower/upper confidence interval and permutation p-value(s). |

Author(s)

Andrej-Nikolai Spiess

References

Error propagation (in general):

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A very good technical paper describing error propagation by matrix calculation can be found under www.nada.kth.se/~kai-a/papers/arrasTR-9801-R3.pdf.

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JCGM 100:2008 (GUM 1995 with minor corrections).
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Error propagation (in qPCR):

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Marcel Dekker (2002), New York.

Approximating the Shapiro-Wilk W-test for non-normality.

Royston P.

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See Also

Function [ratioalc](#) for error analysis within qPCR ratio calculation.

Examples

```
## From summary data just calculate
## Monte-Carlo and propagated error.
EXPR <- expression(x/y)
x <- c(5, 0.01)
y <- c(1, 0.01)
DF <- cbind(x, y)
RES1 <- propagate(expr = EXPR, data = DF, type = "stat",
                 do.sim = TRUE, verbose = TRUE)

## Do Shapiro-Wilks test on Monte Carlo evaluations
## !maximum 5000 datapoints can be used!
## => p.value on border to non-normality
shapiro.test(RES1$data.Sim[1:5000, 3])
## How about a graphical analysis:
qqnorm(RES1$data.Sim[, 3])

## Using raw data
## If data is of unequal length,
## use qpcR::cbind.na to avoid replication!
## Do permutations (swap x and y values)
```

```

## and simulations.
EXPR <- expression(x*y)
x <- c(2, 2.1, 2.2, 2, 2.3, 2.1)
y <- c(4, 4, 3.8, 4.1, 3.1)
DF <- qpcR::cbind.na(x, y)
RES2 <- propagate(EXPR, DF, type = "raw", do.perm = TRUE,
                  do.sim = TRUE, verbose = TRUE)
RES2$summary

## Example in Annex H.1 from the GUM manual
## (see 'References'), an end gauge calibration
## study
EXPR <- expression(ls + d - ls * (da * the + as * dt))
ls <- c(50000623, 25)
d <- c(215, 9.7)
da <- c(0, 0.58E-6)
the <- c(-0.1, 0.41)
as <- c(11.5E-6, 1.2E-6)
dt <- c(0, 0.029)
DF <- cbind(ls, d, da, the, as, dt)
GUM.1 <- propagate(expr = EXPR, data = DF, type = "stat",
                  do.sim = TRUE, verbose = TRUE)
## => u = 31.71 (GUM says 32).

## For replicate data, using relative
## quantification ratio from qPCR.
## How good is the estimation of the propagated error?
## Done without using covariance in the
## calculation and simulation.
## cp's and efficiencies are tied together
## because they are two observations on the
## same sample!
## As we are using an exponential type function,
## better to logarithmize the x-axis.
EXPR <- expression((E1^cp1)/(E2^cp2))
E1 <- c(1.73, 1.75, 1.77)
cp1 <- c(25.77,26.14,26.33)
E2 <- c(1.72,1.68,1.65)
cp2 <- c(33.84,34.04,33.33)
DF <- cbind(E1, cp1, E2, cp2)
RES3 <- propagate(EXPR, DF, type = "raw", do.sim = TRUE,
                  do.perm = FALSE, verbose = TRUE, logx = TRUE)
## STRONG deviation from normality!
shapiro.test(RES3$data.Sim[1:5000, 5])
qqnorm(RES3$data.Sim[, 5])

## Same setup as above but also
## using a permutation approach
## for resampling the confidence interval.
## Cp's and efficiencies are tied together
## because they are two observations on the
## same sample!
## Similar to what REST2008 software does.

```

```

RES4 <- propagate(EXPR, DF, type = "raw", do.sim = TRUE,
                  perm.crit = NULL, do.perm = TRUE,
                  ties = c(1, 1, 2, 2), logx = TRUE, verbose = TRUE)
RES4$summary
## p-value of 0 in perm < init indicates that not a single
## exchange of group memberships resulted in a smaller ratio!

## Proof that covariance of Monte-Carlo
## simulated dataset is the same as from
## initial data
RES4$covMat
cov(RES4$data.Sim[, 1:4])
all.equal(RES4$covMat, cov(RES4$data.Sim[, 1:4]))

## Error propagation in a linear model
## using the covariance matrix from summary.lm
## Estimate error of y for x = 7
x <- 1:10
set.seed(123)
y <- x + rnorm(10, 0, 1) ##generate random data
mod <- lm(y ~ x)
summ <- summary(mod)
## make matrix of parameter estimates and standard error
DF <- t(coef(summ)[, 1:2])
colnames(DF) <- c("b", "m")
CM <- vcov(mod) ## take var-cov matrix
colnames(CM) <- c("b", "m")
RES5 <- propagate(expression(m*7 + b), DF, type = "stat", use.cov = CM)
RES5

## In a x/y regime, when does the propagated error start to
## deviate from normality if error of denominator increases?
## Watch increasing deviation in qqnorm-plot!
## Not run:
x <- c(5, 1)
for (i in seq(0, 0.2, by = 0.01)) {
  y <- c(1, i)
  DF <- cbind(x, y)
  RES6 <- propagate(expression(x/y), DF, type = "stat",
                    do.sim = TRUE, plot = FALSE, verbose = TRUE,
                    logx = TRUE)
  qqnorm(RES6$data.Sim[, 3])
}

## End(Not run)

```

Description

Displays the latest changes (new functions, bug fixes etc.) of the different package versions in a text window.

Usage

```
qpcR.news(...)
```

Arguments

... arguments to be passed to [file.show](#). Usually needs no entry.

Value

None.

Author(s)

Andrej-Nikolai Spiess

Examples

```
qpcR.news()
```

qpcR_functions

The nonlinear/mechanistic models implemented in qpcR

Description

A summary of all available models implemented in this package.

Usage

```
17  
16  
15  
14  
13  
b7  
b6  
b5  
b4  
b3  
expGrowth  
mak2  
mak3  
mak3n  
chag
```

Details

The following nonlinear sigmoidal models are implemented:

l7:

$$f(x) = c + k1 \cdot x + k2 \cdot x^2 + \frac{d - c}{(1 + \exp(b(\log(x) - \log(e))))^f}$$

l6:

$$f(x) = c + k \cdot x + \frac{d - c}{(1 + \exp(b(\log(x) - \log(e))))^f}$$

l5:

$$f(x) = c + \frac{d - c}{(1 + \exp(b(\log(x) - \log(e))))^f}$$

l4:

$$f(x) = c + \frac{d - c}{1 + \exp(b(\log(x) - \log(e)))}$$

l3:

$$f(x) = \frac{d}{1 + \exp(b(\log(x) - \log(e)))}$$

b7:

$$f(x) = c + k1 \cdot x + k2 \cdot x^2 + \frac{d - c}{(1 + \exp(b(x - e)))^f}$$

b6:

$$f(x) = c + k \cdot x + \frac{d - c}{(1 + \exp(b(x - e)))^f}$$

b5:

$$f(x) = c + \frac{d - c}{(1 + \exp(b(x - e)))^f}$$

b4:

$$f(x) = c + \frac{d - c}{1 + \exp(b(x - e))}$$

b3:

$$f(x) = \frac{d}{1 + \exp(b(x - e))}$$

expGrowth:

$$f(x) = a \cdot \exp(b \cdot x) + c$$

The following mechanistic models are implemented:

mak2:

$$F_n = F_{n-1} + k \cdot \log \left(1 + \left(\frac{F_{n-1}}{k} \right) \right) + Fb$$

mak3 & mak3n:

$$F_n = F_{n-1} + k \cdot \log \left(1 + \left(\frac{F_{n-1}}{k} \right) \right) + (slope \cdot n + Fb)$$

chag:

$$F_n = 1 - (1 - F_{n-1}) \cdot \left(\frac{1 - b \cdot F_{n-1}}{1 + (a - 2) \cdot b \cdot F_{n-1}} \right)^{\frac{1}{a-1}}$$

mak2 and mak3 are two mechanistic models developed by Gregory Boggy (see references). chag was developed by Alexander Chagovetz and James Keener.

The mechanistic models are a completely different approach in that the response value (Fluorescence) is not a function of the predictor value (Cycles), but a function of the preceding response value, that is, $F_n = f(F_{n-1})$. These are also called 'recurrence relations' or 'iterative maps'.

