Package 'chipPCR'

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Type Package

Title Toolkit of Helper Functions to Pre-Process Amplification Data

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Description A collection of functions to pre-process amplification curve data from polymerase chain reaction (PCR) or isothermal amplification reactions. Contains functions to normalize and baseline amplification curves, to detect both the start and end of an amplification reaction, several smoothers (e.g., LOWESS, moving average, cubic splines, Savitzky-Golay), a function to detect false positive amplification reactions and a function to determine the amplification efficiency. Quantification point (Cq) methods include the first (FDM) and second approximate derivative maximum (SDM) methods (calculated by a 5-point-stencil) and the cycle threshold method. Data sets of experimental nucleic acid amplification systems ('VideoScan HCU', capillary convective PCR (ccPCR)) and commercial systems are included. Amplification curves were generated by helicase dependent amplification (HDA), ccPCR or PCR. As detection system intercalating dyes (EvaGreen, SYBR Green) and hydrolysis probes (TaqMan) were used. For more information see: Roediger et al. (2015) <doi:10.1093/bioinformatics/btv205>.

License GPL-3

URL https://github.com/PCRuniversum/chipPCR

BugReports https://github.com/PCRuniversum/chipPCR/issues

Depends R (>= 3.0.0), methods

Suggests drc, knitr, markdown, qpcR, RDML, rmarkdown, spelling, testthat, tinytex, xtable

VignetteBuilder knitr, rmarkdown

Imports lmtest, MASS, outliers, ptw, quantreg, Rfit, robustbase, shiny, signal

NeedsCompilation no

Repository CRAN

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R **topics documented:**

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chipPCR-package   *Toolkit of functions to pre-process amplification data*

Description

A toolkit of functions to pre-process amplification curve data. Amplification data can be obtained from conventional PCR reactions or isothermal amplification reactions. Contains functions to normalize and baseline amplification curves, a routine to detect the start of an amplification reaction, several smoothers for amplification data, a function to distinguish positive and negative amplification reactions and a function to determine the amplification efficiency. The smoothers are based on LOWESS, moving average, cubic splines, Savitzky-Golay and others. In addition the first approximate approximate derivative maximum (FDM) and second approximate derivative maximum (SDM) can be calculated by a 5-point-stencil as quantification points from real-time amplification curves. chipPCR contains data sets of experimental nucleic acid amplification systems including the 'VideoScan' 'HCU' and a capillary convective PCR (ccPCR) system. The amplification data were generated by helicase dependent amplification (HDA) or polymerase chain reaction (PCR) under various temperature conditions. As detection system intercalating dyes (EvaGreen,

SYBR Green) and hydrolysis probes (TaqMan) were used. The latest source code is available via:
https://github.com/PCRuniversum/chipPCR

Details

Package:	chipPCR
Type:	Package
Version:	0.0.8-12
Date:	2017-06-22
License:	GPL-3

bg.max can be used to remove missing values in amplification curve data. amptester tests if an amplification is positive. fixNA is used to impute missing values from a data column. CPP can be used to normalize curve data, to remove background, to remove outliers and further steps. Contains further functions to smooth the data by different functions including LOWESS, Moving Average, Friedman's SuperSmoother, Cubic Spline and Savitzky-Golay smoothing.

For more exhaustive description see the vignette (vignette("chipPCR")).

Author(s)

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References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Nucleic acid detection based on the use of microbeads: a review. S. Roediger, C. Liebsch, C. Schmidt, W. Lehmann, U. Resch-Genger, U. Schedler, P. Schierack. *Microchim Acta* 2014:1–18. DOI: 10.1007/s00604-014-1243-4

Roediger S, Boehm A, Schimke I. Surface Melting Curve Analysis with R. *The R Journal* 2013;5:37–53.

Spiess, A.-N., Deutschmann, C., Burdukiewicz, M., Himmelreich, R., Klat, K., Schierack, P., Roediger, S., 2014. Impact of Smoothing on Parameter Estimation in Quantitative DNA Amplification Experiments. *Clinical Chemistry* clinchem.2014.230656. doi:10.1373/clinchem.2014.230656

See Also

qpcR.news.

Examples

```
# Example: A simple function to test for a background range.
# Data were taken form the chipPCR C17 data set.
data(C17)
plot(C17[, 2], C17[,  3], xlab = "time [min]", ylab = "Fluorescence",
      pch = 20)
res <- bg.max(C17[, 2], C17[, 3], bg.corr = 1.4, bg.start = 3)
abline(v = c(slot(res, "bg.start"), slot(res, "bg.stop")), col = c(1,2))
abline(h = slot(res, "fluo"), col = "blue")
```

AmpSim   *Amplification curve simulator*

Description

This function is a simple simulator of an amplification reaction based on a 5-parameter Richards function. This simplified approach was chosen because it is impossible to model the shape of any amplification curve. An implementation of realistic models is ambitious and not conclusively addressed in the literature. First, they have to take "all" random effects of noise into consideration and second, they need to be generic enough to cover all amplification processes. More sophisticated mechanistic models and simulations have been proposed elsewhere *mehra_2005, cobbs_2012*. This approach of AmpSim is similar to the pcrsim function from the *qpcR* package, which offers simulations of sigmoidal qPCR data with goodness-of-fit analysis by Ritz and Spiess 2008.

Usage

```
AmpSim(cyc = 1:35, b.eff = -25, bl = 0.05, ampl = 1, Cq = 20,
noise = FALSE, nnl = 0.025, nnl.method = "constant")
```

Arguments

cyc	is a vector containing the cycle values.
b.eff	can be used to adjust the amplification efficiency.
bl	is used to define the base level (minimum) of the background range.
ampl	defines the plateau (maximum) of the amplification reaction.
Cq	defines approximately the quantification point (Cq) of the amplification reaction.
noise	adds some noise to the amplification reaction.
nnl	level of noise during the amplification reaction.
nnl.method	trend of noise level during the amplification reaction. "constant" uses same noise of amplification, "decreasing" leads to less noise at the end of the amplification reaction, and "increasing" leads to more noise at the end of the amplification reaction.

Details

AmpSim is a simple simulator for amplification reaction. This function has several parameters which can be used to simulate the amplification curve. b.eff and Cq are most connected with another. Thus changing one of them will change both values. Cq can be used to define an approximate Cq value. The expression "approximate Cq value" is used here because the actual Cq value is dependent on the users preferred method (e.g., Cy0 method, Second Derivative Maximum (SDM) method, threshold method). AmpSim can be used to compare an experimental system to a predicted model. Moreover it can be used to simulate data with noise, missing values (NA), signal-to-noise ratios, photo-bleaching and other influences on a PCR reaction.

Value

A list where one element represents the cycle (cyc) and one represents the fluorescence (fluo) of a simulated qPCR experiment.

Author(s)

Stefan Roediger, Michal Burdukiewicz

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Ritz, C., Spiess, A.-N.: qpcR: an R package for sigmoidal model selection in quantitative real-time polymerase chain reaction analysis. *Bioinformatics* 24(13), 1549–1551 (2008). doi:10.1093/bioinformatics/btn227. PMID: 18482995.

See also qpcR.news

Examples

```
# Example one
# Simulate a qPCR reaction with AmpSim for 40 cycles.
# Use an in-silico dilution of the template be adjusting
# the Cq parameter. A change of 3.32 cycles corresponds
# approximately to a 10-fold dilution.
default.par <- par(no.readonly = TRUE)
par(mfrow = c(2,1))
plot(NA, NA, xlim = c(1,40), ylim = c(0.01,2), xlab = "Cycles",
     ylab = "Fluorescence", main = "In-silco Dilution Experiment")
cycle.dilution <- seq(18, 35, 3.32)
for (i in 1:6) {
  lines(AmpSim(cyc = 1:40, b.eff = -25, bl = 0.01, ampl = 2,
      Cq = cycle.dilution[i]), type = "b", col = i, pch = 20)
}

# Example two
# Simulate a qPCR reaction with AmpSim for 40 cycles and some noise.
```

```
plot(NA, NA, xlim = c(1,40), ylim = c(0.01,2.2), xlab = "Cycles",
     ylab = "Fluorescence",
     main = "In-silco Dilution Experiment with Some Noise")
cycle.dilution <- seq(18, 35, 3.32)
for (i in 1:6) {
  lines(AmpSim(cyc = 1:40, b.eff = -25, bl = 0.01, ampl = 2,
      Cq = cycle.dilution[i], noise = TRUE, nnl = 0.05),
      type = "b", col = i, pch = 20)
}
par(mfrow = c(1,1))

# Example three
# Apply constant, increasing, decreasing nose to
# amplification data.

par(mfrow = c(3,1))
method <- c("constant", "increase", "decrease")
for (j in 1:3){
    plot(NA, NA, xlim = c(1,40), ylim = c(0.02,2.2), xlab = "Cycles",
ylab = "Fluorescence",
main = paste("In-silco Dilution Experiment with noise method: ",
   method[j]))
   cycle.dilution <- seq(18, 35, 3.32)
   for (i in 1:6) {
     lines(AmpSim(cyc = 1:40, b.eff = -25, bl = 0.02, ampl = 2,
   Cq = cycle.dilution[i], noise = TRUE, nnl = 0.08,
   nnl.method = method[j]), type = "b", col = i, pch = 20)
   }
}
par(default.par)
```

AmpSim.gui   *Amplification Curve Simulation Graphical User Interface*

Description

Launches graphical user interface that allows simulating and analyzing amplification reactions. This function will open the GUI in a webpage of the default browser. All parameters of the AmpSim function can be used. In addition to this, the GUI shows some information calculated by the bg.max in a summary field and a plot below the simulated amplification curve.

Usage

```
AmpSim.gui()
```

Value

No return value, called for side effects

Warning

Any ad-blocking software may be cause of malfunctions.

Author(s)

Stefan Roediger, Michal Burdukiewicz.

See Also

AmpSim, bg.max

Examples

```
# The code chunk below will fail if the web browser is not installed. if on UNIX platform try:
# as.vector(Sys.getenv("R_BROWSER"))
# Invoke the shiny AmpSim app in the default browser
## Not run:
#do not execute using example(), it breaks the sequence of the plots in shiny app
AmpSim.gui()

## End(Not run)
```

---

amptest *Class* "amptest"

Description

An S4 class containing the output amptester function.

Value

An object of amptest class containing result of the test as well as the original data.

Slots

.Data: "numeric" is a vector containing the fluorescence values.

decisions: "logical" contains outcomes of various tests. shap.noisy is presence of noise, lrt.test states if data are likely from a amplification curve and both tht.dec and tht.dec defines if the amplification is "positive" or "negative".

noiselevel: "numeric" user-defined threshold for a significant amplification signal.

background: range of the background signal in the amplification curve.

polygon: The pco test determines if the points in an amplification curve (like a polygon) are in a "clockwise" order. The sum over the edges result in a positive value if the amplification curve is "clockwise" and is negative if the curve is counter-clockwise.

slope.ratio: ratio of the slopes at the start and the end of exponential phase..

Methods

summary `signature(object = "amptest")`: prints summary of the object. Silently returns `vector` of all calculated parameters.

show `signature(object = "amptest")`: prints only `.Data` slot of the object.

plot `signature(object = "amptest")`: plots input data and graphical interpretation of `link{amptester}` tests' results.

Author(s)

Stefan Roediger, Michal Burdukiewicz

See Also

amptester

Examples

```
# Compare a positive and a negative amplification reaction.
# First simulate positive reaction (fluo.pos) and than the
# negative reaction (fluo.neg).
# Simulation of an amplifiaction curve with some noise and a high signal.

fluo.pos <- AmpSim(cyc = 1:40, noise = TRUE)[, 2]
ampt.pos <- amptester(fluo.pos, manual = TRUE, background = c(0, 15),
      noiselevel = 0.15)

# Plot amplification curve and result of amptester
plot(fluo.pos, xlab = "Cycles", ylab = "RFU", pch = 19, ylim = c(0, 1))
lines(ampt.pos, col = 2, pch = 19, type = "b")
legend(5, 1, c("Raw data", "amptester output"),
      col = c(1,2,3), bty = "n", pch = c(19, 19))
# Borders for background calculation
abline(v = c(0,15), col = 2)
# Level for background threshold
abline(h = 0.15, col = 3, lty = 2)
text(5, 0.18, "Noise threshold")
# Summary of amptester results
summary(ampt.pos)

# Simulation of an amplifiaction curve with high noise and a low signal.

fluo.neg <- AmpSim(cyc = 1:40, noise = TRUE, ampl = 0.13, nnl = 0.4)[, 2]
ampt.neg <- amptester(fluo.neg, manual = TRUE, background = c(0, 15),
      noiselevel = 0.15)

# Plot amplification curve and result of amptester
plot(fluo.neg, xlab = "Cycles", ylab = "RFU", pch = 19, ylim = c(0, 1))
lines(ampt.neg, col = 2, pch = 19, type = "b")
legend(5, 1, c("Raw data", "amptester output"),
      col = c(1,2,3), bty = "n", pch = c(19, 19))
# Borders for background calculation
```

```
abline(v = c(0,15), col = 2)
# Level for background threshold
abline(h = 0.15, col = 3, lty = 2)
text(5, 0.18, "Noise threshold")
# Summary of amptester results
summary(ampt.neg)
#plot amptester results
plot(ampt.neg)
```

---

amptester   *Amplification test*

Description

The `amptester` function tests if an amplification is significant.

Usage

```
amptester(y, manual = FALSE, noiselevel = 0.08, background = NULL)
```

Arguments

y	is a vector containing the fluorescence values.
manual	switches between a statistical test (based on a Wilcoxon rank sum test (`wilcox.test`)) or manual test for a positive amplification signal.
noiselevel	can be set to a user defined value as threshold for a significant amplification signal.
background	is the range of the background signal in the amplification curve. The values can be added by the user or taken from the `bg.max` function. Ignored if `manual` is TRUE.

Details

This function tries to estimate if data indicates an amplification process. Several instances of tests are included. The first involves a semiautomatic test if the range of the background is lower than the range of the assumed signal. To differ between the ranges an instance of `bg.max` is used. Herein, this function assumes that an amplification takes place in case the signal of the amplification is larger than the `median` + 5 * `mad` than the background. The automatic test uses a Wilcoxon rank sum test `wilcox.test` to compare the first and the last elements of the data. The input values are delivered by `head` and `tail`, respectively. For other methods please refer to the references listed below. Instead of assigning a zero to negative amplification reaction uses the current implementation of `amptester` very small random values. This is because some post function might fail in case all values are set to zero.

FIRST TEST - Shapiro test (SHt)

This is a simple test based on the hypothesis that in case amplification curve data come from noise is the distribution similar to a normal distribution. The Shapiro's normality test is used to test this hypothesis. If p is >= 5e-04, then the distribution of the curve data indicates noise (no amplification).

SECOND TEST - Resids growth test (RGt)

This tests if fluorescence values in a linear phase are stable. Whenever no amplification occurs, fluorescence values quickly deviate from linear model. Their standardized residuals will be strongly correlated with their value. For real amplification curves, situation is much more stable. Noise (that means deviations from linear model) in background do not correlate strongly with the changes in fluorescence. The decision is based on the threshold value (here 0.5).

THIRD TEST - Linear Regression test (LRt)

This test determines the R^2 by a linear regression. The R^2 are determined from a run of circa 15 percent range of the data. If a sequence of more than six R^2s is larger than 0.8 is found that is likely a nonlinear signal. This is a bit counterintuitive because R^2 of nonlinear data should be low.

FOURTH TEST (MANUAL) - Threshold test (THt)

This a commonly employed method. In the manual test one needs to define a fixed threshold. If the amplification curve signal exceeds the threshold than the amplification reaction is positive. Waring: This method will report positive amplification reaction if a negative amplification has a positive trend.