The implementation of these models in the 'qpcR' package is the following:

- 1) In case of mak2 or mak3, all cycles up from the second derivative maximum of a four-parameter log-logistic model (l4) are chopped off. This is because these two models do not fit to a complete sigmoidal curve. For chag and mak3n, the sigmoidal curve is also rescaled between [0, 1].
- 2) A grid of sensible starting values is created for all parameters in the model.
- 3) For each combination of starting parameters, the model is fit by nonlinear least-squares (Levenberg-Marquardt by default).
- 4) The acquired parameters are collected in a parameter matrix together with the residual sum-of-squares (RSS) of the fit.
- 5) The parameter combination is selected that delivered the lowest RSS.
- 6) These parameters are transferred to [pcrfit](#), and the data is refitted.
- 7) Parameter D0 can be used directly to calculate expression ratios, hence making the use of threshold cycles and efficiencies expendable.

Some parameters for this function can be set in [parMAK](#), see also examples there and in [pcrfit](#).

The functions are defined as a list containing the following items:

\$expr the function as an expression for the fitting procedure.
 \$fct the function defined as $f(x, \text{parm})$.
 \$ssfct the self-starter function.
 \$d1 the first derivative function.
 \$d2 the second derivative function.
 \$inv the inverse function.
 \$expr.grad the function as an expression for gradient calculation.
 \$inv.grad the inverse functions as an expression for gradient calculation.
 \$parnames the parameter names.
 \$name the function name.
 \$type the function type as a character string.

Author(s)

Andrej-Nikolai Spiess

References

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BMC Bioinformatics (2006), **7**:107

Comprehensive algorithm for quantitative real-time polymerase chain reaction.

Zhao S & Fernald RD.

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4-parameter log-logistic; 5-parameter logistic/log-logistic:

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Spiess AN, Feig C & Ritz C.

BMC Bioinformatics (2008), **29**:221.

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Standardized determination of real-time PCR efficiency from a single reaction set-up.

Tichopad A, Dilger M, Schwarz G & Pfaffl MW.

Nucleic Acids Research (2003), **31**:e122.

Comprehensive algorithm for quantitative real-time polymerase chain reaction.

Zhao S & Fernald RD.

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mak2, mak3, mak3n:

A Mechanistic Model of PCR for Accurate Quantification of Quantitative PCR Data.

Boggy GJ & Woolf PJ.

PLoS ONE (2010), **5**(8): e12355.

chag:

Kinetic models of qPCR as applied to the problem of low copy numbers.

Chagovetz AM & Keener JP.

Poster presented at the qPCR 2011 Symposium in Munich.

Can be downloaded at <http://www.dr-spiess.de/qpcR/other/Chago.pdf>.

Examples

```
m1 <- pcrfit(reps, 1, 2, b3)
m2 <- pcrfit(reps, 1, 2, b5)
m3 <- pcrfit(reps, 1, 2, l6)
m4 <- pcrfit(reps, 1, 2, l7)

## get the second derivative
## curve of m2
d2 <- b5$d2(m2$DATA[, 1], coef(m2))
plot(m2)
lines(d2, col = 2)
```

ratiobatch

Calculation of ratios in a batch format for multiple genes/samples

Description

For multiple qPCR data from type 'pcrbatch', this function calculates ratios between samples, using normalization against one or more reference gene(s), if supplied. By default, multiple reference genes are averaged according to Vandesompele *et al.* (2002). The input can be single qPCR data or (more likely) data containing replicates. This is essentially a version of [ratiocalc](#) that can handle multiple reference genes and genes-of-interest with multiple (replicated) samples as found in large-scale qPCR runs such as 96- or 384-Well plates. The results are automatically stored as a file or copied into the clipboard. A boxplot representation for all Monte-Carlo simulations, permutations and error propagations including 95% confidence intervals is also given.

Usage

```
ratiobatch(data, group = NULL, plot = TRUE,
           combs = c("same", "across", "all"),
           type.eff = "mean.single", which.cp = "cpD2",
           which.eff = "sli", refmean = TRUE,
           dataout = "clip", verbose = TRUE, ...)
```

Arguments

| | |
|-----------|--|
| data | multiple qPCR data generated by pcrbatch . |
| group | a character vector defining the replicates (if any) of control/treatment samples and reference genes/genes-of-interest. See 'Details'. |
| plot | logical. If TRUE, plots are displayed for the diagnostics and analysis. |
| combs | type of combinations between different samples (i.e. r1s1:g1s2). See 'Details'. |
| type.eff | type of efficiency averaging used. Same as in ratiocalc . |
| which.eff | efficiency obtained from which method. Same as in ratiocalc . |
| which.cp | threshold cycle obtained from which method. Same as in ratiocalc . |
| dataout | where to store the result dataframe. Either it is copied to the clipboard (default) or any path + filename such as c:\temp\result.txt. |

| | |
|---------|---|
| refmean | logical. If TRUE, multiple reference are averaged before calculating the ratios. See 'Details'. |
| verbose | logical. If TRUE, the steps of analysis are shown in the console window |
| ... | other parameters to be passed to ratiocalc . |

Details

Similar to [ratiocalc](#), the replicates of the 'pcrbatch' data columns are to be defined as a character vector with the following abbreviations:

"g1s1": gene-of-interest #1 in treatment sample #1

"g1c1": gene-of-interest #1 in control sample #1

"r1s1": reference gene #1 in treatment sample #1

"r1c1": reference gene #1 in control sample #1

There is no distinction between the different technical replicates so that three different runs of gene-of-interest #1 in treatment sample #2 are defined as c("g1s2", "g1s2", "g1s2").

Example:

1 control sample with 2 genes-of-interest (2 technical replicates), 2 treatment samples with 2 genes-of-interest (2 technical replicates):

"g1c1", "g1c1", "g2c1", "g2c1", "g1s1", "g1s1", "g1s2", "g1s2", "g2s1", "g2s1", "g2s2", "g2s2"

The ratios are calculated for all pairwise 'rc:gc' and 'rs:gs' combinations according to:

For all control samples $i = 1 \dots I$ and treatment samples $j = 1 \dots J$, reference genes $k = 1 \dots K$ and genes-of-interest $l = 1 \dots L$, calculate

Without reference genes:

$$\frac{E_{g_i s_j}^{cp_{g_i s_j}}}{E_{g_k c_l}^{cp_{g_k c_l}}}$$

With reference genes:

$$\frac{E_{g_i s_j}^{cp_{g_i s_j}}}{E_{g_k c_l}^{cp_{g_k c_l}}} / \frac{E_{r_m s_n}^{cp_{r_m s_n}}}{E_{r_o c_p}^{cp_{r_o c_p}}}$$

For the mechanistic model mak3 the following is calculated:

Without reference genes:

$$\frac{D0_{g_i s_j}}{D0_{g_k c_l}}$$

With reference genes:

$$\frac{D0_{g_i s_j}}{D0_{g_k c_l}} / \frac{D0_{r_m s_n}}{D0_{r_o c_p}}$$

Efficiencies can be taken from the individual curves or averaged from the replicates as described in the documentation to [ratiocalc](#). It is also possible to give external efficiencies (i.e. acquired by some calibration curve) to the function. See 'Examples'. The different combinations of type.eff, which.eff and which.cp can yield very different results in ratio calculation. We observed a relatively stable setup which minimizes the overall variance using the combination

```

type.eff = "mean.single" # averaging efficiency across replicates
which.eff = "sli" # taking efficiency from the sliding window method
which.cp = "sig" # using the second derivative maximum of a sigmoidal fit

```

This is also the default setup in the function. The lowest variance can be obtained for the threshold cycles if the asymmetric 5-parameter 15 model is used in the `pcrbatch` function.

There are three different combination setups possible when calculating the pairwise ratios:

`combs = "same"`: reference genes, genes-of-interest, control and treatment samples are the same, i.e. $i = k, m = o, j = n, l = p$.

`combs = "across"`: control and treatment samples are the same, while the genes are combined, i.e. $i \neq k, m \neq o, j = n, l = p$.

`combs = "all"`: reference genes, genes-of-interest, control and treatment samples are all combined, i.e. $i \neq k, m \neq o, j \neq n, l \neq p$.

The last setting rarely makes sense and is very time-intensive. `combs = "same"` is the most common setting, but `combs = "across"` also makes sense if different genes-of-interest and reference gene combinations should be calculated for the same samples.