FOURTH TEST (AUTOMATIC) - Threshold test (THt)

Takes the first 20 percent and the last 15 percent of any input data set (amplification curve) and perform a Wilcoxon rank sum tests with the head (nh) and tail (nt). This test is recommended over the manual THt. Warning: This method may report positive amplification reaction if a negative amplification has a positive trend.

FIFTH TEST - Signal level test (SLt)

The meaningfulness of an amplification curve reaction can be tested by comparison of the signals 1) A robust "sigma" rule by median + 2 * mad 2) comparison of the signal/noise ratio. If less than 1.25 (25 percent) signal increase it is likely that nothing happened during the reaction. Waring: This method may report positive amplification reaction if a negative amplification has a positive trend.

SIXTH TEST - pco test (pco)

This test determines if the points in an amplification curve (like a polygon, in particular non-convex polygons) are in a "clockwise" order. The sum over the edges result in a positive value if the amplification curve is "clockwise" and is negative if the curve is counter-clockwise ((x2 - x1)(y2 + y1)). From experience is noise positive and "true" amplification curves "highly" negative. This test depends on the definition of a threshold.

SEVENTH TEST - Slope ratio (SlR)

Uses the inder function to find the approximated first derivative maximum, second derivative minimum and the second derivative maximum. Next the raw fluorescence at the approximated second derivative minimum and the second derivative maximum are taken from the original data set. The fluorescence intensities are normalized to the maximum fluorescence of this data. This data is used for a linear regression. Where the slope is used.

Value

An object of amptest class containing result of the test as well as the original data.

Author(s)

Stefan Roediger, Michal Burdukiewicz

References

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Tichopad, A., Dilger, M., Schwarz, G. and Pfaffl, M. W. Standardized determination of real-time PCR efficiency from a single reaction set-up *Nucleic Acids Research*, 2003, Vol. 31(20), pp. e122

Wilhelm, J., Pingoud, A. and Hahn, M. SoFAR: software for fully automatic evaluation of real-time PCR data *BioTechniques*, 2003, Vol. 34(2), pp. 324-332

Zhao, S. and Fernald, R. D. Comprehensive Algorithm for Quantitative Real-Time Polymerase Chain Reaction *Journal of computational biology: a journal of computational molecular cell biology*, 2005, Vol. 12(8), pp. 1047-1064

Examples

```
# First example
# Arrange graphs in orthogonal matrix and set parameter for the plot.
default.par <- par(no.readonly = TRUE)
par(las = 0, bty = "n", cex.axis = 1.5, cex.lab = 1.5,
    font = 2, cex.main = 1.8, oma = c(1,1,1,1))

# Simulation of an amplification curve with 40 cycles and a Cq of
# circa 28. The amplification curve of "pos" (positive) has low
# noise and the amplification curve of "neg" (negative) has high
# noise.

pos <- AmpSim(cyc = 1:40, Cq = 28, noise = TRUE, nnl = 0.03)
neg <- AmpSim(cyc = 1:40, Cq = 28, noise = TRUE, nnl = 0.8)

# Plot the raw data of the simulations.

par(fig = c(0,0.5,0.5,1))
plot(NA, NA, xlim = c(1, 40), ylim = c(0, 2.1), xlab = "Cycles",
     ylab = "Fluorescence", main = "qPCR - Raw data", type = "b")
mtext("A", cex = 2, side = 3, adj = 0, font = 2)
points(pos, col = 1, typ = "b", pch = 19)
points(neg, col = 2, typ = "b", pch = 20)
legend(1, 2, c("Positive", "Negative Control (noise)"),
```

```
            pch = c(19,20), col = c(1,2), lwd = 2, bty = "n")

# Plot data again after an analysis by ampteser. "neg" is set to small
# random numbers, while "pos" remains unchanged.

par(fig = c(0,0.5,0,0.5), new = TRUE)
plot(NA, NA, xlim = c(1, 40), ylim = c(0, 2.1), xlab = "Cycles",
     ylab = "Fluorescence", main = "qPCR - amptester", type = "b")
points(amptester(pos[, 2]), col = 1, type = "b", pch = 19)
points(amptester(neg[, 2]), col = 2, type = "b", pch = 20)
legend(1, 2, c("Positive", "Negative Control (noise)"),
       pch = c(19,20), col = c(1,2), lwd = 2, bty = "n")

# Use of amptester for time-dependent measurements. Amplification curves
# from the capillaryPCR data set were processed in a loop. The results of
# amptester are added to the raw data.

par(fig = c(0.5,1,0,1), new = TRUE)
colors <- rainbow(8)
plot(NA, NA, xlim = c(0,80), ylim = c(0,1300), xlab = "Time [min]",
     ylab = "Voltage (micro V)", main = "ccPCR")
mtext("B", cex = 2, side = 3, adj = 0, font = 2)
sapply(c(1,3,5,7), function(i) {
    xy.tmp <- cbind(capillaryPCR[1:750, i], capillaryPCR[1:750, i + 1])

# Use amptester to analyse the amplification curve.
# Note: The decisions of amptester can be invoked via res.ampt@decisions
# in the present example.

    res.ampt <- amptester(xy.tmp[, 2])

# Use the "decisions" of amptester in a logic to automatically decide if an
# amplification reaction is positive. In this example linear regression test
# (lrt.test) and the threshold test (tht.dec) are used.

    res.ampt <- ifelse(res.ampt@decisions[2] == TRUE &&
        res.ampt@decisions[4] == TRUE, "positve", "negative")

# Plot the amplification curve with the decisions.
    lines(xy.tmp[, 1], xy.tmp[, 2], type = "b", pch = 20, col = colors[i])
    text(75, max(na.omit(xy.tmp[, 2])), res.ampt, cex = 1.3, col = colors[i])
  }
)
# Second Example
# Example to test an amplification reaction.
# Simulate first a positive amplification curve with 45 cycles and than a
# negative amplification curve with 45 cycles. The negative amplification
# curve is created from a normal distribution
#
fluo.neg <- rnorm(45)
fluo.pos <- AmpSim(cyc = 1:45, Cq = 45, ampl = 40, noise = TRUE,
   nnl = 0.03)[, 2]
```

```
plot(NA, NA, xlim = c(1, 45), ylim = c(-1, 45), xlab = "Cycles",
     ylab = "Fluorescence",
     main = "Simulation of a qPCR with 45 Cycles", type = "b")
points(amptester(fluo.pos), type = "b", pch = 20)
points(amptester(fluo.neg), type = "b", col = "red", pch = 20)
points(1:45, fluo.neg, col = "red")

legend(1,40, c("Positive", "Negative Control (noise)",
        "noise pattern"), pch = c(20,20,1), col = c(1,2,2), lwd = 2)
par(default.par)
```

---

amptester.gui   *Amplification Test Graphical User Interface*

Description

amptester.gui is a graphical user interface for the amptester function. This function can be used
for a fast and convenient analysis of amplification curve data. In addition it is possible to analyze
the Cq (quantification cycle) and to perform a report generation of the analyzed data.

Usage

```
amptester.gui()
```

Value

An object of amptest class containing result of the test as well as the original data.

Warning

Any ad-blocking software may be cause of malfunctions.

Author(s)

Stefan Roediger, Michal Burdukiewicz.

See Also

AmpSim, bg.max

Examples

```
# The code chunk below will fail if the web browser is not installed. if on UNIX platform try:
# as.vector(Sys.getenv("R_BROWSER"))
# Invoke the shiny AmpSim app in the default browser
## Not run:
#do not execute using example(), it breaks the sequence of the plots in shiny app
amptester.gui()

## End(Not run)
```

---

bg   *Class* "bg"

Description

An S4 class containing the output `bg.max` function.

Value

An object containing result of the `bg.max` function.

Slots

`.Data:` "matrix" which columns represent respectively cycle number, raw fluorescence data, first derivative and second derivative.

`bg.start:` "numeric" value representing start of the background range.

`bg.stop:` "numeric" value representing end of the background range.

`bg.corr:` "numeric" a value which helps to tweak on the suggested background value of `bg.max`.

`fluo:` "numeric" a value of fluorescence at the end of amplification.

`amp.stop:` "numeric" value representing end of the amplification .

Methods

plot signature(x = "bg"): plots background information. See `plot.bg`

show signature(object = "bg"): prints only `.Data` slot of the object.

summary signature(object = "bg"): prints information about object prettier than show and allows easy access to some slots. See `summary.bg`

Author(s)

Stefan Roediger, Michal Burdukiewicz

See Also

`bg.max`, `plot.bg`, `summary.bg`

Examples

```
res <- AmpSim(cyc = 1:40, Cq = 25)
tmp <- bg.max(res)
summary(tmp)
plot(tmp)
```

---

bg.max   *Simple function to detect and correct the background range*

Description

[bg.max](bg.max) detects and corrects background noise. The detection is made without any assumptions regarding the model of this function.

Usage

```
## S4 method for signature 'numeric,numeric'
bg.max(x, y, bg.corr = 1.3, bg.start = 2,
  inder.approx = TRUE)

## S4 method for signature 'matrix,missing'
bg.max(x, y, bg.corr = 1.3, bg.start = 2,
  inder.approx = TRUE)

## S4 method for signature 'data.frame,missing'
bg.max(x, y, bg.corr = 1.3, bg.start = 2,
  inder.approx = TRUE)
```

Arguments

x	is a vector containing the time or cycle values or data frame/matrix containing cycle in the first column and fluorescence values in the second column.
y	is a vector containing the fluorescence values. Used only if x is also a vector.
bg.corr	a value which helps to tweak on the suggested background value of bg.max.
bg.start	a user defined value for the start of the background range.
inder.approx	a logical value defining if data should be numerically derived by inder function. If FALSE, derivatives are calculated by the predict.smooth.spline.

Details

Background range herein refers to a level of fluorescence measured before any specific amplification is detectable. The raw data (e.g., fluorescence intensity) measured after each step (cycle or time point) follow a non-linear progress. The background is assumed to be constant for the entire measurement. The algorithm of bg.max is based on the assumption that during the linear ground phase the signal difference of successive cycles is approximately constant. After transition to the early exponential phase the signal changes drastically. First data are smoothed by Friedman's 'super smoother' (as found in supsmu. Thereof the approximate first and second derivative are calculated by a five-point stencil inder.

The difference of cycles at the maxima of the first and second approximate derivative as well as a correction factor are used to estimate the range before the exponential phase. This function finds the background range without modeling the relationship between signal and cycle number. The start of the background range is defined be a fixed value. Since many signals tend to overshot in the

first cycles a default value of 3 is chosen. `bg.max` tries also to estimate the end of an amplification reaction.

Value

An object of `bg` class containing predicted background range as well as other parameters.

Author(s)

Stefan Roediger, Michal Burdukiewicz

References

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Examples

```
# First example: Test for the background of an amplification reaction.
default.par <- par(no.readonly = TRUE)
par(mfrow = c(2,1))
res <- AmpSim(cyc = 1:40, Cq = 25)
background <- bg.max(res)
plot(background, main = "Estimation of the Background Range\n
in Absence of Noise")
res.noise <- AmpSim(cyc = 1:40, Cq = 25, noise = TRUE)
background.noise <- bg.max(res.noise)
plot(background.noise, main = "Estimation of the Background Range\n
in Presence of Noise")
par(mfrow = c(1,1))
```

```
# Second example: A simple function to test for a background range.
# Data were taken form the chipPCR C17 data set.
# Note that the not the time but the "cycle number" was
# used to calculate the background range.
data(C17)
plot(C17[, 2], C17[,  3], xlab = "Cycle", ylab = "RFU",
     main = "Estimate the begin of the Amplification\n of a HDA",
     pch = 20)
res <- bg.max(C17[, 2:3], bg.corr = 1.4, bg.start = 1)
abline(v = c(slot(res, "bg.start"), slot(res, "bg.stop")),
       col = c(1,2))
abline(h = slot(res, "fluo"), col = "blue")

# Third example: Test for the background of an amplification reaction.
# Simulate amplification curves with different quantification points
# within 40 cycles.
cyc <- seq(1, 40, 1)

# Use a five parameter model to simulate amplification curves
b <- -15; c <- 0.02; d <- 1
# Define the different quantification points with a difference of
# circa 3.32 cycles
e <- seq(21, 35, 3.32)

# Plot the amplification curves and the estimated background ranges.
plot(NA, NA, xlim = c(1, 40), ylim = c(0, 1), xlab = "Cycles",
     ylab = "Fluorescence")

for (i in 1:length(e)) {
  fluo <- c + (d - c)/(1 + exp(b * (log(cyc) - log(e[i]))))
  points(cyc, fluo, type = "b", col = i, pch = 20)
  res <- bg.max(cyc, fluo, bg.corr = 1.4, bg.start = 1)
  abline(v = slot(res, "bg.stop"), col = i)
  abline(h = slot(res, "fluo"), col = i)
}
par(default.par)
```

C126EG595   *qPCR Experiment for the Amplification of HPRT1 Using the Bio-Rad iQ5 thermo cycler*

Description

A quantitative PCR (qPCR) with the DNA binding dye (EvaGreen) (Mao et al. 2007) was performed in a Bio-Rad iQ5 thermo cycler. The cycle-dependent increase of the fluorescence was quantified at the annealing step (59.5 degrees Celsius).

Usage

```
data(C126EG595)
```

Format

A data frame with 40 observations on the following 97 variables. The first column ("Cycle") contains the number of cycles and consecutive columns contain the replicates ("A01" to "H12").

Details

HPRT1 was amplified in the Bio-Rad iQ5. The the change of fluorescence was simultaneously monitored for the Hydrolysis probe of HPRT1 and EvaGreen. The primer sequences for HPRT1 were taken from Roediger et al. (2013). A 10 micro L qPCR reaction was composed of 250 nM primer (forward and reverse), qPCR Mix (according to the manufactures recommendations), 1 micro L template (HPRT1 amplification product), 60 nM hydrolysis probe probe for HPRT1. EvaGreen was used at 0.5 x final. During the amplification was monitored 59.5 degrees Celsius.

Source

Stefan Roediger, Claudia Deutschmann (BTU Cottbus - Senftenberg)

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Mao, F., Leung, W.-Y., Xin, X., 2007. Characterization of EvaGreen and the implication of its physicochemical properties for qPCR applications. *BMC Biotechnol*. 7, 76.

Examples

```
data(C126EG595)
tmp  <- C126EG595

plot(NA,NA, xlim = c(1,40), ylim = c(min(tmp[, 2:ncol(tmp)]),
   max(tmp[, 2:ncol(tmp)])), xlab = "Cycle", ylab = "RFU (FAM)",
   main = "Amplification monitored at \n58.5 degrees Celsius (annealing step)")
apply(tmp[, 2:ncol(tmp)], 2,
      function(x) lines(tmp[1:nrow(tmp),1],x))
```

C126EG685   *qPCR Experiment for the Amplification of HPRT1 Using the Roche Light Cycler 1.5*

Description

A quantitative PCR (qPCR) with the DNA binding dye (EvaGreen) (Mao et al. 2007) was performed in the Roche Light Cycler 1.5 thermo cycler. The cycle-dependent increase of the fluorescence was quantified at the elongation step (68.5 degrees Celsius).

Usage

```
data(C126EG685)
```

Format

A data frame with 40 observations on the following 97 variables. The first column ("Cycles") contains the number of cycles and consecutive columns contain the replicates ("A01" to "H12").

Details

MLC-2v was amplified in the Roche Light Cycler 1.5. The the change of fluorescence was simultaneously monitored for the Hydrolysis probe of MLC-2v and EvaGreen. The primer sequences for MLC-2v were taken from Roediger et al. (2013). A 10 micro L qPCR reaction was composed of 250 nM primer (forward and reverse), qPCR Mix (according to the manufactures recommendations), 1 micro L template (MLC-2v amplification product), 60 nM hydrolysis probe probe for MLC-2v. EvaGreen was used at 0.5 x final. The amplification was monitored at 68.5 degrees Celsius (elongation step).

Source

Claudia Deutschmann & Stefan Roediger, BTU Cottbus - Senftenberg, Senftenberg, Germany

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Mao, F., Leung, W.-Y., Xin, X., 2007. Characterization of EvaGreen and the implication of its physicochemical properties for qPCR applications. *BMC Biotechnol*. 7, 76.

Examples

```
data(C126EG685)
tmp <- C126EG685

plot(NA,NA, xlim = c(1,40), ylim = c(min(tmp[, 2:ncol(tmp)]),
    max(tmp[, 2:ncol(tmp)])), xlab = "Cycle",
    ylab = "RFU (FAM)",
    main = "Amplification monitored at \n68.5 degrees Celsius (elongation
step)")

apply(tmp[, 2:ncol(tmp)], 2,
      function(x) lines(tmp[1:nrow(tmp),1],x))
```

| C127EGHP | *qPCR Experiment for the Amplification of MLC-2v Using the Roche Light Cycler 1.5* |

Description

Quantitative PCR (qPCR) with a hydrolysis probe (Cy5/BHQ2) and DNA binding dye (EvaGreen) (Mao et al. 2007) was performed in the Roche Light Cycler 1.5 thermo cycler. The cycle-dependent increase of the fluorescence was quantified at the annealing step.

Usage

```
data(C127EGHP)
```

Format

A data frame with 40 observations on the following 66 variables. The first columns ("index") contains index of a sample and second column ("Cycle") contains the number of cycle. Consecutive columns EG1-EG32 contains fluorescence data for Eva Green dye. Consecutive columns HP1-HP32 contains data for hydrolysis probe.

Details

MLC-2v was amplified in the Roche Light Cycler 1.5. The the change of fluorescence was simultaneously monitored for the Hydrolysis probe of MLC-2v and EvaGreen. The primer sequences for MLC-2v were taken from Roediger et al. (2013). A 10 micro L qPCR reaction was composed of 250 nM primer (forward and reverse), qPCR Mix (according to the manufactures recommendations), 1 micro L template (MLC-2v amplification product), 60 nM hydrolysis probe probe for MLC-2v. EvaGreen was used at 0.5 x final. During the amplification was monitored 59.5 degrees Celsius.

Source

Claudia Deutschmann & Stefan Roediger, BTU Cottbus - Senftenberg, Senftenberg, Germany

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Mao, F., Leung, W.-Y., Xin, X., 2007. Characterization of EvaGreen and the implication of its physicochemical properties for qPCR applications. *BMC Biotechnol.* 7, 76.

Examples

```
str(C127EGHP)
data(C127EGHP)
tmp <- C127EGHP

default.par <- par(no.readonly = TRUE)
par(mfrow = c(2,1))
plot(NA, NA, xlim = c(1,40), ylim = c(0,10), xlab = "Cycle",
     ylab = "Fluorescence", main = "MLC-2v qPCR - EvaGreen")
  for (i in 3:34) {
    points(tmp[, 2], tmp[, i], type = "l", col = i)
  }

plot(NA, NA, xlim = c(1,40), ylim = c(0,10), xlab = "Cycle",
     ylab = "Fluorescence", main = "MLC-2v qPCR - Hydrolysis probe")
  for (i in 35:66) {
    points(tmp[, 2], tmp[, i], type = "l", col = i)
  }
par(default.par)
```

C17   *Helicase Dependent Amplification of HPRT1 at Different Tempera-*
                                  tures using the 'VideoScan' Platform 2.0

Description

A Helicase Dependent Amplification (HDA) of HPRT1 (Homo sapiens hypoxanthine phosphoribo-syltransferase 1) was performed at different temperatures in the 'VideoScan' Platform 2.0 (similar to Roediger et al. (2013)). The HDA was performed at 55, 60 and 65 degrees Celsius. The optimal temperature for a HDA is circa 65 degrees Celsius. Lower temperatures will affect the slope and plateau of the HDA amplification curve.

Usage

```
data(C17)
```

Format

A data frame with 125 observations on the following 5 variables.

C17.t Elapsed time during HDA in seconds.

C17.cycle a numeric vector

C17.T55 Time-dependent fluorescence at 55 degrees Celsius

C17.T60 Time-dependent fluorescence at 60 degrees Celsius

C17.T65 Time-dependent fluorescence at 65 degrees Celsius

Details

To perform an isothermal amplification in 'VideoScan' 2.0, standard conditions for the IsoAmp(R) III Universal tHDA Kit (Biohelix) were used. The reaction was composed of 12.5 micro L buffer A containing 1.25 micro L 10x reaction buffer, 150 nM primer (forward and reverse), 0.75 micro L template (synthetic) and A. bidest which was covered with 50 micro L mineral oil. The primer sequences for HPRT1 were taken from Roediger et al. (2013). Preincubation: This mixture was incubated for 2 min at 95 degree. Celsius and immediately placed on ice. 12.5 micro L of reaction buffer B which was composed of 1.25 micro L 10x buffer, 40 mM NaCl, 5 mM MgSO4, 1.75 micro L dNTPs, 0.2 x EvaGreen, 1 micro L Enzyme mix and A. bidest. The fluorescence measurement in 'VideoScan' 'HCU' started directly after adding buffer B at 55, 60 or 65 degrees Celsius and revealed optimal conditions for the amplification when using 60 or 65 degrees Celsius. Temperature profile (after Preincubation): - 60 seconds at 65 degrees Celsius - 11 seconds at 55 degrees Celsius && Measurement

Source

Claudia Deutschmann & Stefan Roediger, BTU Cottbus - Senftenberg, Senftenberg, Germany

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Examples

```
data(C17)
plot(NA, NA, xlim = c(0,5000), ylim = c(0,1.2), xlab = "Time [sec]",
     ylab = "Fluorescence",
     main = "Temperature dependency of HDA amplification reactions")
  points(C17[, 1], C17[, 3], type = "b", col = 1, pch = 20)
  points(C17[, 1], C17[, 4], type = "b", col = 2, pch = 20)
  points(C17[, 1], C17[, 5], type = "b", col = 3, pch = 20)
legend(2000, 0.4, c("55 degrees Celsius", "60 degrees Celsius",
       "65 degrees Celsius"),
col = c(1,2,3), pch = rep(20,3))
```

C316.amp   *qPCR Experiment for the Amplification of adk Using the Bio-Rad iQ5 thermo cycler*

Description

A quantitative real-time PCR of adk was performed.

Usage

```
data("C316.amp")
```

Format

A data frame with 40 observations on the following 97 variables. The first column ("Cycle") contains the number of cycles and consecutive columns contain the replicates ("A01" to "H12").

Details

adk was amplified in the Bio-Rad iQ5. The change of fluorescence was simultaneously monitored (EvaGreen, Mao et al. 2007). The primer sequences for adk were taken from this study.

gDNA: 28.43 ng/microL DNA concentration, 260/280 ratio= 1.96

adk fw: CTCAGGCTCAGTTCATCATGGA adk rv: AGTTTGCCAGCATCCATAATGTC

PCR conditions: 10 minutes at 95 degrees Celsius 40 x 30 seconds at 95 degrees Celsius 45 seconds at 59 degrees Celsius 45 seconds at 68 degrees Celsius

Source

Stefan Roediger, Claudia Deutschmann, Claudia Zelck (BTU Cottbus - Senftenberg)

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Mao, F., Leung, W.-Y., Xin, X., 2007. Characterization of EvaGreen and the implication of its physicochemical properties for qPCR applications. *BMC Biotechnol*. 7, 76.

Examples

```
data(C316.amp)
str(C316.amp)
```

| C316.melt.hr | *Melting Curves (resolution: 0.2 degrees Celsius / step) for the Gene adk Using the Bio-Rad iQ5 thermo cycler* |

Description

A melting curve for adk was performed.

Usage

```
data("C316.melt.hr")
```

Format

A data frame with 40 observations on the following 97 variables. The first column ("T") contains the temperature (resolution: 0.2 degrees Celsius / step) and consecutive columns contain the replicates ("A01" to "H12").

Details

adk was amplified in the Bio-Rad iQ5. After PCR, the temperature-dependent change of fluorescence was simultaneously monitored (EvaGreen, Mao et al. 2007). The primer sequences for adk were taken from this study.

adk fw: CTCAGGCTCAGTTCATCATGGA adk rv: AGTTTGCCAGCATCCATAATGTC

PCR conditions: `C316.amp`

Source

Stefan Roediger, Claudia Deutschmann, Claudia Zelck (BTU Cottbus - Senftenberg)

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Mao, F., Leung, W.-Y., Xin, X., 2007. Characterization of EvaGreen and the implication of its physicochemical properties for qPCR applications. *BMC Biotechnol*. 7, 76.

Examples

```
data(C316.melt.hr)
str(C316.melt.hr)
```

C316.melt.lr	*Melting Curves (resolution: 0.5 degrees Celsius / step) for the Gene adk Using the Bio-Rad iQ5 thermo cycler*

Description

A melting curve for adk was performed.

Usage

```
data("C316.melt.lr")
```

Format

A data frame with 40 observations on the following 97 variables. The first column ("T") contains the temperature (resolution: 0.5 degrees Celsius / step) and consecutive columns contain the replicates ("A01" to "H12").

Details

adk was amplified in the Bio-Rad iQ5. After PCR, the temperature-dependent change of fluorescence was simultaneously monitored (EvaGreen, Mao et al. 2007). The primer sequences for adk were taken from this study.

adk fw: CTCAGGCTCAGTTCATCATGGA adk rv: AGTTTGCCAGCATCCATAATGTC

PCR conditions: `C316.amp`

Source

Stefan Roediger, Claudia Deutschmann, Claudia Zelck (BTU Cottbus - Senftenberg)

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Mao, F., Leung, W.-Y., Xin, X., 2007. Characterization of EvaGreen and the implication of its physicochemical properties for qPCR applications. *BMC Biotechnol*. 7, 76.

Examples

```
data(C316.melt.lr)
str(C316.melt.lr)
```

| C317.amp | *qPCR Experiment for the Amplification of adk Using the Bio-Rad CFX96 thermo cycler* |

Description

A quantitative real-time PCR of adk was performed.

Usage

```
data("C317.amp")
```

Format

A data frame with 40 observations on the following 97 variables. The first column ("Cycle") contains the number of cycles and consecutive columns contain the replicates ("A01" to "H12").

Details

adk was amplified in the Bio-Rad CFX96. The change of fluorescence was simultaneously monitored (EvaGreen, Mao et al. 2007). The primer sequences for adk were taken from this study.

gDNA: 28.43 ng/microL DNA concentration, 260/280 ratio= 1.96

adk fw: CTCAGGCTCAGTTCATCATGGA adk rv: AGTTTGCCAGCATCCATAATGTC

PCR conditions: 10 minutes at 95 degrees Celsius 40 x 30 seconds at 95 degrees Celsius 45 seconds at 59 degrees Celsius 45 seconds at 68 degrees Celsius

Source

Stefan Roediger, Claudia Deutschmann, Claudia Zelck (BTU Cottbus - Senftenberg)

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Mao, F., Leung, W.-Y., Xin, X., 2007. Characterization of EvaGreen and the implication of its physicochemical properties for qPCR applications. *BMC Biotechnol*. 7, 76.

Examples

```
data(C317.amp)
str(C317.amp)
```

C317.melt.hr   *Melting Curves (resolution: 0.1 degrees Celsius / step) for the Gene adk Using the Bio-Rad CFX96 thermo cycler*

Description

A melting curve for adk was performed.

Usage

```
data("C317.melt.hr")
```

Format

A data frame with 40 observations on the following 97 variables. The first column ("Temperature") contains the temperature (resolution: 0.1 degrees Celsius / step) and consecutive columns contain the replicates ("A1" to "H12").

Details

adk was amplified in the Bio-Rad CFX96. After PCR, the temperature-dependent change of fluorescence was simultaneously monitored (EvaGreen, Mao et al. 2007). The primer sequences for adk were taken from this study.

adk fw: CTCAGGCTCAGTTCATCATGGA adk rv: AGTTTGCCAGCATCCATAATGTC

PCR conditions: `C317.amp`

Source

Stefan Roediger, Claudia Deutschmann, Claudia Zelck (BTU Cottbus - Senftenberg)

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Mao, F., Leung, W.-Y., Xin, X., 2007. Characterization of EvaGreen and the implication of its physicochemical properties for qPCR applications. *BMC Biotechnol*. 7, 76.

Examples

```
data(C317.melt.hr)
str(C317.melt.hr)
```

C317.melt.lr	*Melting Curves (resolution: 0.5 degrees Celsius / step) for the Gene adk Using the Bio-Rad CFX96 thermo cycler*

Description

A melting curve for adk was performed.

Usage

```
data("C317.melt.lr")
```

Format

A data frame with 40 observations on the following 97 variables. The first column ("Temperature") contains the temperature (resolution: 0.5 degrees Celsius / step) and consecutive columns contain the replicates ("A1" to "H12").

Details

adk was amplified in the Bio-Rad CFX96. After PCR, the temperature-dependent change of fluorescence was simultaneously monitored (EvaGreen, Mao et al. 2007). The primer sequences for adk were taken from this study.

adk fw: CTCAGGCTCAGTTCATCATGGA adk rv: AGTTTGCCAGCATCCATAATGTC

PCR conditions: `C317.amp`

Source

Stefan Roediger, Claudia Deutschmann, Claudia Zelck (BTU Cottbus - Senftenberg)

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Mao, F., Leung, W.-Y., Xin, X., 2007. Characterization of EvaGreen and the implication of its physicochemical properties for qPCR applications. *BMC Biotechnol*. 7, 76.

Examples

```
data(C317.melt.lr)
str(C317.melt.lr)
```

C54	*qPCR Experiment for the amplification of MLC-2v using the 'VideoScan' heating/cooling-unit*

Description

qPCR Experiment for the amplification of MLC-2v using the 'VideoScan' heating/cooling-unit

Usage

```
data(C54)
```

Format

A data frame with 56 observations on the following 4 variables.

Cycle Cycle number

D1 Stock concentration of input cDNA

D2 1/10 diluted stock cDNA

D3 1/100 diluted stock cDNA

Details

The aim was to amplify MLC-2v in the 'VideoScan' and to monitor with a hydrolysis probe for MLC-2v. The primer sequences for MLC-2v were taken from Roediger et al. (2013). The amplification was detected in solution of the '1 HCU' (see Roediger et al. 2013 for details). A 20 micro L PCR reaction was composed of 250 nM primer (forward and reverse), 1x Maxima Probe qPCR Master Mix (Fermentas), 1 micro L template (MLC-2v amplification product in different dilutions), 50 nM hydrolysis probe probe for MLC-2v and A. bidest. During the amplification, fluorescence was measured at 59.5 degree Celsius. The Cy5 channel was used to monitor the MLC-2v specific hydrolysis probe. Input stock cDNA was used undiluted (D1). D2 was 1/1000 and D3 1/1000000 diluted in A. bidest. The D1, D2, and D3 have different numbers measure points and D2 contains a missing value at cycle 37.