From version 1.3-6, `ratiobatch` has the option of averaging several reference genes, as described in Vandesompele *et al.* (2002). Threshold cycles and efficiency values for any i reference genes with j replicates are averaged before calculating the ratios using the averaged value μ_r for all reference genes in a control/treatment sample. The overall error σ_r is obtained by error propagation. The whole procedure is accomplished by function `refmean`, which can be used as a stand-alone function, but is most conveniently used inside `ratiobatch` setting `refmean = TRUE`. See also Example #2 in 'Examples'. For details about reference gene averaging by `refmean`, see there. The default setting is `refmean = TRUE`, so that the number of reference genes is checked by `refmean`. If none or only one per sample is found, the data is analyzed without using reference gene averaging/error propagation.

Value

A list with the following components:

| | |
|----------------------|--|
| <code>resList</code> | a list with the results from the combinations as list items. |
| <code>resDat</code> | a dataframe with the results in columns. |

Both `resList` and `resDat` have as names the combinations used for the ratio calculation. If `plot = TRUE`, a boxplot matrix from the Monte-Carlo simulations, permutations and error propagations is given including 95% confidence intervals as coloured horizontal lines.

Note

This function can be used quite conveniently when the raw fluorescence data from the 96- or 384-well runs come from Excel with 'Cycles' in the first column and run descriptions as explained above in the remaining column descriptions (such as 'r1c6'). Examples for a proper format can be found under <http://www.dr-spiess.de//qpcR//datasets.html>. This data may then be imported into R by `dat <- pcrimport()`.

Author(s)

Andrej-Nikolai Spiess

References

Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes.

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F.
Genome Biol (2002), **3**: research0034-research0034.11.

Examples

```
## Not run:
## One control sample, two treatment samples,
## one gene-of-interest, two reference genes,
## two replicates each. Replicates are averaged,
## but reference genes not, so that we have 4 ratios.
DAT1 <- pcrbatch(reps, fluo = 2:19, model = 15)
GROUP1 <- c("r1c1", "r1c1", "r2c1", "r2c1", "g1c1", "g1c1",
           "r1s1", "r1s1", "r1s2", "r1s2", "r2s1", "r2s1",
           "r2s2", "r2s2", "g1s1", "g1s1", "g1s2", "g1s2")
RES1 <- ratiobatch(DAT1, GROUP1, refmean = FALSE)

## Same as above, but now we average the two
## reference genes, so that we have 2 ratios
RES2 <- ratiobatch(DAT1, GROUP1, refmean = TRUE)

## Two control samples, one treatment sample,
## one gene-of-interest, one reference gene,
## no replicates. Use same efficiency E = 2.
DAT3 <- pcrbatch(reps, fluo = 2:7, model = 15)
GROUP3 <- c("r1c1", "r1c2", "g1c1", "g1c2",
           "r1s1", "g1s1")
RES3 <- ratiobatch(DAT3, GROUP3, which.eff = 2)

## One control sample, one treatment sample,
## three genes-of-interest, no reference gene,
## three replicates. Using efficiency from sigmoidal model.
DAT4 <- pcrbatch(reps, fluo = 2:19, model = 15)
GROUP4 <- c("g1c1", "g1c1", "g1c1", "g2c1", "g2c1", "g2c1", "g3c1", "g3c1", "g3c1",
           "g1s1", "g1s1", "g1s1", "g2s1", "g2s1", "g2s1", "g3s1", "g3s1", "g3s1")
RES4 <- ratiobatch(DAT4, GROUP4, which.eff = "sig")

## Using a mechanistic model (mak3).
## BEWARE: type.eff must be "individual"!
DAT5 <- pcrbatch(reps, fluo = 2:19, do.mak = "mak3")
GROUP5 <- c("r1c1", "r1c1", "r2c1", "r2c1", "g1c1", "g1c1",
           "r1s1", "r1s1", "r1s2", "r1s2", "r2s1", "r2s1",
           "r2s2", "r2s2", "g1s1", "g1s1", "g1s2", "g1s2")
RES5 <- ratiobatch(DAT5, GROUP5, which.eff = "mak",
                  type.eff = "individual")

## Using external efficiencies from a
## calibration curve. Can be supplied by the
## user from external calibration (or likewise),
```

```
## but in this example acquired by function 'calib'.
m11 <- modlist(reps, model = 15)
DIL <- rep(10^(6:0), each = 4)
EFF <- calib(refcurve = m11, dil = DIL)$eff
pba <- pcrbatch(m11)
GROUP6 <- c(rep("g1s1", 4), rep("g1s2", 4),
            rep("g1s3", 4), rep("g1s4", 4),
            rep("g1s5", 4), rep("g1s6", 4),
            rep("g1c1", 4))
RES6 <- ratiobatch(pba, GROUP6, which.eff = EFF)

## End(Not run)
```

ratiocalc

Calculation of ratios from qPCR runs with/without reference genes

Description

For multiple qPCR data from type 'pcrbatch', this function calculates ratios between two samples (control/treatment) of a gene-of-interest, using normalization against a reference gene, if supplied. The input can be single qPCR data or (more likely) data containing replicates. Errors and confidence intervals for the obtained ratios can be calculated by Monte-Carlo simulation, a permutation approach similar to the popular REST software and by error propagation. Statistical significance for the ratios is calculated by a permutation approach of randomly reallocated vs. non-reallocated data. See 'Details'.

Usage

```
ratiocalc(data, group = NULL, which.eff = c("sig", "sli", "exp", "mak"),
          type.eff = c("individual", "mean.single", "median.single",
                    "mean.pair", "median.pair"),
          which.cp = c("cpD2", "cpD1", "cpE", "cpR", "cpT", "Cy0"),
          ...)
```

Arguments

| | |
|-----------|---|
| data | multiple qPCR data generated by pcrbatch . |
| group | a character vector defining the replicates (if any) of control/treatment samples and reference genes/genes-of-interest. See 'Details'. |
| which.eff | efficiency calculated by which method. Defaults to sigmoidal fit. See output of pcrbatch . Alternatively, a fixed numeric value between 1 and 2 for all runs or a vector of external efficiencies with one element per run. |
| type.eff | type of efficiency pre-processing prior to error analysis. See 'Details'. |
| which.cp | type of threshold cycles to be used for the analysis. See output of efficiency . Alternatively, a vector of external threshold cycles with one element per run. |
| ... | other parameters to be passed to propagate . |

Details

The replicates for the data columns are to be defined as a character vector with the following abbreviations:

"gs": gene-of-interest in treatment sample
 "gc": gene-of-interest in control sample
 "rs": reference gene in treatment sample
 "rc": reference gene in control sample

There is no distinction between the different runs of the same sample, so that three different runs of a gene-of-interest in a treatment sample are defined as c("gs", "gs", "gs"). The error analysis calculates statistics from ALL replicates, so that a further sub-categorization of runs is superfluous. NOTE: If the setup consists of different sample or gene combinations, use [ratiobatch!](#)

Examples:

No replicates: NULL.

2 runs with 2 replicates each, no reference gene: c("gs", "gs", "gs", "gs", "gc", "gc", "gc", "gc").

1 run with two replicates each and reference gene: c("gs", "gs", "gc", "gc", "rs", "rs", "rc", "rc").

type.eff defines the pre-processing of the efficiencies before being transferred to [propagate](#). The qPCR community sometimes uses single efficiencies, or averaged over replicates etc., so that different settings were implemented. In detail, these are the following:

"individual": The individual efficiencies from each run are used.

"mean.single": Efficiencies are averaged over all replicates.

"median.single": Same as above but median instead of mean.

"mean.pair": Efficiencies are averaged from all replicates of treatment sample AND control.

"median.pair": Same as above but median instead of mean.

Efficiencies can be taken from the individual curves or averaged from the replicates as described in the documentation to [ratiocalc](#). The different combinations of type.eff, which.eff and which.cp can yield very different results in ratio calculation. We observed a relatively stable setup which minimizes the overall variance using the combination

type.eff = "mean.single" # averaging efficiency across replicates

which.eff = "sli" # taking efficiency from the sliding window method

which.cp = "sig" # using the second derivative maximum of a sigmoidal fit

The ratios are calculated according to the following formulas:

Without reference gene:

$$\frac{E_{gs}^{cp_{gs}}}{E_{gc}^{cp_{gc}}}$$

With reference gene:

$$\frac{E_{gs}^{cp_{gs}}}{E_{gc}^{cp_{gc}}} / \frac{E_{rs}^{cp_{rs}}}{E_{rc}^{cp_{rc}}}$$

The permutation approach permutes threshold cycles and efficiency replicates within treatment and control samples. The treatment/control samples (and their respective efficiencies) are tied together, which is similar to the popular REST software approach ("pairwise-reallocation test"). Ratios are calculated for each permutation and compared to ratios obtained if samples were randomly reallocated from the treatment to the control group. Three p-values are calculated from all permutations that gave a higher/equal/lower ratio than the original data. The resulting p-values are thus an indication for the significance in any direction AGAINST the null hypothesis that ratios calculated by permutation are just by chance.