Source

Stefan Roediger, Claudia Deutschmann

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Examples

```
data(C54)
str(C54)
plot(NA, NA, xlim = c(0,56), ylim = c(0, 0.7), xlab = "Cycle",
     ylab = "refMFI")
apply(C54[, c(2:4)], 2, function(x) lines(C54[, 1], x))
```

C60.amp   *qPCR Experiment for the Amplification of MLC-2v and Vimentin (as decadic dilutions) Using the Roche Light Cycler 1.5*

Description

Dilution experiment and metling curve (`C60.melt`) for the human genes MLC-2v and Vimentin (see Roediger et al. 2013) using the Roch Light Cycler 1.5.

Usage

```
data(C60.amp)
```

Format

A data frame with 45 observations on the following 33 variables.

`Index` Index of Cycles

`Vim.0.1` Vimentin water control

`Vim.0.2` Vimentin water control

`Vim.1.1` Vimentin 10^-3 diluted

`Vim.1.2` Vimentin 10^-3 diluted

`Vim.2.1` Vimentin 10^-4 diluted

`Vim.2.2` Vimentin 10^-4 diluted

`Vim.3.1` Vimentin 10^-5 diluted

`Vim.3.2` Vimentin 10^-5 diluted

`Vim.4.1` Vimentin 10^-6 diluted

`Vim.4.2` Vimentin 10^-6 diluted

`Vim.5.1` Vimentin 10^-7 diluted

`Vim.5.2` Vimentin 10^-7 diluted

`Vim.6.1` Vimentin 10^-8 diluted

`Vim.6.2` Vimentin 10^-8 diluted

`Vim.7.1` Vimentin 10^-9 diluted

`Vim.7.2` Vimentin 10^-9 diluted

`MLC2v.1.1` MLC-2v 10^-3 diluted

`MLC2v.1.2` MLC-2v 10^-3 diluted

`MLC2v.2.1` MLC-2v 10^-4 diluted

`MLC2v.2.2` MLC-2v 10^-4 diluted

`MLC2v.3.1` MLC-2v 10^-5 diluted

`MLC2v.3.2` MLC-2v 10^-5 diluted

`MLC2v.4.1` MLC-2v 10^-6 diluted

`MLC2v.4.2` MLC-2v 10^-6 diluted

`MLC2v.5.1` MLC-2v 10^-7 diluted

`MLC2v.5.2` MLC-2v 10^-7 diluted

`MLC2v.6.1` MLC-2v 10^-8 diluted

`MLC2v.6.2` MLC-2v 10^-8 diluted

`MLC2v.7.1` MLC-2v 10^-9 diluted

`MLC2v.7.2` MLC-2v 10^-9 diluted

`MLC2v.0.1` MLC-2v water control

`MLC2v.0.2` MLC-2v water control

Details

MLC-2v and Vimentin were amplified in the Roche Light Cycler 1.5. Decadic dilutions of the input cDNA were prepared. The change of fluorescence was simultaneously monitored with EvaGreen. The primer sequences for MLC-2v were taken from Roediger et al. (2013). A 10 micro L qPCR reaction was composed of 250 nM primer (forward and reverse), Roche qPCR Master-Mix (according to the manufactures recommendations) and 1 micro L input DNA. EvaGreen was used at 1x final. During the amplification was monitored 58 degrees Celsius. Temperature profile:

95 deg C for 8 minutes 40 x 95 deg C for 10 sec 58 deg C for 15 sec 69 deg C for 25 sec

Source

Stefan Roediger, Claudia Deutschmann (BTU Cottbus - Senftenberg)

References

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Mao, F., Leung, W.-Y., Xin, X., 2007. Characterization of EvaGreen and the implication of its physicochemical properties for qPCR applications. *BMC Biotechnol*. 7, 76.

Examples

```
str(C60.amp)
matplot(C60.amp[, 1], C60.amp[, c(3,5,7)], type = "l",
        xlab = "Cycle", ylab = "RFU")
```

C60.melt   *Melt Curves MLC-2v and Vimentin for the qPCR Experiment C60.amp*
                                  Using the Roche Light Cycler 1.5

Description

Melting curves were continuously monitored with the Light Cycler 1.5 (Roche). For details on sample preparation refer to C60.amp.

Usage

```
data(C60.melt)
```

Format

A data frame with 128 observations on the following 65 variables. Refer to C60.amp for details of the specific samples.

`Index` Index of elements

`Vim.0.1.T` Temperature

`Vim.0.1.F` Fluorescence

`Vim.0.2.T` Temperature

`Vim.0.2.F` Fluorescence

`Vim.1.1.T` Temperature

`Vim.1.1.F` Fluorescence

`Vim.1.2.T` Temperature

`Vim.1.2.F` Fluorescence

`Vim.2.1.T` Temperature

`Vim.2.1.F` Fluorescence

`Vim.2.2.T` Temperature

`Vim.2.2.F` Fluorescence

`Vim.3.1.T` Temperature

`Vim.3.1.F` Fluorescence

`Vim.3.2.T` Temperature

`Vim.3.2.F` Fluorescence

`Vim.4.1.T` Temperature

`Vim.4.1.F` Fluorescence

`Vim.4.2.T` Temperature

`Vim.4.2.F` Fluorescence

`Vim.5.1.T` Temperature

`Vim.5.1.F` Fluorescence

`Vim.5.2.T` Temperature

`Vim.5.2.F` Fluorescence

`Vim.6.1.T` Temperature

`Vim.6.1.F` Fluorescence

`Vim.6.2.T` Temperature

`Vim.6.2.F` Fluorescence

`Vim.7.1.T` Temperature

`Vim.7.1.F` Fluorescence

`Vim.7.2.T` Temperature

`Vim.7.2.F` Fluorescence

`MLC2v.1.1.T` Temperature

`MLC2v.1.1.F` Fluorescence

`MLC2v.1.2.T` Temperature

`MLC2v.1.2.F` Fluorescence

`MLC2v.2.1.T` Temperature

`MLC2v.2.1.F` Fluorescence

`MLC2v.2.2.T` Temperature

`MLC2v.2.2.F` Fluorescence

`MLC2v.3.1.T` Temperature

`MLC2v.3.1.F` Fluorescence

`MLC2v.3.2.T` Temperature

`MLC2v.3.2.F` Fluorescence

`MLC2v.4.1.T` Temperature

`MLC2v.4.1.F` Fluorescence

`MLC2v.4.2.T` Temperature

`MLC2v.4.2.F` Fluorescence

`MLC2v.5.1.T` Temperature

`MLC2v.5.1.F` Fluorescence

`MLC2v.5.2.T` Temperature

`MLC2v.5.2.F` Fluorescence

`MLC2v.6.1.T` Temperature

`MLC2v.6.1.F` Fluorescence

`MLC2v.6.2.T` Temperature

`MLC2v.6.2.F` Fluorescence

`MLC2v.7.1.T` Temperature

`MLC2v.7.1.F` Fluorescence

`MLC2v.7.2.T` Temperature

`MLC2v.7.2.F` Fluorescence

`MLC2v.0.1.T` Temperature

`MLC2v.0.1.F` Fluorescence

`MLC2v.0.2.T` Temperature

`MLC2v.0.2.F` Fluorescence

Details

Melting curves were continuously monitored with the Light Cycler 1.5 (Roche).

Source

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References

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Mao, F., Leung, W.-Y., Xin, X., 2007. Characterization of EvaGreen and the implication of its physicochemical properties for qPCR applications. *BMC Biotechnol*. 7, 76.

Examples

```
str(C60.melt)
matplot(C60.melt[, c(2,4,6)], C60.melt[, c(3,5,7)], type = "l",
        xlab = "Temperature", ylab = "RFU")
```

C67	*Helicase Dependent Amplification of HPRT1 with different input DNA quantities using the Bio-Rad iQ5 thermo cycler*

Description

A Helicase Dependent Amplification (HDA) of HPRT1 (Homo sapiens hypoxanthine phosphoribosyltransferase 1) was performed at three different input DNA quantities using the Bio-Rad iQ5 thermo cycler. The HDA was performed at 65 degrees Celsius. The optimal temperature for a HDA is circa 65 degrees Celsius. Lower temperatures will affect the slope and plateau of the HDA amplification curve.

Usage

```
data(C67)
```

Format

A data frame with 43 observations on the following 6 variables.

Cycles.C67 a numeric vector containing the cycle numbers

t.C67 a numeric vector containing the time elapsed between the cycles. The time was calculated by the cycle duration of one iQ5 thermocycler step (71 seconds / step).

D1 Dilution 1.

D2 Dilution 2.

D3 Dilution 3.

D4 Dilution 4.

Details

To perform an isothermal amplification in 'VideoScan', standard conditions for the IsoAmp(R) III Universal tHDA Kit (Biohelix) were used. The reaction was composed of 12.5 micro L buffer A containing 1.25 micro L 10x reaction buffer, 150 nM primer (forward and reverse), 0.75 micro L template (synthetic) and A. bidest which was covered with 50 micro L mineral oil. The primer sequences for HPRT1 were taken from Roediger et al. (2013). Preincubation: This mixture was incubated for 2 min at 95 degree. Celsius and immediately placed on ice. 12.5 micro L of reaction buffer B which was composed of 1.25 micro L 10x buffer, 40 mM NaCl, 5 mM MgSO4, 1.75 micro L dNTPs, 0.2 x EvaGreen, 1 micro L Enzyme mix and A. bidest. The fluorescence measurement started directly after adding buffer B and the preincubation step. Temperature profile if the iQ5 thermo cycler (after Preincubation): - 60 seconds at 65 degrees Celsius - 11 seconds at 55 degrees Celsius && Measurement

Source

Claudia Deutschmann & Stefan Roediger, BTU Cottbus - Senftenberg, Senftenberg, Germany

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology.* 133:33–74, 2013.

Examples

```
data(C67)
matplot(C67[, -c(1,2)], type = "l", xlab = "Time [sec]", ylab = "RFU")
```

C81   *Helicase Dependent Amplification of pCNG1 using the 'VideoScan'*
                             Platform

Description

A Helicase Dependent Amplification (HDA) of pCNG1 was performed. The 'VideoScan' Platform (Roediger et al. (2013)) was used to monitor the amplification. The HDA was performed at 65 degrees Celsius. Two concentrations of input DNA were used.

Usage

```
data(C81)
```

Format

A data frame with 351 observations on the following 5 variables.

Cycle Cycles HDA measurements.

t.D1 Dilution 1, elapsed time during HDA in seconds.

MFI.D1 Dilution 1, fluorescence.

t.D2 Dilution 2, elapsed time during HDA in seconds.

MFI.D2 Dilution 2, fluorescence.

Details

To perform an isothermal amplification in 'VideoScan', standard conditions for the IsoAmp(R) III Universal tHDA Kit (Biohelix) were used. The reaction was composed of reaction mix A)10 micro L A. bidest, 1.25 micro L 10xbuffer, 0.75 micro L primer(150 nM final), 0.5 micro L template plasmid. Preincubation: This mixture was incubated for 2 min at 95 degree. Celsius and immediately placed on ice. Reaction mix B) 5 micro L A. bidest., 1.25 micro L 10x buffer, 2 micro L NaCl, 1.25 micro L MgSO4, 1.75 micro L dNTPs, 0.25 micro L EvaGreen, 1 micro L enzyme mix. The mix was covered with 50 micro L mineral oil. The fluorescence measurement in 'VideoScan' 'HCU' started directly after adding buffer B at 65 degrees Celsius. A 1x (D1) and a 1:10 dilution (D2) were tested. Temperature profile (after Preincubation): - 60 seconds at 65 degrees Celsius - 11 seconds at 55 degrees Celsius && Measurement

Source

Claudia Deutschmann & Stefan Roediger, BTU Cottbus - Senftenberg, Senftenberg, Germany

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Examples

```
data(C81)
# First example
# Comparison of Lowess, Moving average and splines to smooth amplification curve
# data of
# HDA for pCNG1.

plot(NA, NA, xlim = c(0, 120), ylim = c(0, 1.2), xlab = "Time [min]",
     ylab = "Fluorescence", main = "VideScan HCU HDA amplification - Raw data")
  points(C81[, 2]/60, C81[, 3], type = "b", col = 1, pch = 20)
  points(C81[, 4]/60, C81[, 5], type = "b", col = 2, pch = 20)
legend(2000, 0.4, c("D1", "D2"), col = c(1,2), pch = rep(20,2))
```

C85	*Helicase Dependent Amplification of Vimentin using the 'VideoScan' Platform*

Description

A Helicase Dependent Amplification (HDA) of Vimentin (Vim) was performed. The 'VideoScan' Platform (Roediger et al. (2013)) was used to monitor the amplification. The HDA was performed at 65 degrees Celsius. Three concentrations of input DNA (D1, D2, D3) were used.

Usage

```
data(C85)
```

Format

A data frame with 301 observations on the following 5 variables.

Cycle Cycles HDA measurements.

t.D1 Dilution 1, elapsed time during HDA in seconds.

MFI.D1 Dilution 1, fluorescence.

t.D2 Dilution 2, elapsed time during HDA in seconds.

MFI.D2 Dilution 2, fluorescence.

t.D3 Dilution 3, elapsed time during HDA in seconds.

MFI.D3 Dilution 3, fluorescence.

Details

To perform an isothermal amplification in 'VideoScan', standard conditions for the IsoAmp(R) III Universal tHDA Kit (Biohelix) were used. Primers and templates are described in Roediger et al. (2013). The reaction was composed of reaction mix A)10 micro L A. bidest, 1.25 micro L 10xbuffer, 0.75 micro L primer(150nM final), 0.5 micro L template plasmid. Preincubation: This mixture was incubated for 2 min at 95 degree. Celsius and immediately placed on ice. Reaction mix B) 5 micro L A. bidest., 1.25 micro L 10x buffer, 2 micro L NaCl, 1.25 micro L MgSO4, 1.75 micro L dNTPs, 0.25 micro L EvaGreen, 1 micro L enzyme mix. The mix was covered with 50 micro L mineral oil. The fluorescence measurement in 'VideoScan' 'HCU' started directly after adding buffer B at 65 degrees Celsius. A 1x (D1), a 1:10 dilution (D2) and a 1:100 (D3) dilution were tested. Temperature profile (after Preincubation): - 60 seconds at 65 degrees Celsius - 11 seconds at 55 degrees Celsius && Measurement

Source

Claudia Deutschmann & Stefan Roediger, BTU Cottbus - Senftenberg, Senftenberg, Germany

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Examples

```
data(C85)
# First example
plot(NA, NA, xlim = c(0,85), ylim = c(0,1), xlab = "Time [min]",
     ylab = "Fluorescence", main = "HDA amplification")
points(C85[, 2]/60, C85[, 3], type = "b", col = 1, pch = 20)
points(C85[, 4]/60, C85[, 5], type = "b", col = 2, pch = 20)
points(C85[, 6]/60, C85[, 7], type = "b", col = 3, pch = 20)
legend(40, 0.5, c("D1, 1x", "D2, 1:10", "D3, 1:100"), col = c(1:3),
pch = rep(20,3))

# Second example
plot(NA, NA, xlim = c(0,30), ylim = c(0,0.8), xlab = "Time [min]",
     ylab = "Fluorescence", main = "HDA amplification")
points(C85[, 2]/60, C85[, 3], type = "b", col = 1, pch = 20)
points(C85[, 2]/60, smoother(C85[, 2]/60, C85[, 3],
     method = list("savgol")), type = "b", col = 2, pch = 20)
points(C85[, 2]/60, smoother(C85[, 2]/60, C85[, 3],
     method = list("smooth")), type = "b", col = 3, pch = 20)
points(C85[, 2]/60, smoother(C85[, 2]/60, C85[, 3],
     method = list("mova")), type = "b", col = 4, pch = 20)

legend(1, 0.8, c("D1, raw", "D1, savgol", "D1, smooth", "D1, mova"),
col = c(1:4), pch = rep(20,4))

# Third example
# Comparison of Lowess, Moving average and splines to smooth amplification
# curve data of
# a HDA using the 'VideoScan' 'HCU' for amplification and monitoring.

xrange <- 2:2400
plot(NA, NA, xlim = c(0,85), ylim = c(0.4, 0.8), xlab = "Time [min]",
     ylab = "RFI", main = "Raw data")
points(C85[, 2]/60, C85[, 3], type = "b", col = 1, pch = 20)
points(C85[, 4]/60, C85[, 5], type = "b", col = 2, pch = 20)
points(C85[, 6]/60, C85[, 7], type = "b", col = 3, pch = 20)
legend("topleft", c("D1, 1x", "D2, 1:10", "D3, 1:100"), col = c(1:3),
pch = rep(20,3))

mtext("A", cex = 2, side = 3, adj = 0, font = 2)

plot(NA, NA, xlim = c(0,40), ylim = c(-0.05, 0.3), xlab = "Time [min]",
     ylab = "RFI", main = "Moving average")
movaww <- seq(1,17,4)
```

```
for (i in 1:length(movaww)) {
  for (j in c(2,4,6)) {
    tmp <- data.frame(na.omit(C85[xrange, j])/60, na.omit(C85[xrange, j + 1]))
    tmp.out <- smoother(tmp[, 1], tmp[, 2], method = list(mova = list(movaww = movaww[i])),
bg.outliers = TRUE)
    lines(data.frame(tmp[, 1], tmp.out), type = "l", pch = 20, cex = 0.5,
  col = i)
    }
}
mtext("B", cex = 2, side = 3, adj = 0, font = 2)
legend("topleft", c("D1, 1x", "D2, 1:10", "D3, 1:100"), col = c(1:3),
pch = rep(20,3))
legend("bottomright", 6, paste("movaww : ", movaww), pch = 20, lwd = 2,
col = 1:length(movaww))

plot(NA, NA, xlim = c(0,40), ylim = c(-0.05, 0.3), xlab = "Time [min]",
     ylab = "RFI", main = "Cubic Spline")
df.fact <- seq(0.5,0.9,0.1)
for (i in 1:length(df.fact)) {
  for (j in c(2,4,6)) {
    tmp <- data.frame(na.omit(C85[xrange, j])/60, na.omit(C85[xrange, j + 1]))
    tmp.out <- smoother(tmp[, 1], tmp[, 2],
  method = list(smooth = list(df.fact = df.fact[i])),
  bg.outliers = TRUE)

    lines(data.frame(tmp[, 1], tmp.out), type = "l", pch = 20,
    cex = 0.5, col = i)
    }
}

mtext("C", cex = 2, side = 3, adj = 0, font = 2)
legend("topleft", c("D1, 1x", "D2, 1:10", "D3, 1:100"), col = c(1:3),
pch = rep(20,3))
legend("bottomright", paste("df.fact : ", df.fact), pch = 20, lwd = 2,
col = 1:length(df.fact))

plot(NA, NA, xlim = c(0,40), ylim = c(-0.05, 0.3), xlab = "Time [min]",
ylab = "RFI", main = "Lowess")
f <- seq(0.01,0.2,0.04)
for (i in 1:length(f)) {
  for (j in c(2,4,6)) {
    tmp <- data.frame(na.omit(C85[xrange, j])/60, na.omit(C85[xrange, j + 1]))
    tmp.out <- smoother(tmp[, 1], tmp[, 2], method = list(lowess = list(f = f[i])),
  bg.outliers = TRUE)

    lines(data.frame(tmp[, 1], tmp.out), type = "l", pch = 20, cex = 0.5,
    col = i)
    }
    }

    mtext("D", cex = 2, side = 3, adj = 0, font = 2)
    legend("topleft", c("D1, 1x", "D2, 1:10", "D3, 1:100"), col = c(1:3),
    pch = rep(20,3))
```

```
      legend("bottomright", paste("f : ", f), pch = 20, lwd = 2, col = 1:length(f))

plot(NA, NA, xlim = c(0,40), ylim = c(-0.05, 0.3), xlab = "Time [min]",
ylab = "RFI", main = "Friedman's\n''super smoother''")
span <- seq(0.01,0.05,0.01)
for (i in 1:length(span)) {
  for (j in c(2,4,6)) {
    tmp <- data.frame(na.omit(C85[xrange, j])/60, na.omit(C85[xrange, j + 1]))
    tmp.out <- smoother(tmp[, 1], tmp[, 2], method = list(supsmu = list(span = span[i])),
  bg.outliers = TRUE)

    lines(data.frame(tmp[, 1], tmp.out), type = "l", pch = 20, cex = 0.5,
col = i)
    }
    }

    mtext("E", cex = 2, side = 3, adj = 0, font = 2)
    legend("topleft", c("D1, 1x", "D2, 1:10", "D3, 1:100"), col = c(1:3),
pch = rep(20,3))
    legend("bottomright", paste("span : ", span), pch = 20, lwd = 2, col = 1:length(span))

plot(NA, NA, xlim = c(0,40), ylim = c(-0.05, 0.3), xlab = "Time [min]",
ylab = "RFI", main = "Savitzky-Golay")

for (j in c(2,4,6)) {
  tmp <- data.frame(na.omit(C85[xrange, j])/60, na.omit(C85[xrange, j + 1]))
  tmp.out <- smoother(tmp[, 1], tmp[, 2], method = list("savgol"),
bg.outliers = TRUE)

  lines(data.frame(tmp[, 1], tmp.out), type = "l", pch = 20, cex = 0.5,
col = 1)
  }

  mtext("F", cex = 2, side = 3, adj = 0, font = 2)
  legend("bottomright", paste("/ : ", NULL), pch = 20, lwd = 2, col = 1:length(span))
```

---

capillaryPCR	*capillary convective PCR*

Description

The capillary convective PCR (ccPCR) is a modified device of the ccPCR system proposed by Chou et al. 2011.

Usage

```
data(capillaryPCR)
```

Format

A data frame with 1844 observations on the following 10 variables.

t.121205 Elapsed time during amplification

ED.121205 a numeric vector

t.121128 Elapsed time during amplification

ED.121128 a numeric vector

t.121130.1 Elapsed time during amplification

ED.121130.1 a numeric vector

t.121130.2 Elapsed time during amplification

ED.121130.2 a numeric vector

t.121130.3 Elapsed time during amplification

ED.121130.3 a numeric vector

Details

Modified version of the capillary convective tube isothermal heater heater by Chou et al. 2011. As heating system a conventional block heat was used. On the top of the heating block, we placed for the uptake of the capillaries an aluminum block (8 mm height) in which four holes (3.2 mm diameter and 3.0 mm depth with round shaped bottom) were drilled. The capillaries are regular 100 micro L Roche LightCycler(R). These glass capillaries have a round shaped closed bottom (2.3 mm inner diameter and 3.2 mm outer diameter). An "ESE-Log" detector (QIAGEN Lake Constance) was used for the real time fluorescent measurements, which was mounted in a distance of 5-10 mm next to the capillary. The PCR was performed with SYBR(R) Green fluorescent intercalating dye. Thereof the ESE-Log has in one channel the excitation at 470 nm and the detection at 520 nm. The data was recorded by the FL Digital Software (QIAGEN Lake Constance) and the exported text based raw data.

Source

Ralf Himmelreich, IMM, Mainz, Germany

References

Chou, W., Chen, P., Miao Jr, M., Kuo, L., Yeh, S. and Chen, P. (2011). Rapid DNA amplification in a capillary tube by natural convection with a single isothermal heater. Biotech. 50, 52-57.

Examples

```
default.par <- par(no.readonly = TRUE)
# First example
data(capillaryPCR)
plot(NA, NA, xlim = c(0,80), ylim = c(0,1300), xlab = "Time [min]",
     ylab = "Voltage [micro V]", main = "ccPCR - Raw Data")

for (i in c(1,3,5,7)) {
  lines(capillaryPCR[, i], capillaryPCR[, i+1], type = "b", pch = 20)
```

```
}

abline(h = 290, v = c(18, 23, 35))
legend(60,800, c("Run 1", "Run 2", "Run 3", "Control"), pch = 20, lwd = 2)

# Second example
par(mfrow = c(2,1))

type <- c("mova", "spline", "savgol")
plot(NA, NA, xlim = c(0,80), ylim = c(0,1100), xlab = "Time [min]",
     ylab = "Voltage [micro V]", main = "ccPCR with mova,
     spline and savgol")
for (i in 1:3) {
  for (j in c(1,3,5,7)) {
      tmp <- data.frame(na.omit(capillaryPCR[, j]),
na.omit(capillaryPCR[, j+1]))
      tmp.sm <- smoother(tmp[, 1], tmp[, 2], method = list(type[i]))
      lines(data.frame(tmp[, 1], tmp.sm), type = "b", pch = 20, cex = 0.5,
    col = i)
  }
}

abline(h = 200, v = c(17.5, 21.3, 32.9))
legend(0, 1000, c("mova", "spline", "savgol"), pch = 20, lwd = 2,
col = c(1:3))

plot(NA, NA, xlim = c(10,40), ylim = c(50,300), xlab = "Time [min]",
     ylab = "Voltage [micro V]", main = "ccPCR with mova,
     spline and savgol")
for (i in 1:3) {
  for (j in c(1,3,5,7)) {
      tmp <- data.frame(na.omit(capillaryPCR[, j]),
na.omit(capillaryPCR[, j+1]))
      tmp.sm <- smoother(tmp[, 1], tmp[, 2], method = list(type[i]))
      lines(data.frame(tmp[, 1], tmp.sm), type = "b", pch = 20, cex = 0.5,
    col = i)
  }
}
abline(h = 200, v = c(17.5, 21.3, 32.9))
legend(10, 300, c("mova", "spline", "savgol"), pch = 20, lwd = 2,
col = c(1:3))

par(mfrow = c(1,1))

# Third example
method <- c("lowess","mova","savgol","smooth","spline", "supsmu")

plot(NA, NA, xlim = c(0,100), ylim = c(-50,1100), xlab = "Time [min]",
     ylab = "Voltage [micro V]", main = "capillary convective PCR")
for (i in 1:length(method)) {
  for (j in c(1,3,5,7)) {
      tmp <- data.frame(na.omit(capillaryPCR[, j]),
na.omit(capillaryPCR[, j+1]))
```

```
        tmp.sm <- smoother(tmp[, 1], tmp[, 2], method = list(method[i]))
        lines(data.frame(tmp[, 1], tmp.sm), type = "l", pch = 20, cex = 0.5,
    col = i)
  }
}
legend(0,1000, method, pch = 20, lwd = 2, col = 1:length(method))


par(fig = c(0.5,1,0.25,0.8), new = TRUE)
plot(NA, NA, xlim = c(10,40), ylim = c(50,300), xlab = "Time [min]",
     ylab = "Voltage [micro V]", main = "")
for (i in 1:length(method)) {
  for (j in c(1,3,5,7)) {
      tmp <- data.frame(na.omit(capillaryPCR[, j]),
na.omit(capillaryPCR[, j+1]))
      tmp.sm <- smoother(tmp[, 1], tmp[, 2], method = list(method[i]))
      lines(data.frame(tmp[, 1], tmp.sm), type = "l", pch = 20, cex = 0.5,
    col = i)
  }
}
legend(0,1000, method, pch = 20, lwd = 2, col = 1:length(method))

# Fourth example
# Comparison of Lowess, Moving average and splines to smooth amplification
# curve data of
# a capillary convective PCR.

plot(NA, NA, xlim = c(10,40), ylim = c(50, 300), xlab = "Time [min]",
     ylab = "Voltage [micro V]", main = "ccPCR - Moving average")
movaww <- seq(1,17,4)
for (i in 1:length(movaww)) {
  for (j in c(1,3,5,7)) {
    tmp <- data.frame(na.omit(capillaryPCR[, j]),
      na.omit(capillaryPCR[, j+1]))
    tmp.out <- smoother(tmp[, 1], tmp[, 2],
                 method = list(mova = list(movaww = movaww[i])))

    lines(data.frame(tmp[, 1], tmp.out), type = "l", pch = 20,
  cex = 0.5, col = i)
  }
}
text(10,300, "A", cex = 3)
legend(25,200, paste("movaww : ", movaww), pch = 20, lwd = 2,
col = 1:length(movaww))

plot(NA, NA, xlim = c(10,40), ylim = c(50, 300), xlab = "Time [min]",
     ylab = "Voltage [micro V]", main = "ccPCR - Cubic Spline")
df.fact <- seq(0.5,0.9,0.1)
for (i in 1:length(df.fact)) {
  for (j in c(1,3,5,7)) {
    tmp <- data.frame(na.omit(capillaryPCR[, j]),
      na.omit(capillaryPCR[, j+1]))
    tmp.out <- smoother(tmp[, 1], tmp[, 2], method = list(smooth =
```

```
      list(df.fact = df.fact[i])))

      lines(data.frame(tmp[, 1], tmp.out), type = "l", pch = 20,
  cex = 0.5, col = i)
   }
}
text(10,300, "B)", cex = 3)
legend(30,200, paste("df.fact : ", df.fact), pch = 20, lwd = 2,
col = 1:length(df.fact))


plot(NA, NA, xlim = c(10,40), ylim = c(50, 300), xlab = "Time [min]",
     ylab = "Voltage [micro V]", main = "ccPCR - Lowess")
f <- seq(0.01,0.2,0.04)
for (i in 1:length(f)) {
  for (j in c(1,3,5,7)) {
    tmp <- data.frame(na.omit(capillaryPCR[, j]),
      na.omit(capillaryPCR[, j+1]))
    tmp.out <- smoother(tmp[, 1], tmp[, 2], method = list(lowess = list(f = f[i])))

    lines(data.frame(tmp[, 1], tmp.out), type = "l", pch = 20,
  cex = 0.5, col = i)
   }
}
text(10,300, "C)", cex = 3)
legend(30,200,  paste("f : ", f), pch = 20, lwd = 2, col = 1:length(f))

plot(NA, NA, xlim = c(10,40), ylim = c(50, 300), xlab = "Time [min]",
     ylab = "Voltage [micro V]",
     main = "ccPCR - Friedman's ''super smoother''")
span <- seq(0.01,0.05,0.01)
for (i in 1:length(span)) {
  for (j in c(1,3,5,7)) {
    tmp <- data.frame(na.omit(capillaryPCR[, j]),
      na.omit(capillaryPCR[, j+1]))
    tmp.out <- smoother(tmp[, 1], tmp[, 2],
                 method = list(supsmu = list(span = span[i])))

    lines(data.frame(tmp[, 1], tmp.out), type = "l", pch = 20,
  cex = 0.5, col = i)
   }
}
text(10,300, "D)", cex = 3)
legend(25,200,  paste("span : ", f), pch = 20, lwd = 2, col = 1:length(span))

par(default.par)
```

Description

Quantitative PCR (qPCR) with a hydrolysis probe and DNA binding dye (EvaGreen) (Mao et al. 2007) was performed in the 'VideoScan' heating cooling unit. The cycle-dependent increase of the fluorescence was quantified at three different temperatures in order to estimate temperature dependent effects.

Usage

```
data(CD74)
```

Format

A data frame with 60 observations on the following 19 variables. refMFI (referenced Mean Fluorescence Intensity), fluorescence. Dilution A, 1x; Dilution B, 1:10; Dilution B, 1:100.