If the mechanistic mak3/mak3n/chag models are used in [pcrbatch](#), this is automatically recognized and the ratio calculation is conducted from the $D0$ values of the model:

Without reference gene:

$$\frac{D0_{gs}}{D0_{gc}}$$

With reference gene:

$$\frac{D0_{gs} / D0_{rs}}{D0_{gc} / D0_{rc}}$$

Confidence values are returned for all three methods (Monte Carlo, permutation, error propagation) as follows:

Monte-Carlo: From the evaluations of the Monte-Carlo simulated data.

Permutation: From the evaluations of the within-group permuted data.

Propagation: From the propagated error, assuming normality.

Value

A list with the following components:

data: the data that was transferred to [propagate](#) for the error analysis.

data.Sim, data.Perm, data.Prop, derivs, covMat: The complete output from [propagate](#).

summary: a summary of the results obtained from the Monte Carlo simulation, permutation and error propagation.

Note

The error calculated from qPCR data by [propagate](#) often seems quite high. This largely depends on the error of the exponent (i.e. threshold cycles) of the exponential function. The error usually decreases when setting `use.cov = TRUE` in the `...` part of the function. It can be debated anyhow, if the variables 'efficiency' and 'threshold cycles' have a covariance structure. As the efficiency is deduced at the second derivative maximum of the sigmoidal curve, variance in the second should show an effect on the first, such that the use of a var-covar matrix might be feasible. It is also commonly encountered that the propagated error is much higher when using reference genes, as the number of partial derivative functions increases.

Author(s)

Andrej-Nikolai Spiess

References

Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method.

Livak KJ & Schmittgen TD.
Methods (2001), **25**: 402-428.

Standardized determination of real-time PCR efficiency from a single reaction set-up.

Tichopad A, Dilger M, Schwarz G, Pfaffl MW.
Nucleic Acids Res (2003), **31**: e122.

Validation of a quantitative method for real time PCR kinetics.

Liu W & Saint DA.
Biochem Biophys Res Commun (2002), **294**: 347-53.

Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR.

Pfaffl MW, Horgan GW, Dempfle L.
Nucl Acids Res (2002), **30**: e36.

Examples

```
## Only treatment sample and control,
## no reference gene, 4 replicates each.
## Individual efficiencies for error calculation.
DAT1 <- pcrbatch(reps, fluo = 2:9, model = 14)
GROUP1 <- c("gs", "gs", "gs", "gs", "gc", "gc", "gc", "gc")
RES1 <- ratiocalc(DAT1, group = GROUP1, which.eff = "sli",
                 type.eff = "individual", which.cp = "cpD2")
RES1$summary

## Gets even better using averaged efficiencies
## over all replicates.
## p-value indicates significant upregulation in
## comparison to randomly reallocated
## samples (similar to REST software)
RES2 <- ratiocalc(DAT1, GROUP1, which.eff = "sli",
                 type.eff = "mean.single", which.cp = "cpD2")
RES2$summary

## failed fits and sigmoidal outliers
## using 'testdat' set.
## GROUP is automatically reduced to
## runs that passed checking.
DAT3 <- pcrbatch(testdat[, 1:9], fluo = 2:9, model = 15)
GROUP3 <- c("gs", "gs", "gs", "gs", "gc", "gc", "gc", "gc")
RES3 <- ratiocalc(DAT3, GROUP3, which.eff = "sli",
                 type.eff = "individual", which.cp = "cpD2")
RES3$summary

## Not run:
```

```

## Using reference data.
## Toy example is same data as above
## but replicated as reference such
## that the ratio should be 1.
DAT4 <- pcrbatch(reps, fluo = c(2:9, 2:9), model = 14)
GROUP4 <- c("gs", "gs", "gs", "gs",
            "gc", "gc", "gc", "gc",
            "rs", "rs", "rs", "rs",
            "rc", "rc", "rc", "rc")
RES4 <- ratiocalc(DAT4, GROUP4, which.eff = "sli",
                 type.eff = "mean.single", which.cp = "cpD2")
RES4$summary

## Using one of the mechanistic models
## => ratios are calculated from the replicate
## D0 values. Without/with reference gene.
DAT5 <- pcrbatch(reps, fluo = 2:9, do.mak = "mak3")
GROUP5 <- c("gs", "gs", "gs", "gs", "gc", "gc", "gc", "gc")
RES5 <- ratiocalc(DAT5, GROUP5, which.eff = "mak")
RES5$summary

## Example without replicates
## => no Monte-Carlo simulations
## and no plots.
DAT6 <- pcrbatch(reps, fluo = 2:5, model = 14)
GROUP6 <- c("gs", "gc", "rs", "rc")
RES6 <- ratiocalc(DAT6, GROUP6, which.eff = "sli",
                 type.eff = "individual", which.cp = "cpD2")
RES6$summary

## Using external efficiencies
DAT7 <- pcrbatch(reps, fluo = 2:9, model = 15)
GROUP7 <- c("gs", "gs", "gs", "gs", "gc", "gc", "gc", "gc")
EFF7 <- rep(c(1.72, 1.76), c(4, 4))
RES7 <- ratiocalc(DAT7, GROUP7, which.eff = EFF7,
                 type.eff = "individual", which.cp = "cpD2")
RES7$summary

## Using external efficiencies AND
## external threshold cycles
DAT8 <- pcrbatch(reps, fluo = 2:9, model = 15)
GROUP8 <- c("gs", "gs", "gs", "gs", "gc", "gc", "gc", "gc")
EFF8 <- rep(c(1.72, 1.76), c(4, 4))
CP8 <- c(15.44, 15.33, 14.84, 15.34, 18.89, 18.71, 18.13, 17.22)
RES8 <- ratiocalc(DAT8, GROUP8, which.eff = EFF8,
                 type.eff = "individual", which.cp = CP8)
RES8$summary

## Compare 'propagate' to REST software
## using the data from the REST 2008
## manual (http://rest.gene-quantification.info/),
## Have to create dataframe with values as we do
## not use 'pcrbatch', but external cp's & eff's!

```

```

## Ties define random reallocation of crossing points
## keeping controls and samples together.
## See help for 'propagate'.
EXPR <- expression((2.01^(cp.gc - cp.gs)/1.97^(cp.rc - cp.rs)))
cp.rc <- c(26.74, 26.85, 26.83, 26.68, 27.39, 27.03, 26.78, 27.32)
cp.rs <- c(26.77, 26.47, 27.03, 26.92, 26.97, 26.97, 26.07, 26.3, 26.14, 26.81)
cp.gc <- c(27.57, 27.61, 27.82, 27.12, 27.76, 27.74, 26.91, 27.49)
cp.gs <- c(24.54, 24.95, 24.57, 24.63, 24.66, 24.89, 24.71, 24.9, 24.26, 24.44)
DAT9 <- qPCR::cbind.na(cp.rc, cp.rs, cp.gc, cp.gs)
RES9 <- propagate(EXPR, DAT9, do.sim = TRUE, do.perm = TRUE,
                  ties = c(1, 2, 1, 2), verbose = TRUE)

RES9$summary

## End(Not run)

```

refmean

Averaging of multiple reference genes

Description

This function averages the expression of several reference genes before calculation of gene expression ratios by [ratiocalc](#) or [ratiobatch](#). The method is similar to that described in Vandesompele *et al.* (2002), but uses **arithmetic** averaging of threshold cycles/efficiencies and **not geometric** averaging of relative expression values. This is equivalent, as discussed in 'Details' and as shown in 'Examples'. An essential extension of this method is, that if replicates for the reference genes are supplied, the threshold cycles and efficiencies are subjected to error propagation prior to ratio calculation. The propagated error is then included in the calculation of the gene expression ratios, as advocated in Nordgard *et al.* (2006).

Usage

```

refmean(data, group, which.eff = c("sig", "sli", "exp", "mak"),
        type.eff = c("individual", "mean.single"),
        which.cp = c("cpD2", "cpD1", "cpE", "cpR", "cpT", "Cy0"),
        verbose = TRUE, ...)

```

Arguments

| | |
|-----------|--|
| data | multiple qPCR data generated by pcrbatch . |
| group | a character vector defining the replicates (if any) of control/treatment samples and reference genes/genes-of-interest. See 'Details' |
| which.eff | efficiency calculated by which method. Defaults to sigmoidal fit. Can also be a value such as 1.8, as shown in 'Examples'. See ratiocalc . |
| type.eff | using individual or averaged efficiencies for the replicates of a reference gene. See 'Details'. |
| which.cp | type of threshold cycles to be used for the analysis. Defaults to cpD2. See ratiocalc . |

verbose logical. If TRUE, the steps of analysis are shown in the console window.
 ... parameters to be supplied to `propagate`.

Details

As in `rationbatch`, the samples are to be defined as a character vector in the style of "g1s1", "g1c1", "r1s1" and "r1c1" etc. If `refmean` is used as a standalone function or switched on in `rationbatch` using `refmean = TRUE`, different reference genes per control/treatment samples are averaged when supplied either as single runs or as replicates.

Examples (omitting genes-of-interest in control/treatment samples):

2 reference genes, 2 replicates each:

`c("r1s1", "r1s1", "r2s1", "r2s1", "r1c1", "r1c1", "r2c1", "r2c1", ...)`.

3 reference genes, no replicates:

`c("r1s1", "r2s1", "r3s1", "r1c1", "r2c1", "r3c1", ...)`

Averaging of multiple reference genes is accomplished the following way:

Given i reference genes with j replicates in a sample, all replicates r_{ij} are used for calculating mean μ_{r_i} and standard deviation σ_{r_i} of the threshold cycles and efficiencies. The overall (grand) mean μ_r and propagated error σ_r is calculated using `propagate` with first-order Taylor expansion including covariance: $\sigma_r = F_{r_i} C_{r_i} F_{r_i}^T$. Finally, a vector of length $L = n(r_{ij})$ containing equidistant numbers $X = (x_1, x_2, x_3, \dots, x_L)$ with mean μ_r and standard deviation σ_r is generated for a new overall reference gene r_1 . This is done using the internal function `qpcR:::makeStat` which calculates a shifted (μ_r) and scaled (σ_r) Z-transformation on a vector $x_1 \dots x_L$:

$$Z_i = \mu_r + \frac{(x_i - \bar{X})}{\sigma_X} \cdot \sigma_r$$

The new Z_i threshold cycle and efficiency values replace all values of r_{ij} in data. When using `rationbatch`, this modified data is then used for the ratio calculation, again using `propagate` to calculate errors for ratios using the Z_i values as mentioned above.

By using logarithmic identities (http://en.wikipedia.org/wiki/Logarithmic_identities), it can be shown that the **geometric** mean can be transformed to the **arithmetic** mean by logarithmation (assuming constant E):

$$\left(\prod_{i=1}^n E^{x_i} \right)^{\frac{1}{n}} = \frac{1}{n} \cdot \log_E \left(\prod_{i=1}^n E^{x_i} \right) = \frac{1}{n} \sum_{i=1}^n x_i$$

Hence, **arithmetic** averaging of the threshold cycles **BEFORE** ratio calculation is the same as doing **geometric** averaging on relative quantities **AFTER** ratio calculation. This is demonstrated in 'Examples' and also mentioned in the `geNorm` manual (http://medgen.ugent.be/~jvdesomp/genorm/geNorm_manual.pdf) in Q8 (page 12).

When setting `type.eff = "individual"` (default), all efficiencies from replicates of a reference gene in a control/treatment sample $E(r_{ij})$ are used for calculating mean $\mu_{E(r_i)}$ and standard deviation $\sigma_{E(r_i)}$, the latter being used for calculating a propagated error for all reference gene efficiencies $\sigma_{E(r)}$. If `type.eff = "mean.single"`, all $E(r_{ij})$ values from the replicates are set to the same value $\mu_{E(r_i)}$, that is, there is no variation assumed between the different $E(r_{ij})$. In this case, $\sigma_{E(r_i)} = 0$, so that no error of the replicates is propagated to $\sigma_{E(r)}$. This results in smaller overall errors of the output, but it can be debated if this is a realistic approach, hence both settings were implemented.

which `eff` can be supplied with an efficiency value such as 1.8, which is then used as the efficiency for all reference runs $E(r_{ij})$.

Value

The same dataset data which was supplied to the function, but with modified threshold cycle/efficiency values in which the values are created per sample in a way, that they have the mean of all averaged reference genes and the same standard deviation as obtained by error propagation. See 'Details' for a more thorough explanation. Furthermore, a modified label vector "NAME_mod" is written to the global environment (if "NAME" was supplied for group) in which the different reference gene labels are aggregated, i.e. `c("r1c1", "r2c1", "r3c1")` will become `c("r1c1", "r1c1", "r1c1")`. This new label vector is also attached as an attribute to the output data and can be obtained by `attr(RES1, "group")`.

Note

The function checks stringently if the same number of different reference genes are used for control and treatment samples, although the number of replicates may differ.

Example:

`GROUP <- c("r1c1", "r1c1", "r2c1", "r2c1", "r1s1", "r2s1")` will work (2 reference genes in control/treatment samples), but `GROUP <- c("r1c1", "r2c1", "r3c1", "r1s1", "r1s1", "r1s2", "r1s2", "r2s1", "r2s1")` will not work (3 reference genes in controls, only 2 in treatment samples). Also, when no or only one reference genes are detected, the original data is not averaged and returned unchanged.

Author(s)

Andrej-Nikolai Spiess

References

Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes.

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F.
Genome Biol (2002), **3**: research0034-research0034.11.

Error propagation in relative real-time reverse transcription polymerase chain reaction quantification models: the balance between accuracy and precision.

Nordgard O, Kvaloy JT, Farnen RK, Heikkilä R.
Anal Biochem (2006), **356**: 182-193.

See Also

In [ratiobatch](#), reference gene averaging can be done automatically by setting `refmean = TRUE`. See there.

Examples

```

## Not run:
## Replacing the reference gene values by
## averaged ones in the original data.
## => RES1 is new dataset.
## => GROUP1_mod in global environment is
## new labeling vector.
DAT1 <- pcrbatch(reps, fluo = 2:19, model = 15)
GROUP1 <- c("r1c1", "r1c1", "r2c1", "r2c1", "g1c1", "g1c1",
           "r1s1", "r1s1", "r1s2", "r1s2", "r2s1", "r2s1",
           "r2s2", "r2s2", "g1s1", "g1s1", "g1s2", "g1s2")
RES1 <- refmean(DAT1, GROUP1, which.eff = "sig", which.cp = "cpD2")

## Using three reference genes without replicates
## and then 'ratiobatch'.
## This can also be called in 'ratiobatch' directly
## with parameter 'refmean = TRUE'. See there.
## In this example, already averaged dataset and
## new labeling vector are supplied to 'ratiobatch',
## so one has to set 'refmean = FALSE'
DAT2 <- pcrbatch(reps, fluo = 2:9, model = 15)
GROUP2 <- c("r1c1", "r2c1", "r3c1", "g1c1", "r1s1", "r2s1", "r3s1", "g1s1" )
RES2 <- refmean(DAT2, GROUP2, which.eff = "sig", which.cp = "cpD2")
ratiobatch(RES2, GROUP2_mod, refmean = FALSE)

## Comparison between 'refmean' ct-value arithmetic averaging
## and 'geNorm' relative quantities geometric averaging
## using data from the geNorm manual (2008), page 6.
## We will use HK1-HK3 as in the manual (no replicates).
## First we create a 'pcrbatch' dataset and then
## override the ct values with those of the manual and all
## efficiencies with E = 2. Sample A is considered as control sample.
DAT3 <- pcrbatch(reps, fluo = 2:17, model = 15)
DAT3[8, -1] <- c(32.10, 27.00, 34.90, 23.00,
               33.30, 28.40, 36.10, 24.20,
               31.00, 27.50, 34.00, 26.35,
               30.50, 28.20, 33.00, 25.45)
DAT3[1, -1] <- 2
GROUP3 <- c("r1c1", "r2c1", "r3c1", "g1c1",
           "r1s1", "r2s1", "r3s1", "g1s1",
           "r1s2", "r2s2", "r3s2", "g1s2",
           "r1s3", "r2s3", "r3s3", "g1s3")
RES3 <- refmean(DAT3, GROUP3, which.eff = "sig", which.cp = "cpD2")
ratiobatch(RES3, GROUP3_mod, which.cp = "cpD2",
           which.eff = "sig", refmean = FALSE)

## Results:
## r1c1:g1c1:r1s1:g1s1 refmean 1.0497
## geNorm 1.0472 (2.351/2.245)
## r1c1:g1c1:r1s2:g1s2 refmean 0.0693
## geNorm 0.0695 (0.156/2.245)
## r1c1:g1c1:r1s3:g1s3 refmean 0.1081
## geNorm 0.1074 (0.241/2.245)

```

```
## Slight differences are due to rounding.  
## End(Not run)
```

| | |
|---------|---|
| rep2mod | <i>Converts a 'replist' back to a 'modlist'</i> |
|---------|---|

Description

This function is essentially the reverse of `modlist`. An object of class 'replist' is converted back to a 'modlist', with the original grouping structure attached.