Cycle PCR cycles

EG.30.A refMFI at 30 degrees Celsius, EvaGreen, Dilution A

EG.59.5.A refMFI at 59.5 degrees Celsius, EvaGreen, Dilution A

EG.68.5.A refMFI at 68.5 degrees Celsius, EvaGreen, Dilution A

HP.30.A refMFI at 30 degrees Celsius, hydrolysis probe, Dilution A

HP.59.5.A refMFI at 59.5 degrees Celsius, hydrolysis probe, Dilution A

HP.68.5.A refMFI at 68.5 degrees Celsius, hydrolysis probe, Dilution A

EG.30.B refMFI at 30 degrees Celsius, EvaGreen, Dilution B

EG.59.5.B refMFI at 59.5 degrees Celsius, EvaGreen, Dilution B

EG.68.5.B refMFI at 68.5 degrees Celsius, EvaGreen, Dilution B

HP.30.B refMFI at 30 degrees Celsius, hydrolysis probe, Dilution B

HP.59.5.B refMFI at 59.5 degrees Celsius, hydrolysis probe, Dilution B

HP.68.5.B refMFI at 68.5 degrees Celsius, hydrolysis probe, Dilution B

EG.30.C refMFI at 30 degrees Celsius, EvaGreen, Dilution C

EG.59.5.C refMFI at 59.5 degrees Celsius, EvaGreen, Dilution C

EG.68.5.C refMFI at 68.5 degrees Celsius, EvaGreen, Dilution C

HP.30.C refMFI at 30 degrees Celsius, hydrolysis probe, Dilution C

HP.59.5.C refMFI at 59.5 degrees Celsius, hydrolysis probe, Dilution C

HP.68.5.C refMFI at 68.5 degrees Celsius, hydrolysis probe, Dilution C

Details

The aim was to amplify MLC-2v in the 'VideoScan' platform while the intercalating dye EvaGreen and a hydrolysis probe for MLC-2v were used simultaneously. The primer sequences for MLC-2v were taken from Roediger et al. (2013). The amplification was detected in solution of the '1 HCU' (see Roediger et al. 2013 for details). A 20 micro L PCR reaction was composed of 500 nM primer (forward and reverse), 1x Maxima Probe qPCR Master Mix (Fermentas), 1 micro L template (MLC-2v amplification product in different dilutions), 50 nM hydrolysis probe probe for

MLC-2v, 0.5 x EvaGreen and A. bidest. During the amplification, fluorescence was measured at 3 different temperatures, at 59.5 degrees Celsius the annealing temperature, at 68.5 degree Celsius the elongation temperature and at 30 degrees Celsius. The FAM channel was used to monitor EvaGreen and the Cy5 channel to monitor the MLC-2v specific hydrolysis probe.

Source

Claudia Deutschmann & Stefan Roediger, BTU Cottbus - Senftenberg, Senftenberg, Germany

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Mao, F., Leung, W.-Y., Xin, X., 2007. Characterization of EvaGreen and the implication of its physicochemical properties for qPCR applications. *BMC Biotechnol*. 7, 76.

Examples

```
# First example
# Comparison of smoothers and filter on amplification curves
# Amplification curves were measured at three temperature (30,
# 59.5, 68.5 degrees Celsius) using the 'VideoScan' 'HCU' (see
# Roediger et al. 2013 for details). MLC-2v was amplified.
# The change of fluorescence was monitored by the intercalating
# dye EvaGreen and hydrolysis probes.

data(CD74)
default.par <- par(no.readonly = TRUE)
par(mfrow = c(1,2))
plot(NA, NA, xlim = c(1,30), ylim = c(0,2), xlab = ”Cycle”,
     ylab = ”MFI”, main = ”VideoScan HCU\nRaw Data”)
lim <- 1:30
for (j in c(2:4)) {
  for (i in seq(j,19,6)) {
    lines(CD74[lim, 1], CD74[lim, i], col = 1)
  }
}
for (j in c(5:7)) {
  for (i in seq(j,19,6)) {
    lines(CD74[lim, 1], CD74[lim, i], col = 2)
  }
}

plot(NA, NA, xlim = c(1,30), ylim = c(0,1.8), xlab = ”Cycle”,
     ylab = ”MFI”, main = ”VideoScan HCU\nSmoothed Data”)
lim <- 1:30
for (j in c(2:4)) {
  for (i in seq(j,19,6)) {
```

```
    lines(CD74[lim, 1], smoother(CD74[lim, 1], CD74[lim, i], trans = TRUE),
  col = 1)
  }
}
for (j in c(5:7)) {
  for (i in seq(j,19,6)) {
    lines(CD74[lim, 1], smoother(CD74[lim, 1], CD74[lim, i], trans = TRUE),
  col = 2)
  }
}
par(default.par)
```

CD75   *Helicase Dependent Amplification in the 'VideoScan' 'HCU'*

Description

Helicase Dependent Amplification in the 'VideoScan' 'HCU' of HPRT1 (Homo sapiens hypoxan-
thine phosphoribosyltransferase 1)

Usage

```
data(CD75)
```

Format

A data frame with 93 observations on the following 6 variables. The data frame contains three
replicates of a HDA for HPRT1.

CD75.t1 Elapsed time during HDA in seconds.

CD75.F1 Time-dependent fluorescence during HDA.

CD75.t2 Elapsed time during HDA in seconds.a numeric vector

CD75.F2 Time-dependent fluorescence during HDA

CD75.t3 Elapsed time during HDA in seconds.

CD75.F3 Time-dependent fluorescence during HDA.

Details

To perform an isothermal amplification in 'VideoScan', standard conditions for the IsoAmp(R) III
Universal tHDA Kit (Biohelix) were used. The reaction was composed of 12.5 micro L buffer A
containing 1.25 micro L 10x reaction buffer, 150 nM primer (forward and reverse), 0.75 micro L
template (synthetic) and A. bidest which was covered with 50 micro L mineral oil. The primer
sequences for HPRT1 were taken from Roediger et al. (2013). Preincubation: This mixture was
incubated for 2 min at 95 degree. Celsius and immediately placed on ice. 12.5 micro L of reaction
buffer B which was composed of 1.25 micro L 10x buffer, 40 mM NaCl, 5 mM MgSO4, 1.75 micro
L dNTPs, 0.2 x EvaGreen, 1 micro L Enzyme mix and A. bidest. The fluorescence measurement
in 'VideoScan' 'HCU' started directly after adding buffer B at 55, 60 or 65 degrees Celsius and

revealed optimal conditions for the amplification when using 60 or 65 degree Celsius. Temperature profile (after Preincubation): - 60 seconds at 65 degrees Celsius - 11 seconds at 55 degrees Celsius && Measurement

Source

Claudia Deutschmann & Stefan Roediger, BTU Cottbus - Senftenberg, Senftenberg, Germany

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Examples

```
data(CD75)
matplot(CD75[1:80, c(1,3,4)], CD75[1:80, c(2,4,6)], type = "l",
        xlab = "Temperature", ylab = "RFU")
```

---

chipPCR.datasets   *Overview for data sets of the* chipPCR *package*

Description

The chipPCR package contains numerous data sets from commercial and experimental technologies for the amplification of nucleic acids. The data sets include results from qPCR experiments with the 'VideoScan' 'HCU' (Roediger et al. 2013), Bio-Rad (iQ5, CFX96), capillary convective PCR (ccPCR) and Roche Light Cycler 1.5. Real-time monitored amplification reactions were performed using standard amplification methods (qPCR, based on Taq polymerase) and quantitative isothermal amplification (qIA). In selected data sets are melting curves and dilution series available. Most of the data sets have equidistant measure points. However, some datasets have none homogeneous measure points as indicated below.

capillary convective PCR (ccPCR)

capillaryPCR: The capillary convective PCR (ccPCR) is a modified device of the ccPCR system proposed by Chou et al. 2013.

standard qPCR - commercial thermo cyclers

C60.amp: qPCR Experiment for the Amplification of MLC-2v and Vimentin (as decadic dilutions) Using the Roche Light Cycler 1.5.

C60.melt: Melt Curves MLC-2v and Vimentin for the qPCR experiment C60.amp using the Roche Light Cycler 1.5

C126EG595: A quantitative PCR (qPCR) with the DNA binding dye (EvaGreen) (Mao et al. 2007) was performed in a Bio-Rad iQ5 thermo cycler. The cycle-dependent increase of the fluorescence was quantified at the elongation step (59.5 degrees Celsius).

C126EG685: A quantitative PCR (qPCR) with the DNA binding dye (EvaGreen) (Mao et al. 2007) was performed in a Bio-Rad iQ5 thermo cycler. The cycle-dependent increase of the fluorescence was quantified at the elongation step (68.5 degrees Celsius).

C127EGHP: Quantitative PCR (qPCR) with a hydrolysis probe (Cy5/BHQ2) and DNA binding dye (EvaGreen) (Mao et al. 2007) performed in the Roche Light Cycler 1.5 thermo cycler.

VIMCFX96_60: Human vimentin amplification curve data (measured during annealing phase at 60 degrees Celsius) for 96 replicate samples in a Bio-Rad CFX96 thermo cycler.

VIMCFX96_69: Human vimentin amplification curve data (measured during elongation phase at 69 degrees Celsius) for 96 replicate samples in a Bio-Rad CFX96 thermo cycler.

VIMCFX96_meltcurve: Human vimentin melting curve data for 96 replicate samples in a Bio-Rad CFX96 thermo cycler.

VIMiQ5_595: Human vimentin amplification curve data (measured during annealing phase at 59.5 degrees Celsius) for 96 replicate samples in a Bio-Rad iQ5 thermo cycler.

VIMiQ5_685: Human vimentin amplification curve data (measured during elongation phase at 68.5 degrees Celsius) for 96 replicate samples in a Bio-Rad iQ5 thermo cycler.

VIMiQ5_melt: Human vimentin melting curve data for 96 replicate samples in a Bio-Rad iQ5 thermo cycler.

standard qPCR - experimental thermo cyclers

C54: qPCR Experiment in the 'VideoScan' heating/cooling-unit for the amplification using different concentrations of MLC-2v input cDNA quantities.

CD74: Quantitative PCR with a hydrolysis probe and DNA binding dye (EvaGreen) for MLC-2v measured at 59.5 degrees Celsius (annealing temperature), 68.5 degrees Celsius (elongation temperature) and at 30 degrees Celsius.

Simulations

Eff625: Highly replicate number amplification curves with an approximate amplification efficiency of 62.5 percent at cycle number 18. The data were derived from a simulation such as the AmpSim function.

Eff750: Highly replicate number amplification curves with an approximate amplification efficiency of 75 percent at cycle number 18. The data were derived from a simulation such as the AmpSim function.

Eff875: Highly replicate number amplification curves with an approximate amplification efficiency of 87.5 percent at cycle number 18. The data were derived from a simulation such as the AmpSim function.

Eff1000: Highly replicate number amplification curves with an approximate amplification efficiency of 100 percent at cycle number 18. The data were derived from a simulation such as the AmpSim function.

Isothermal Amplification - Helicase Dependent Amplification

C67: A Helicase Dependent Amplification (HDA) of HPRT1 (Homo sapiens hypoxanthine phosphoribosyltransferase 1), performed at different input DNA quantities using the Bio-Rad iQ5 thermo cycler.

CD75: Helicase Dependent Amplification in the 'VideoScan' 'HCU' of HPRT1 (Homo sapiens hypoxanthine phosphoribosyltransferase 1) measured at at 55, 60 or 65 degrees Celsius.

C81: Helicase Dependent Amplification (HDA) of pCNG1 using the 'VideoScan' Platform (Roediger et al. (2013)). The HDA was performed at 65 degree Celsius. Two concentrations of input DNA were used.

C85: Helicase Dependent Amplification (HDA) of Vimentin (Vim) in the 'VideoScan' Platform (Roediger et al. (2013)). The HDA was performed at 65 degree Celsius with three dilutions of input DNA.

Source

Stefan Roediger

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Examples

```
data(VIMiQ5_melt)

tmp <- VIMiQ5_melt

plot(NA, NA, xlim = c(55,95), ylim = c(0, 40000),
    xlab = "Temperature (degrees Celsius)",ylab = "RFU",
    main = "Melting curve in iQ5 (Bio-Rad)")
apply(tmp[, 2:ncol(tmp)], 2,
      function(x) lines(tmp[1:nrow(tmp),1],x))

Fmean <- rowMeans(tmp[, 2:ncol(tmp)])
lines(tmp[1:nrow(tmp),1], Fmean, col = "red", lwd = 3)

legend(55, 4000, c("Raw", "Mean"), pch = c(19,19), col = c(1,2))
```

CPP   *Curve Pre-processor*

Description

CPP encompasses a set of functions to pre-process an amplification curve. The pre-processing includes options to normalize curve data, to remove background, to remove outliers in the background range and to test if an amplification is significant.

Usage

```
## S4 method for signature 'numeric,numeric'
CPP(x, y, smoother = TRUE, method = "savgol",
trans = FALSE, method.reg = "lmrob",
bg.outliers = FALSE, median = FALSE,
method.norm = "none", qnL = 0.03, amptest = FALSE,
manual = FALSE, nl = 0.08, bg.range = NULL, ...)

## S4 method for signature 'matrix,missing'
CPP(x, y, smoother = TRUE, method = "savgol",
        trans = FALSE, method.reg = "lmrob",
        bg.outliers = FALSE, median = FALSE,
        method.norm = "none", qnL = 0.03, amptest = FALSE,
        manual = FALSE, nl = 0.08, bg.range = NULL, ...)

## S4 method for signature 'data.frame,missing'
CPP(x, y, smoother = TRUE,
    method = "savgol", trans = FALSE,
    method.reg = "lmrob", bg.outliers = FALSE,
    median = FALSE, method.norm = "none",
    qnL = 0.03, amptest = FALSE,
    manual = FALSE, nl = 0.08, bg.range = NULL, ...)
```

Arguments

x	is a vector containing the time or cycle values or a matrix or data frame containing both time or cycle values and fluorescence.
y	is a vector containing the fluorescence values. Omitted if x is a data frame or matrix.
smoother	logical parameter which indicates if smoother should be used.
method	a vector of names defining which smoothing method should be used. The Savitzky-Golay smoothing filter is the default smoother. Use "lowess" for LOWESS smoother (locally-weighted polynomial regression), "mova" for moving average, "savgol" for Savitzky-Golay smoothing filter, "smooth" for cubic spline smooth, "spline" for standard cubic spline smooth, "supsmu" for Friedman's SuperSmoother, "whit1" for weighted Whittaker smoothing with a first order finite difference penalty, "whit2" for weighted Whittaker smoothing with a second order finite difference penalty or "all" for all implemented smoothing algorithms.
trans	defines if the slope of the background range in a curve should be corrected by a linear regression.
method.reg	defines the method ("rfit", "lmrob", "rq") for the robust linear regression. If equal to "least", CPP uses linear regression.
bg.outliers	is a logical argument which to remove outliers in the background range.
median	If set to TRUE, median is used instead of mean in outlier replacement. The mean is used by default.

method.norm	is a argument to use a "none", "minm", "max", "lugn", or "zscore" normalization.
qnL	is the quantile to be used for the quantile normalization.
amptest	is a logical operator which is used to set a test for a positive amplification.
manual	is used to test for a fixed threshold value of the background.
nl	is a value used as fixed threshold value for the background.
bg.range	is a numeric vector of length 2 pointing the background range. If NULL, the background range is calculated by bg.max function.
...	dot operator for diverse arguments of smoother for details).

Details

CPP uses the bg.max function to estimate automatically the start of the amplification process. In the background range there is often noise which makes it harder to determine a meaningful background value. Therefore CPP can optionally remove outliers by finding the value with largest difference from the mean as provided by the rm.outlier function. This function also tries to prevent calculations of non amplified signals.