Usage

```
rep2mod(r1)
```

Arguments

`r1` a 'replist' containing replicates.

Details

The returned 'modlist' has an attribute 'group' attached, which is a vector containing the original grouping (number of groups, number of replicates). This can be queried by `attr(name, "group")`.

Value

A 'modlist' containing all single runs.

Author(s)

Andrej-Nikolai Spiess

Examples

```
## create a 'modlist'  
m11 <- modlist(reps, 1, 2:5, 14)  
  
## convert into 'replist'  
r11 <- replist(m11, group = rep(1, 4))  
par(mfrow = c(2, 1))  
plot(r11, main = "A 'replist'")  
  
## convert back to a 'modlist'  
m12 <- rep2mod(r11)  
plot(m12, main = "A 'modlist'")
```

| | |
|---------|--|
| replist | <i>Amalgamation of single data models into a model containing replicates</i> |
|---------|--|

Description

Starting from a 'modlist' containing qPCR models from single data, `replist` amalgamates the models according to the grouping structure as defined in `group`. The result is a 'replist' with models obtained from fitting the replicates by `pcrfit`. A kinetic outlier detection and removal option is included.

Usage

```
replist(object, group = NULL, check = "none",
        checkPAR = parKOD(), remove = c("none", "KOD"),
        names = c("group", "first"), doFit = TRUE, opt = FALSE,
        optPAR = list(sig.level = 0.05, crit = "ftest"),
        verbose = TRUE, ...)
```

Arguments

| | |
|-----------------------|--|
| <code>object</code> | an object of class 'modlist'. |
| <code>group</code> | a vector defining the replicates for each group. |
| <code>check</code> | which method to use for kinetic outlier detection. Either none or any of the methods in <code>KOD</code> . |
| <code>checkPAR</code> | parameters to be supplied to the check method, see <code>KOD</code> . |
| <code>remove</code> | which runs to remove. Either none or those that failed from the method defined in <code>check</code> . |
| <code>names</code> | how to name the grouped fit. Either 'group_1, ...' or the first name of the replicates. |
| <code>doFit</code> | logical. If set to FALSE, the replicate data is only aggregated without doing a refitting. See 'Details'. |
| <code>opt</code> | logical. Should model selection be applied to the final model? |
| <code>optPAR</code> | parameters to be supplied to <code>mselect</code> . |
| <code>verbose</code> | if TRUE, the analysis is printed to the console. |
| <code>...</code> | other parameters to be supplied to <code>mselect</code> . |

Details

As being defined by `group`, the 'modlist' is split into groups of runs and these amalgamated into a nonlinear model. Runs which have failed to be fitted by `modlist` are automatically removed and `group` is updated (that is, the corresponding entries also removed) prior to fitting the replicate model by `pcrfit`. Model selection can be applied to the final replicate model by setting `opt = TRUE` and changing the parameters in `optPAR`. If `check` is set to any of the methods in "KOD", kinetic outliers

are identified and optionally removed, if `remove` is set to "KOD".
If `doFit = FALSE`, the replicate data is only aggregated and no refitting is done. This is useful when plotting replicate data by some grouping vector. See 'Examples'.

Value

An object of class 'replis' containing the replicate models of class 'nls'/'pcrfit'.

Author(s)

Andrej-Nikolai Spiess

See Also

[modlist](#), [pcrfit](#).

Examples

```
## convert 'modlist' into 'replis'
m11 <- modlist(reps, model = 14)
r11 <- replis(m11, group = gl(7, 4))
plot(r11)
summary(r11[[1]])

## optimize model based on Akaike weights
r12 <- replis(m11, group = gl(7, 4), opt = TRUE,
             optPARS = list(crit = "weights"))
plot(r12)

## remove kinetic outliers,
## use first replicate name for output
m13 <- modlist(reps, model = 14)
r13 <- replis(m13, group = gl(7, 4), check = "uni1",
             remove = "KOD", names = "first")
plot(r13, which = "single")

## Not run:
## just aggregation and no
## refitting
m14 <- modlist(reps, model = 14)
r14 <- replis(m14, group = gl(7, 4), doFit = FALSE)
plot(r14, which = "single")

## Scenario 1:
## automatic removal of runs that failed to
## fit during 'modlist' by using
## 'testdat' set
m15 <- modlist(testdat, model = 15)
r15 <- replis(m15, gl(6, 4))
plot(r15, which = "single")
```

```

## Scenario 2:
## automatic removal of runs that failed to
## fit during 'replist':
## samples F3.1-F3.4 is set to 1.
dat1 <- reps
m16 <- modlist(dat1)
m16[[9]]$DATA[, 2] <- 1
m16[[10]]$DATA[, 2] <- 1
m16[[11]]$DATA[, 2] <- 1
m16[[12]]$DATA[, 2] <- 1
r16 <- replist(m16, gl(7, 4))
plot(r16, which = "single")

## End(Not run)

```

repsdat

qPCR dilution experiments from the package author

Description

reps: A dilution experiment with seven 10-fold dilutions of the cDNA, and four replicates for each dilution.

reps2: A dilution experiment of two different cDNAs with five 4-fold dilutions of the cDNA, and three replicates for each dilution.

reps3: A dilution experiment with six 4-fold dilutions of the cDNA, and three replicates for each dilution.

Usage

```

reps
reps2
reps3

```

Format

reps: A data frame with the PCR cycles and 28 qPCR runs with four replicates of seven 10-fold dilutions. The replicates are defined by FX.Y (X = dilution number, Y = replicate number).

reps2: A data frame with the PCR cycles and 30 qPCR runs with three replicates of five 4-fold dilutions. The replicates are defined by FX.Y.Z (X = cDNA number, Y = dilution number, Z = replicate number).

reps3: A data frame with the PCR cycles and 21 qPCR runs with three replicates of six 4-fold dilutions. The replicates are defined by FX.Y (X = dilution number, Y = replicate number).

Details

The real-time PCR was conducted with primers for the S27a housekeeping gene in a Lightcycler 1.0 instrument (Roche Diagnostics) for reps or in a MXPro3000P instrument (Stratagene) for reps2, reps3. reps3 was ROX-normalized.

Source

Andrej-Nikolai Spiess & Nadine Mueller, Institute for Hormone and Fertility Research, Hamburg, Germany.

Examples

```
m1 <- pcrfit(reps, 1, 2, 15)
plot(m1)
```

resplot *An (overlaid) residuals barplot*

Description

A plotting function which displays a barplot of the residuals. The bars are colour-coded with heat colours proportional to the residual value. As default, the residuals are displayed together with the points of the fitted PCR curve.

Usage

```
resplot(object, overlay = TRUE, ylim = NULL, ...)
```

Arguments

| | |
|---------|---|
| object | an object of class 'pcrfit'. |
| overlay | logical. If TRUE, the residuals are plotted on top of the fitted curve, else alone. |
| ylim | graphical ylim values for tweaking the scale and position of the barplot overlay. |
| ... | any other parameters to be passed to barplot . |

Details

If replicate data is present in the fitted curve, the residuals from all replicates i, j are summed up from the absolute values: $Y_i = \sum |\hat{\epsilon}_{i,j}|$.

Value

A plot as described above.

Author(s)

Andrej-Nikolai Spiess

Examples

```
## create l5 model and plot residuals
## in a good position
m1 <- pcrfit(reps, 1, 2, 15)
resplot(m1, ylim = c(-0.5, 0.5))

## Not run:
## using replicates
m2 <- pcrfit(reps, 1, 2:5, 15)
resplot(m2)

## End(Not run)
```

resVar

Residual variance of a fitted model

Description

Calculates the residual variance for objects of class `nls`, `lm`, `glm`, `drc` or any other models from which `coef` and `residuals` can be extracted.

Usage

```
resVar(object)
```

Arguments

`object` a fitted model.

Details

$$resVar = \frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{n - p}$$

where n is the number of response values and p the number of parameters in the model.

Value

The residual variance of the fit.

Author(s)

Andrej-Nikolai Spiess

Examples

```
m1 <- pcrfit(reps, 1, 2, 15)
resVar(m1)
```

| | |
|------|--|
| RMSE | <i>Root-mean-squared-error of a fitted model</i> |
|------|--|

Description

Calculates the root-mean-squared-error (RMSE) for objects of class `nls`, `lm`, `glm`, `drc` or any other models from which `residuals` can be extracted.

Usage

```
RMSE(object, which = NULL)
```

Arguments

| | |
|---------------------|--|
| <code>object</code> | a fitted model. |
| <code>which</code> | a subset of the curve to be used for RMSE calculation. If not defined, the complete curve is used. |

Details

$$RMSE = \sqrt{(y_i - \hat{y}_i)^2}$$

Value

The root-mean-squared-error from the fit or a part thereof.

Author(s)

Andrej-Nikolai Spiess

Examples

```
## for a part of the curve
m1 <- pcrfit(reps, 1, 2, 15)
RMSE(m1, 10:15)
```

Rsq

*R-square value of a fitted model***Description**

Calculates the R^2 value for objects of class `nls`, `lm`, `glm`, `drc` or any other models from which `fitted` and `residuals` can be extracted. Since version 1.2-9 it calculates a weighted R^2 if the object has an item `object$weights` containing weighting values.

Usage

```
Rsq(object)
```

Arguments

`object` a fitted model.

Details

Uses the most general definition of R^2 :

$$R^2 \equiv 1 - \frac{RSS}{TSS}$$

where

$$RSS = \sum_{i=1}^n w_i \cdot (y_i - \hat{y}_i)^2$$

and

$$TSS = \sum_{i=1}^n w_i \cdot (y_i - \bar{y})^2$$

using the weighted mean

$$\bar{y} = \frac{\sum_{i=1}^n w_i x_i}{\sum_{i=1}^n w_i}$$

Value

The R^2 value of the fit.

Author(s)

Andrej-Nikolai Spiess

Examples

```
m1 <- pcrfit(reps, 1, 2, 15)
Rsq(m1)
```

| | |
|--------|--|
| Rsq.ad | <i>Adjusted R-square value of a fitted model</i> |
|--------|--|

Description

Calculates the adjusted R_{adj}^2 value for objects of class `nls`, `lm`, `glm`, `drc` or any other models from which `fitted`, `residuals` and `coef` can be extracted.

Usage

```
Rsq.ad(object)
```

Arguments

`object` a fitted model.

Details

$$R_{adj}^2 = 1 - \frac{n-1}{n-p} \cdot (1 - R^2)$$

with n = sample size, p = number of parameters.

Value

The adjusted R_{adj}^2 value of the fit.

Author(s)

Andrej-Nikolai Spiess

Examples

```
## single model
m1 <- pcrfit(reps, 1, 2, 15)
Rsq.ad(m1)

## compare different models with increasing
## number of parameters
m11 <- lapply(list(13, 14, 15), function(x) pcrfit(reps, 1, 2, x))
sapply(m11, function(x) Rsq.ad(x))
```

`RSS`*Residual sum-of-squares of a fitted model*

Description

Calculates the residual sum-of-squares for objects of class `nls`, `lm`, `glm`, `drc` or any other models from which `residuals` can be extracted. From version 1.3-6, this function uses weights, if object has an item `$weights`.

Usage

```
RSS(object)
```

Arguments

`object` a fitted model.

Details

$$RSS = \sum_{i=1}^n w_i \cdot (y_i - \hat{y}_i)^2$$

Value

The (weighted) residual sum-of-squares from the fit.

Author(s)

Andrej-Nikolai Spiess

Examples

```
m1 <- pcrfit(reps, 1, 2, 15)
RSS(m1)
```

`rutledge`*qPCR dilution experiments from Rutledge (2004)*

Description

A dilution experiment of a 102 bp amplicon with six 10-fold dilutions, with four replicates each in five independent runs.

Usage

```
rutledge
```

Format

A data frame with the PCR cycles and the six 10-fold dilutions, with four replicates each and five independent runs, organized as X.Ry.Z, with X = the dilution number, Y = the run number and Z = the replicate number.

Details

The real-time PCR was conducted for a 102 bp amplicon in an Opticon2 instrument.

Source

Supplemental data 1 to the paper.

References

Sigmoidal curve-fitting redefines quantitative real-time PCR with the prospective of developing automated high-throughput applications.

Rutledge RG.

Nucleic Acids Research (2004), **32**: e178.

Examples

```
data(rutledge)
m1 <- pcrfit(rutledge, 1 , 2, 15)
plot(m1)
```

S27

qPCR data of the S27a transcript in 15 testicular biopsies

Description

The data was obtained from cDNA reverse transcribed from the isolated RNA of 15 different testicular biopsies.

Usage

S27

Format

A data frame with cycle number and 15 different qPCR runs (F1 - F15).

Details

The real-time PCR was conducted with primers for the S27a housekeeping gene in a Lightcycler 1.0 instrument (Roche Diagnostics).

Source

Andrej-Nikolai Spiess & Caroline Feig, Department of Andrology, University Hospital Hamburg, Germany.

Examples

```
m1 <- pcrfit(S27, 1, 2, 15)
plot(m1)
```

sliwin

*Calculation of qPCR efficiency by the 'window-of-linearity' method***Description**

A linear model of Cycles versus $\log(\text{Fluorescence})$ is fit within a sliding window of defined size(s) and within a defined border. Regression coefficients are calculated for each window, and at the point of maximum regression (log-linear range) or least variation in slope, parameters such as PCR efficiency and initial template fluorescence are calculated. From version 1.3-5, an approach "not unlike" to Ruijter et al. (2009) has been implemented, in which baseline values can be iteratively subtracted from the data prior to fitting the sliding window. See 'Details' for more information.

Usage

```
sliwin(object, wsize = 6, basecyc = 1:6, base = 0, border = NULL,
       type = c("rsq", "slope"), plot = TRUE, verbose = TRUE, ...)
```

Arguments

| | |
|---------|---|
| object | an object of class 'pcrfit'. |
| wsize | the size(s) of the sliding window(s), default is 6. A sequence such as 4:6 can be used to optimize the window size. |
| basecyc | if base != 0, which cycles to use for an initial baseline estimation based on the averaged fluorescence values. |
| base | either 0 for no baseline optimization, or a scalar defining multiples of the standard deviation of all baseline points obtained from basecyc. These are iteratively subtracted from the raw data. See 'Details' and 'Examples'. |
| border | either NULL (default) or a two-element vector which defines the border from the take-off point to points nearby the upper asymptote (saturation phase). See 'Details'. |
| type | selection of the window with best baseline + maximum R^2 ("rsq") or best baseline + minimal variance in slope + maximum R^2 ("slope"). |
| plot | if TRUE, the result is plotted with the logarithmized curve, sliding window, regression line and baseline. |
| verbose | logical. If TRUE, more information is displayed in the console window. |
| ... | only used internally for passing the parameter matrix. |

Details

To avoid fits with a high R^2 in the baseline region, some border in the data must be defined. In *sliwin*, this is by default (`base = NULL`) the region in the curve starting at the take-off cycle (*top*) as calculated from `takeoff` and ending at the transition region to the upper asymptote (saturation region). The latter is calculated from the first and second derivative maxima: $asympt = cpD1 + (cpD1 - cpD2)$. If the border is to be set by the user, border values such as `c(-2, 4)` extend these values by $top + border[1]$ and $asympt + border[2]$. The \log_{10} transformed raw fluorescence values are regressed against the cycle number $\log_{10}(F) = n\beta + \epsilon$ and the efficiency is then calculated by $E = 10^{slope}$. For the baseline optimization, 100 baseline values Fb_i are interpolated in the range of the data:

$$F_{min} \leq Fb_i \leq base \cdot \sigma(F_{basecyc[1]} \dots F_{basecyc[2]})$$

and subtracted from F_n . If `type = "rsq"`, the best window in terms of R^2 is selected from all iterations, as defined by `wsiz` and `border`. If `type = "slope"`, the baseline value delivering the smallest variance in the slope of the upper/lower part of the sliding window and highest R^2 is selected. This approach is quite similar to the one in Ruijter et al. (2009) but has to be tweaked in order to obtain the same values as in the 'LinRegPCR' software. Especially the border value has significant influence on the calculation of the best window's efficiency value.

Value

A list with the following components:

| | |
|---------------------|---|
| <code>eff</code> | the optimized PCR efficiency found within the sliding window. |
| <code>rsq</code> | the maximum R-squared. |
| <code>init</code> | the initial template fluorescence F_0 . |
| <code>base</code> | the optimized baseline value. |
| <code>window</code> | the best window found within the borders. |
| <code>parMat</code> | a matrix containing the parameters as above for each iteration. |

Author(s)

Andrej-Nikolai Spiess

References

Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data.