The slope of the background range is often unequal to zero. By setting the parameter trans it is possible to apply a simple correction of the slope. Thereby either a robust linear regression by computing MM-type regression estimators, a nonparametric rank-based estimator or a standard linear regression model. Care is needed when using trans with time series (see lm for details).

Value

A list where each element represents the pre-process data and parameters for the background region (BG) of a qPCR experiment.

Author(s)

Stefan Roediger, Michal Burdukiewicz

See Also

Normalization: normalizer

Smoothing: smoother

Robust linear regression: lm.coefs

Examples

```
# Function to pre-process an amplification curve.
# Take a subset of the C17 data frame.
data(C17)
default.par <- par(no.readonly = TRUE)
par(mfrow = c(2,1))
plot(NA, NA, xlab = "Time [sec]", ylab = "refMFI",
     main = "HDA Raw Data",
     xlim = c(0, 2500), ylim = c(0,1.1), pch = 20)
```

```
for (i in 3:5) {
  lines(C17[1:50, 1], C17[1:50, i], col = i - 2,
type = "b", pch = 20)
}

legend(50, 0.5, c("55 degrees Celsius", "60 degrees Celsius", "65 degrees Celsius"),
col = c(1,2,3), pch = rep(20,3))

# Use CPP to pre-process the data by removing the missing value and
# normalization of the data
plot(NA, NA, xlab = "Time [sec]", ylab = "refMFI",
     main = "Curve Pre-processor Applied to HDA Data",
     xlim = c(0, 2500), ylim = c(0,1.1), pch = 20)
for (i in 3:5) {
  y.cpp <- CPP(C17[2:50, 1], C17[2:50, i], method.norm = "minm",
      bg.outliers = TRUE)$y.norm
  lines(C17[2:50, 1], y.cpp, col = i - 2,
type = "b", pch = 20)
}
legend(50, 1, c("55 degrees Celsius", "60 degrees Celsius", "65 degrees Celsius"),
col = c(1,2,3), pch = rep(20,3))

par(default.par)
```

---

der   *Class* "der"

Description

An S4 class containing the output [inder](#) function.

Value

A class for a matrix of derivatives from amplifications curve data of a qPCR experiment.

Slots

.Data: "matrix" is a matrix containing smoothed data as well as the first and second derivative.

method: "character" used method of smoothing.

Methods

summary signature(object = "der"): calculates and prints approximate first derivative maximum, second derivative maximum, second derivative minimum and second derivative center. See [summary.der](#).

show signature(object = "der"): prints only .Data slot of the object.

Author(s)

Stefan Roediger, Michal Burdukiewicz

See Also

inder

Examples

```
pcr <- AmpSim(cyc = 1:40)
res <- inder(pcr[, 1], pcr[, 2])
sums <- summary(res)
print(sums)
```

---

eff   *Class* "eff"

Description

An S4 class containing the output effcalc function.

Slots

.Data: "matrix" containing the "Concentration", "Location" (mean, median), "Deviation" (standard deviation, median absolute deviation), "Coefficient of Variance" (CV, RSD) sequential in the columns.

amplification.efficiency: "numeric" value representing amplification efficiency.

regression: "lm" the results of the linear regression and .

correlation.test: "htest". the correlation test (Pearson) results.

Methods

plot signature(x = "eff"): plots calculated efficiency. See plot.eff

show signature(object = "eff"): prints only .Data slot of the object.

summary signature(object = "eff"): prints information about object prettier than show.

Author(s)

Stefan Roediger, Michal Burdukiewicz

See Also

effcalc, plot.eff,

Eff1000   *Highly Replicate Number Amplification Curves*

Description

Highly replicate number amplification curves with an approximate amplification efficiency of 100 percent at cycle number 18. The data were derived from a simulation such as the AmpSim function.

Usage

```
data(Eff1000)
```

Format

A data frame with 40 (Cycles) observations on the following 1000 (amplification curves) variables. The columns are all replicates.

Examples

```
data(Eff1000)

plot(NA, NA, xlim = c(1,40), ylim = c(0,max(Eff1000)), xlab = "Cycle",
    ylab = "RFU",
    main = "Amplification Curves with 100 Percent Efficiency")
apply(Eff1000[, 1:ncol(Eff1000)], 2, function(x) lines(1:40,x))

Fmean <- rowMeans(Eff1000[, 1:ncol(Eff1000)])
lines(1:40, Fmean, col = "red", lwd = 3)

legend(1, quantile(unlist(Eff1000), 0.9), c("Raw", "Mean"),
pch = c(19,19), col = c(1,2))
```

Eff625   *Highly Replicate Number Amplification Curves*

Description

Highly replicate number amplification curves with an approximate amplification efficiency of 62.5 percent at cycle number 18. The data were derived from a simulation such as the AmpSim function.

Usage

```
data(Eff625)
```

Format

A data frame with 40 (Cycles) observations on the following 1000 (amplification curves) variables. The columns are all replicates.

Examples

```
data(Eff625)

plot(NA, NA, xlim = c(1,40), ylim = c(0,max(Eff625)), xlab = "Cycle",
    ylab = "RFU",
    main = "Amplification Curves with 62.5 Percent Efficiency")
apply(Eff625[, 1:ncol(Eff625)], 2, function(x) lines(1:40,x))

Fmean <- rowMeans(Eff625[, 1:ncol(Eff625)])
lines(1:40, Fmean, col = "red", lwd = 3)

legend(1, quantile(unlist(Eff625), 0.9), c("Raw", "Mean"),
       pch = c(19,19), col = c(1,2))
```

Eff750   *Highly Replicate Number Amplification Curves*

Description

Highly replicate number amplification curves with an approximate amplification efficiency of 75 percent at cycle number 18. The data were derived from a simulation such as the AmpSim function.

Usage

```
data(Eff750)
```

Format

A data frame with 40 (Cycles) observations on the following 1000 (amplification curves) variables. The columns are all replicates.

Examples

```
data(Eff750)

plot(NA, NA, xlim = c(1,40), ylim = c(0,max(Eff750)), xlab = "Cycle",
    ylab = "RFU",
    main = "Amplification Curves with 75 Percent Efficiency")
apply(Eff750[, 1:ncol(Eff750)], 2, function(x) lines(1:40,x))

Fmean <- rowMeans(Eff750[, 1:ncol(Eff750)])
lines(1:40, Fmean, col = "red", lwd = 3)

legend(1, quantile(unlist(Eff750), 0.9), c("Raw", "Mean"),
pch = c(19,19), col = c(1,2))
```

Eff875   *Highly Replicate Number Amplification Curves*

Description

Highly replicate number amplification curves with an approximate amplification efficiency of 87.5
percent at cycle number 18. The data were derived from a simulation such as the AmpSim function.

Usage

```
data(Eff875)
```

Format

A data frame with 40 (Cycles) observations on the following 1000 (amplification curves) variables.
The columns are all replicates.

Examples

```
data(Eff875)

plot(NA, NA, xlim = c(1,40), ylim = c(0,max(Eff875)), xlab = "Cycle",
    ylab = "RFU",
    main = "Amplification Curves with 87.5 Percent Efficiency")
apply(Eff875[, 1:ncol(Eff875)], 2, function(x) lines(1:40,x))

Fmean <- rowMeans(Eff875[, 1:ncol(Eff875)])
lines(1:40, Fmean, col = "red", lwd = 3)

legend(1, quantile(unlist(Eff875), 0.9), c("Raw", "Mean"),
pch = c(19,19), col = c(1,2))
```

---

effcalc   *Analysis of the amplification efficiency*

Description

effcalc calculates the amplification efficiency of a dilution.

Usage

```
## S4 method for signature 'numeric,numeric'
effcalc(x, y, logx = TRUE, RSD = FALSE, rob = FALSE,
                                    level = 0.95)
## S4 method for signature 'matrix,missing'
effcalc(x, y, logx = TRUE, RSD = FALSE, rob = FALSE,
                                    level = 0.95)
```

```
## S4 method for signature 'matrix,missing'
effcalc(x, y, logx = TRUE, RSD = FALSE, rob = FALSE,
                                level = 0.95)
```

Arguments

x	is the column of a data frame for the concentration (dilution).
y	are multiple columns of Cq values from a data.frame (e.g., [, c(1:n)]).
logx	is a logical parameter used to convert the concentration into a decadic logarithm.
RSD	Setting the option RSD = TRUE shows the relative standard deviation (RSD) in percent.
rob	Using the option rob as TRUE the median and the median absolute deviation (MAD) is plotted instead of the mean and standard deviation.
level	Tolerance/confidence level.

Value

an object of the class eff.

Author(s)

Stefan Roediger

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

S. Mehra and W.-S. Hu. A kinetic model of quantitative real-time polymerase chain reaction. *Biotechnology and bioengineering*, 91(7):848–860, Sept. 2005. ISSN 0006-3592. doi: 10.1002/bit.20555. PMID: 15986490.

M. Guescini, D. Sisti, M. B. Rocchi, L. Stocchi, and V. Stocchi. A new real-time PCR method to overcome significant quantitative inaccuracy due to slight amplification inhibition. *BMC Bioinformatics*, 9(1):326, July 2008. ISSN 1471-2105. doi: 10.1186/1471-2105-9-326. PMID: 18667053.

A. Tichopad, M. Dilger, G. Schwarz, and M. W. Pfaffl. Standardized determination of real-time PCR efficiency from a single reaction set-up. *Nucleic Acids Research*, 31(20):e122, Oct. 2003. ISSN 1362-4962. PMID: 14530455 PMCID: PMC219490.

A. Staalberg, P. Aman, B. Ridell, P. Mostad, and M. Kubista. Quantitative real-time PCR method for detection of b-lymphocyte monoclonality by comparison of kappa and lambda immunoglobulin light chain expression. *Clinical Chemistry*, 49(1):51–59, Jan. 2003. ISSN 0009-9147. PMID: 12507960.

W. Liu and D. A. Saint. A new quantitative method of real time reverse transcription polymerase chain reaction assay based on simulation of polymerase chain reaction kinetics. *Analytical Biochemistry*, 302(1):52–59, Mar. 2002. ISSN 0003-2697. doi: 10.1006/abio.2001.5530. PMID: 11846375.

See Also

eff

plot.eff

---

fixNA   *Impute missing values into a column of amplification data*

Description

fixNA imputes missing values in a single column of data. The imputation is based on a linear approximation by default. However, the data can also be estimated from an approximation by splines.

Usage

```
## S4 method for signature 'numeric,numeric'
fixNA(x, y, spline = TRUE, verbose = FALSE)
## S4 method for signature 'matrix,missing'
fixNA(x, y, spline = TRUE, verbose = FALSE)
## S4 method for signature 'data.frame,missing'
fixNA(x, y, spline = TRUE, verbose = FALSE)
```

Arguments

x	x is a vector containing the time or cycle values or data frame/matrix where second column contains missing data.
y	y is a vector containing missing values. Used only if x is also a vector.
spline	spline if TRUE missing values are imputed by spline approximation as per spline. The default FAlSE is linear a approximation as per approx.
verbose	verbose defines if the number of missing values should be reported.

Details

Amplification data of experimental systems may contain missing values (NA). The NAs may be caused by detector problems, acquisition error or other assorted problems. There are different ways to handle missing values. One approach is to ignore NAs which is generally acceptable. However, in case of further calculation it is often necessary to handle cases of missing values in a way that the next calculation steps can be performed. Missing values can be eliminated by a imputation. Imputation encompasses various approaches. This includes to calculate a location parameter (e.g., mean, median) or other significant values (e.g., minimum, maximum, modus) of a data column. However, in non-linear processes such as amplification processes its is better to estimate the missing values from a trend. fixNA was empirically tested and relies on a linear trend estimation based on the approx function. This approach is useful but may be problematic on the phases other then background or plateau phases of an amplification reaction. The parameter spline on fixNA enables a trend estimation on splines and may be more appropriate in most scenarios. Other smoothing functions such as the Savitzky-Golay smoothing filter have the intrinsic capability to remove missing values [Savitzky and Golay 1964, Eilers 2003].

Author(s)

Stefan Roediger, Michal Burdukiewicz

References

Eilers, P. H. C. Anal. Chem. 2003, 75, 3631–3636. Savitzky, A.; Golay, M. J. E. Anal. Chem. 1964, 36, 1627–1639

See Also

approx, spline.

Examples

```
# Simulate a qPCR reaction with AmpSim with for 40 cycles
res <- AmpSim(cyc = c(1:40))
# Introduce a missing value in the transition between
# the background and the exponential phase
res.NA <- res
res.NA[18, 2] <- NA

default.par <- par(no.readonly = TRUE)


par(mfrow = c(2,2))
plot(res, xlab = "Cycles", ylab = "Fluorescence", type = "b", pch = 20,
     main = "Simulation without missing value")
     abline(v = c(17.5, 18.5), col = "grey")
     abline(h = c(0.09, 0.14), col = "grey")

res.NA.linear <- fixNA(res.NA[, 1], res.NA[, 2], spline = FALSE,
       verbose = FALSE)
plot(res.NA.linear, xlab = "Cycles", ylab = "Fluorescence", type = "b",
     pch = 20, main = "Simulation with linear imputed\n NA
     value during transition")
     abline(v = c(17.5, 18.5), col = "grey")
     abline(h = c(0.09, 0.14), col = "grey")

plot(res.NA, xlab = "Cycles", ylab = "Fluorescence", type = "b", pch = 20,
     main = "Simulation with missing\n value during transition")
     abline(v = c(17.5, 18.5), col = "grey")
     abline(h = c(0.09, 0.14), col = "grey")
res.NA.spline <- fixNA(res.NA[, 1], res.NA[, 2], spline = TRUE,
       verbose = FALSE)
plot(res.NA.spline, xlab = "Cycles", ylab = "Fluorescence", type = "b",
      pch = 20, main = "Simulation with spline imputed\n NA value
      during transition")
     abline(v = c(17.5, 18.5), col = "grey")
     abline(h = c(0.09, 0.14), col = "grey")
par(default.par)
```

---

humanrater   *humanrater, a graphical interface to rate curves*

Description

`humanrater` allows rating a curve for a certain characteristic in the interactive, semi-blind manner. `humanrater` draws individual graphs of a curve and prompts an input field for the user. This function can be used to compare the human rating and the rating of a machine.

Usage

```
humanrater(x, cyc = 1, repeats = 1,
designations = list(y = "yes", a = "ambiguous", n = "not"),
shuffle = TRUE, ...)
```

Arguments

x	is the input data (matrix or data.frame).
cyc	is the index of column containing the cycle data.
repeats	number of repeats to rate the samples.
designations	a named list of length at least 2. See Details.
shuffle	logical, if TRUE sequence of curves is shuffled for purpose of rating.
...	additional arguments to plot function.

Details

A user can specify the list of designations characterizing the curve, where the names of elements specify short designations used during rating. Defaults are y for "yes", a for "ambiguous" and n for "no". The supplied designation list must have length at least two (for example "true"/"false").

Value

A `data.frame` containing the classification and conformity of amplification curves as determined by a human rater.