Ramakers C, Ruijter JM, Deprez RH, Moorman AF.

Neurosci Lett (2003), **339**: 62-65.

Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data.

Ruijter JM, Ramakers C, Hoogaars WM, Karlen Y, Bakker O, van den Hoff MJ, Moorman AF.

Nucleic Acids Res (2009), **37**: e45

Examples

```
## sliding window of size 5 between
## take-off point and upper
## asymptote, no baseline optimization
m1 <- pcrfit(reps, 1, 2, 14)
sliwin(m1, wsize = 5)

## Not run:
## optimizing with window sizes of 4 to 6,
## between 0/+2 from lower/upper border,
## and baseline up to 2 standard deviations
sliwin(m1, wsize = 4:6, border = c(0, 2), base = 2)

## End(Not run)
```

takeoff

Calculation of the qPCR takeoff point

Description

Calculates the first significant cycle of the exponential region (takeoff point) using externally studentized residuals as described in Tichopad *et al.* (2003).

Usage

```
takeoff(object, pval = 0.05, nsig = 3)
```

Arguments

| | |
|--------|--|
| object | an object of class 'pcrfit'. |
| pval | the p-value for the takeoff test. |
| nsig | the number of successive takeoff tests. See 'Details'. |

Details

Takeoff points are calculated essentially as described in the reference below. The steps are:

- 1) Fitting a linear model to background cycles 1 : n , starting with $n = 5$.
- 2) Calculation of the external studentized residuals using `rstudent`, which uses the hat matrix of the linear model and leave-one-out:

$$\langle \hat{\epsilon}_i \rangle = \frac{\hat{\epsilon}_i}{\hat{\sigma}_{(i)} \sqrt{1 - h_{ii}}}, \hat{\sigma}_{(i)} = \sqrt{\frac{1}{n - p - 1} \sum_{\substack{j=1 \\ j \neq i}}^n \hat{\epsilon}_j^2}$$

with h_{ii} being the i th diagonal entry in the hat matrix $H = X(X^T X)^{-1} X^T$.

- 3) Test if the last studentized residual $\langle \hat{\epsilon}_n \rangle$ is an outlier in terms of t-distribution:

$$1 - pt(\langle \hat{\epsilon}_n \rangle, n - p) < 0.05$$

- with n = number of residuals and p = number of parameters.
- 4) Test if the next $n_{sig} - 1$ cycles are also outlier cycles.
 - 5) If so, take cycle number from 3), otherwise $n = n + 1$ and start at 1).

Value

A list with the following components:

- top the takeoff point.
f.top the fluorescence at top.

Author(s)

Andrej-Nikolai Spiess

References

Standardized determination of real-time PCR efficiency from a single reaction set-up.
Tichopad A, Dilger M, Schwarz G & Pfaffl MW.
Nucleic Acids Research (2003), **e122**.

Examples

```
m1 <- pcrfit(reps, 1, 2, 15)
res1 <- takeoff(m1)
plot(m1)
abline(v = res1$top, col = 2)
abline(h = res1$f.top, col = 2)
```

| | |
|---------|--|
| testdat | <i>dataset with noisy data to provoke fitting errors</i> |
|---------|--|

Description

A dilution experiment with six 10-fold dilutions of the cDNA, and four replicates for each dilution. Each third and fourth replicate are made of noisy data that result in failed fits or correct fits but lack of sigmoidal structure, respectively. This dataset is mainly for testing the package functions for fail-safety against fitting errors and alike.

Usage

```
testdat
```

Format

A data frame with the PCR cycles and 24 qPCR runs with four replicates of seven 10-fold dilutions. The replicates are defined by FX.Y (X = dilution number, Y = replicate number). Each FX.3 has noisy data which fails to fit with the 15 model, each FX.4 passes fitting but fails in sigmoidal structure detection by KOD.

Details

The real-time PCR was conducted with primers for the S27a housekeeping gene in a Lightcycler 1.0 instrument (Roche Diagnostics).

Source

Andrej-Nikolai Spiess & Nadine Mueller, Institute for Hormone and Fertility Research, Hamburg, Germany.

Examples

```
## Not run:
## remove only non-fitted runs
m11 <- modlist(testdat, model = 15, remove = "fit")
## remove sigmoidal outliers
m12 <- modlist(testdat, model = 15, check = "uni2", remove = "KOD")

## End(Not run)
```

update.pcrfit

Updating and refitting a qPCR model

Description

Updates and re-fits a model of class 'pcrfit'.

Usage

```
## S3 method for class 'pcrfit'
update(object, ..., evaluate = TRUE)
```

Arguments

| | |
|----------|---|
| object | a fitted model of class 'pcrfit'. |
| ... | arguments to alter in object. |
| evaluate | logical. If TRUE, model is re-fit; otherwise an unevaluated call is returned. |

Value

An updated model of class 'pcrfit' and 'nls'.

Author(s)

Andrej-Nikolai Spiess

See Also

The function `pcrfit` in this package.

Examples

```
m1 <- pcrfit(reps, 1, 2, 14)

## update model
update(m1, model = 15)

## update qPCR run
update(m1, fluo = 20)

## update data
update(m1, data = guescini1)

## update 'optim' method
update(m1, opt.method = "BFGS")
```

vermeulenetal

A large-scale qPCR dataset from Vermeulen et al. (2009)

Description

A subset of a larger dataset from Vermeulen *et al.* (2009). The original dataset consists of 64 genes (59 prognostic genes and 5 reference genes) with 384 runs/gene. The 'qpcR' package includes a subset of the first 20 runs for each of the 64 genes, hence 1280 runs (vermeulen1) and the corresponding dilution data for all 64 genes (vermeulen2).

Usage

```
vermeulen1
vermeulen2
```

Format

vermeulen1: A data frame with 1280 runs named by X.Y, with X = gene name and Y = sample number.

vermeulen2: A data frame with dilution data for all 64 genes, with five 10-fold dilutions and 3 replicates each. Data is in the format X.STD_Y.Z, with X = gene name, Y = copy number and Z = replicate number.

Details

The real-time PCR was conducted in a Lightcycler 480 (Roche) using SybrGreen I chemistry. The investigated genes are AHCY, AKR1C1, ALUsq(Eurogentec), ARHGEF7, BIRC5, CAMTA1, CAMTA2, CD44, CDCA5, CDH5, CDKN3, CLSTN1, CPSG3, DDC, DPYSL3, ECEL1, ELAVL4, EPB41L3, EPHA5, EPN2, FYN, GNB1, HIVEP2, HMBS, HPRT1, IGSF4, INPP1, MAP2K4, MAP7, MAPT, MCM2, MRPL3, MTSS1, MYCN(4), NHLH2, NM23A, NRCAM, NTRK1, ODC1, PAICS, PDE4DIP, PIK3R1, PLAGL1, PLAT, PMP22, PRAME, PRDM2, PRKACB, PRKCZ, PTN, PTPRF, PTPRH, PTPRN2, QPCT, SCG2, SDHA(1), SLC25A5, SLC6A8, SNAPC1, TNFRSF, TYMS, UBC(2), ULK2 and WSB1.

Source

Originally, raw data was available at <http://medgen.ugent.be/jvermeulen>, but site is down. The complete (vermeulen_all) and smaller (vermeulen_sub) datasets can be downloaded from <http://www.dr-spiess.de/qpcR/datasets.html>.

References

Predicting outcomes for children with neuroblastoma using a multigene-expression signature: a retrospective SIOPEN/COG/GPOH study.

Vermeulen J, De Preter K, Naranjo A, Vercruyse L, Van Roy N, Hellemans J, Swerts K, Bravo S, Scaruffi P, Tonini GP, De Bernardi B, Noguera R, Piqueras M, Cañete A, Castel V, Janoueix-Lerosey I, Delattre O, Schleiermacher G, Michon J, Combaret V, Fischer M, Oberthuer A, Ambros PF, Beiske K, Bénard J, Marques B, Rubie H, Kohler J, Pötschger U, Ladenstein R, Hogarty MD, McGrady P, London WB, Laureys G, Speleman F & Vandesompele J. *Lancet Oncol* (2009), **10**:663-671.

Examples

```
## Not run:
## make model list and plot
## with colors for each gene
COL <- rep(rainbow(64), each = 20)
ml <- modlist(vermeulen, model = 14)
plot(ml, col = COL)

## End(Not run)
```

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