Author(s)

Michal Burdukiewicz, Stefan Roediger

Examples

```
testdata <- data.frame(1:35,
AmpSim(Cq = 15, noise = TRUE)[, 2],
AmpSim(Cq = 25, noise = TRUE)[, 2],
rnorm(35),
AmpSim(Cq = 35, noise = TRUE)[, 2],
rnorm(35),
```

```
AmpSim(Cq = 45, noise = TRUE)[, 2])
#we strongly advise against running code below using 'example(humanrater)'
#due to the highly interactive nature of this function (it would not end
#without user's input), it is recommended to just copy lines below into R
#command line
## Not run:
  #check testdata for significance of amplification in two repeats
  human.test1 <- humanrater(testdata, repeats = 2)
  #check testdata for significance of amplification in one repeat and declare more
  #finger friendly (but less obvious) designations
  human.test2 <- humanrater(testdata, repeats = 1, list(q = "yes", w = "no"))

## End(Not run)
```

---

inder   *Interpolate derivatives*

Description

A function inder ("in" + "der" = interpolate derivatives) for interpolating first and second derivatives using the five-point stencil. Therefore this function can be used to estimate the Cq (cycle of quantification) of an amplification curve. First positive derivative also known as First Derivative Maximum (FDM) and the Second Derivative Maximum (SDM) are calculated this way (Ruijter et al. 2013). However, from the mathematical point of view it can also be used to calculate the melting point for melting curve analysis (compare Roediger et al. 2013) provided that the sign of the derivative is changed.

Usage

```
inder(x, y, Nip = 4, logy = FALSE, smooth.method = "spline")
```

Arguments

x	is a numeric vector of independent variable or data frame/matrix containing abscissa and ordinate values.
y	is a vector of dependent variable. Omitted if x is data frame or matrix.
Nip	is a value which defines how often an interpolation takes place at n equally spaced points spanning the interval (default 4). Nip is a such a resolution. A high Nip may improve the precision. Nips less than 2 and higher than 20 are not meaningful for conventional qPCR with up to 50 cycles. However, higher Nips might be appropriate for isothermal amplification reactions.
logy	If logy is TRUE than a semi-decadic log scale graph (corresponds to the linear phase) to illustrate the exponential dynamic of the qPCR amplification is used.
smooth.method	a character vector of length 1 or NULL defining smoothing method used. Currently accepted character values: "spline", "supsmu". If NULL, smoothing is not performed.

Details

The function y = f(x) is numerically derived using the five-point stencil. This method does not require any assumptions regarding the function f.

A smoothing procedure greatly enhances calculating derivative calculation. inder uses two smoothing algorithms best suited for this approach. A smoothing can be omitted by setting smooth.method to NULL, which is advisable in case of the already smoothed data.

Value

An object of [der](#) class.

Author(s)

Stefan Roediger, Michal Burdukiewicz

References

Ruijter JM, Pfaffl MW, Zhao S, et al. (2013) Evaluation of qPCR curve analysis methods for reliable biomarker discovery: bias, resolution, precision, and implications. *Methods* San Diego Calif 59:32–46.

Surface Melting Curve Analysis with R. S. Roediger, A. Boehm and I. Schimke. *The R Journal*. 5(2):37–52, 2013. https://journal.r-project.org

Examples

```
# First example
# Derive sinus
x <- 1:100/10
y <- sin(x)
ders <- inder(x, y)
plot(x, y, type = "l")
lines(ders[, "x"], ders[, "d1y"], col = "red")
lines(ders[, "x"], ders[, "d2y"], col = "green")
legend("topright", c("f(x)", "f'(x)", "f''(x)"), lty = 1, col = c("black",
"red", "green"))

# Second example
# Determine the approximate second derivative maximum
# for a qPCR experiment. SDM,
isPCR <- AmpSim(cyc = 1:40)

res <- inder(isPCR)

plot(isPCR, xlab = "Cycle", ylab = "RFU", ylim = c(-0.15,1),
     main = "Approximate Second Derivative Maximum (SDM)",
     type = "b", pch = 20)

  lines(res[, "x"], res[, "d1y"], col = "blue")
  lines(res[, "x"], res[, "d2y"], col = "red")
  summ <- summary(res, print = FALSE)
```

```
abline(v = c(summ["SDM"], summ["SDm"], summ["SDC"]), col = c(3,4,5))
text(summ["SDM"], 0.5, paste0("SDM ~ ", round(summ["SDM"], 2)),
     cex = 1.5, col = 3)
text(summ["SDC"], 0.7, paste0("SDC ~ ", round(summ["SDC"], 2)),
     cex = 1.5, col = 5)
text(summ["SDm"], 0.9, paste0("SDm ~ ", round(summ["SDm"], 2)),
     cex = 1.5, col = 4)
text(summ["FDM"] + 10, 0.65, paste("FDM ~ ", round(summ["FDM"], 2)),
     cex = 1.5, col = 1)

legend(1, 1, c("raw", "first derivative", "second derivative"),
col = c(1,4,2), lty = c(2,1,1), cex = 1.2)
```

LAMP   *real-time loop-mediated isothermal amplification (LAMP) by Tanner*
                              et al. (2012)

Description

Real-time amplification plot of a Loop-mediated isothermal amplification (LAMP) by Tanner et al.(2012) in a CFX96 Real Time System (Bio-Rad Laboratories, Hercules, CA, USA).

Usage

```
data("LAMP")
```

Format

A data frame with 115 observations on the following 2 variables.

Time Time in minutes

Normalized.RFU RFU (Normalized)

Details

The data were digitized as described by Poisot (2011). The image for data extraction was taken from Figure 4B by Tanner et al.(2012). For further experimental and technical details refer to Tanner et al.(2012).

Source

The original data were taken from Figure 4B by Tanner et al.(2012).

References

Poisot, T. (2011). The digitize Package: Extracting Numerical Data from Scatterplots. *The R Journal* 3, 25–26.

Tanner, N.A., Zhang, Y., and Evans, T.C. (2012). Simultaneous multiple target detection in real-time loop-mediated isothermal amplification. *BioTechniques* 53, 81–89.

Examples

```
plot(LAMP, main = "LAMP by Tanner et al.(2012)", xlab = "Time (minutes)",
        ylab = "RFU (Normalized)", col = "red", type = "l")
abline(h = 0.05, lty = 2, col = "grey")
legend("bottomright", c("E. coli (Cy5)"), col = "red", pch = 19)
```

---

lm.coefs   *Compute linear model coefficients*

Description

Computes linear model using the robust linear regression.

Usage

```
lm.coefs(x, y, method.reg)
```

Arguments

x	a vector of ordinate values.
y	a vector of abscissa values.
method.reg	defines the method ("rfit", "lmrob", "rq", "least") for the linear regression.

Details

`lm.coefs` is a convenient wrapper around few functions performing normal (least squares) and robust linear regression. If the robust linear regression is impossible, `lm.coefs` will give a warning and perform linear regression using the least squares method. This function can be used to calculate the background of an amplification curve. The coefficients of the analysis can be used for a trend based correction of the entire data set.

Value

A data frame with one column and two rows representing coefficients of the linear model.

Author(s)

Stefan Roediger, Michal Burdukiewicz

See Also

rq, rfit, lm, lmrob

Examples

```
plot(VIMCFX96_69[, 1], VIMCFX96_69[, 2], type = "l", xlab = "Cycle",
     ylab = "Fluorescence")
rect(1,0,10,5000)
method <- c("lmrob", "rq", "least", "rfit")
for (i in 1:4) {
  tmp <- lm.coefs(VIMCFX96_69[1:10, 1], VIMCFX96_69[1:10, 2],
  method.reg = method[i])
  abline(a = tmp[1, 1], b = tmp[2, 1], col = i + 1, lwd = 1.5)
}
legend(2, 3000, c("Data", "lmrob", "rq", "least", "rfit"), lty = 1, col = 1:5,
       cex = 1.5)
```

MFIaggr	*Multiple comparison of the cycle dependent variance of the fluorescence*

Description

MFIaggr is used for a fast multiple comparison of the cycle dependent variance of the fluorescence.

Usage

```
## S4 method for signature 'numeric,numeric'
MFIaggr(x, y, cyc = 1, fluo = 2:ncol(x),
    RSD = FALSE, rob = FALSE, llul = c(1,10))
## S4 method for signature 'matrix,missing'
MFIaggr(x, y, cyc = 1, fluo = 2:ncol(x),
    RSD = FALSE, rob = FALSE, llul = c(1,10))
## S4 method for signature 'data.frame,missing'
MFIaggr(x, y, cyc = 1, fluo = 2:ncol(x),
    RSD = FALSE, rob = FALSE, llul = c(1,10))
```

Arguments

x	is the column of a data frame for the cycle or data.frame/matrix with whole data.
y	are multiple columns of fluorescence values from a data.frame (e.g., [, c(1:n)]).
cyc	is the index of column containing the cycle data. Used only if y is missing.
fluo	are the columns containing the fluorescence data. Used only if y is missing.
RSD	Setting the option RSD = TRUE shows the relative standard deviation (RSD) in percent.
rob	Using the option rob as TRUE the median and the median absolute deviation (MAD) are calculated instead of the mean and standard deviation.
llul	is a parameter to define the lower and upper data limit (cycle), aka region of interest (ROI) used for the density and quantile plot.

Value

An object of the class [refMFI](). refMFI means referenced Mean Fluorescence Intensity (Roediger et al. 2013).

Author(s)

Stefan Roediger, Michal Burdukiewicz

References

Roediger S, Boehm A, Schimke I. Surface Melting Curve Analysis with R. *The R Journal* 2013;5:37–53.

Examples

```
# First Example
# Cycle dependent variance of the refMFI using standard measures
# (Mean, Standard Deviation (SD)).
# Use Standard Deviation (SD) in the plot

data(VIMCFX96_60)

MFIaggr(VIMCFX96_60[, 1], VIMCFX96_60[, 2:ncol(VIMCFX96_60)])

#alternative usage
MFIaggr(VIMCFX96_60)

#only second and forth column
plot(MFIaggr(VIMCFX96_60, fluo = c(2, 4)))

# Example
# Use of MFIaggr to test for heteroskedasticity using the Breusch-Pagan
# test. The data were aggregated with the MFIaggr function and assigned to
# the object res. The standard deviation was transformed to the variance.
# The plot shows the cycle dependent variance.
# First cycles 1 to 10 of 96 qPCR replicate amplification curves were
# analyzed. Next the cycles 1 to 40 of the same amplification curve data
# were analyzed. The Breusch-Pagan confirmed the heteroskedasticity in the
# amplification curve data.

default.par <- par(no.readonly = TRUE)
par(mfrow = c(1,2), bty = "n")
res <- MFIaggr(VIMCFX96_60[, 1], VIMCFX96_60[, 2:ncol(VIMCFX96_60)],
       llul = c(1,10))
head(res)
plot(res[, 1], res[, 3]^2, xlab = "Cycle", ylab = "Variance of refMFI",
     xlim = c(1,10), main = "ROI from Cycle 1 to 10", pch = 19, type = "b")
abline(v = c(1,10), col = "grey", lty = 2, lwd = 2)
legend("top", paste0("Breusch-Pagan test p-value: \n", format(summary(res)[5],
       digits = 2)), bty = "n")

res <- MFIaggr(VIMCFX96_60[, 1], VIMCFX96_60[, 2:ncol(VIMCFX96_60)],
```

```
        llul = c(1,40))
head(res)
plot(res[, 1], res[, 3]^2, xlab = "Cycle", ylab = "Variance of refMFI",
     main = "ROI from Cycle 1 to 40", pch = 19, type = "b")
abline(v = c(1,40), col = "grey", lty = 2, lwd = 2)
legend("top", paste0("Breusch-Pagan test p-value: \n", format(summary(res)[5],
       digits = 2)), bty = "n")
par(default.par)
```

MFIaggr.gui   *Multiple Comparison of the Cycle Dependent Variance - Graphical*
                          User Interface

Description

`MFIaggr.gui` is a Graphical User Interface based on the `shiny` package. The core if this GUI is based on the Comparison of the [MFIaggr](#) function. The cycle dependent variance can be analyzed intuitively. This GUI dynamically generates reports.

Usage

```
MFIaggr.gui()
```

Value

No return value, called for side effects.

Warning

Any ad-blocking software may be cause of malfunctions.

Author(s)

Stefan Roediger, Michal Burdukiewicz.

See Also

[MFIaggr](#)

Examples

```
# The code chunk below will fail if the web browser is not installed. if on UNIX platform try:
# as.vector(Sys.getenv("R_BROWSER"))
# Invoke the shiny AmpSim app in the default browser
## Not run:
#do not execute using example(), it breaks the sequence of the plots in shiny app
MFIaggr.gui()

## End(Not run)
```

---

normalizer   *Normalize data*

Description

normalizer normalizes any data set using a chosen method (see Details). It may be used when the data from an experiment have considerable variation regarding the background and plateau signal.

Usage

```
normalizer(y, method.norm = "none", qnL = 0.03)
```

Arguments

y	is a vector containing the fluorescence values.
method.norm	is a argument to use a "none", "minm", "max", "luqn", or "zscore" normalization. See Details.
qnL	is the quantile to be used for the quantile normalization. Ignored if method.norm is not equal to "luqn".

Details

The parameter qnL is a user defined quantile which is used for the quantile normalization. A quantile normalization herein refers to an approach which is less prone to outliers than a normalization based on the minimum and the maximum of an amplification curve. minm does a min-max normalization between 0 and 1 (see Roediger et al. 2013 for explanation). max does a normalization to the maximum value (MFI/max(MFI)). luqn does a quantile normalization based on a symmetric proportion as defined by the qnL parameter (e.g., qnL = 0.03 equals 3 and 97 percent quantiles). zscore performs a z-score normalization with a mean of 0 and a standard deviation of 1.

Value

A vector of normalized fluorescence values.

Author(s)

Stefan Roediger, Michal Burdukiewicz

References

Surface Melting Curve Analysis with R. S. Roediger, A. Boehm and I. Schimke. *The R Journal*. 5(2):37–52, 2013. https://journal.r-project.org

See Also

CPP

Examples

```
normalizer(C17[2L:50, 1], "minm")
```

---

plot.bg   *Plot* bg *objects*

Description

Draws diagnostic plots of amplification reaction.

Usage

```
## S4 method for signature 'bg'
plot(x, what = 1:3, add = FALSE, indicators = TRUE,
    legend = TRUE, stan.labs = TRUE,
    plot.colors = c("black", "red", "blue"), ...)
```

Arguments

x	is a bg object.
what	is a vector specifying what should be plotted. 1 means raw data, 2 means first derivative and 3 means second derivative. Any combination of mentioned values is valid, for example: c(1,2), c(1, 2, 3), c(2, 3). See Details and Examples.
add	is a "logical" argument. If TRUE, plot is added to existing plot. Moreover, enforces indicators = FALSE.
indicators	is a "logical" argument. If FALSE, background start, stop and plateau transition indication lines aren't plotted.
legend	is a "logical" argument. If TRUE, legend is added to the plot.
stan.labs	is a "logical" argument. If TRUE, standard axis labels ("Cycle" and "Fluorescence") are added.
plot.colors	is a vectors of colors used in plot. Must have length 3.
...	Arguments to be passed further to the plot function, such as graphical parameters.

Details

plot.bg is simplified, ready-to-use version of plot.der, which still can be used whenever more flexible function is needed.

Author(s)

Stefan Roediger, Michal Burdukiewicz

See Also

bg, plot.der

Examples

```
#step-by-step plotting bg object

res <- AmpSim(cyc = 1:40, Cq = 25)
background <- bg.max(res)

plot(background)

#above is equivalent of below
plot(rounder(inder(res)), xlab = "Cycles",
     ylab = "Fluorescence",
     pch = 20, legend = FALSE)

abline(v = slot(background, "bg.start"))
  text(slot(background, "bg.start"), 0.2, "Background start", pos = 4)
abline(v = slot(background, "bg.stop"), col = "blue")
  text(slot(background, "bg.stop"), 0.25, "Background stop", pos = 4,
       col = "blue")
abline(v = slot(background, "amp.stop"), col = "green")
  text(slot(background, "amp.stop"), 0.3, "Plateau transition", pos = 4,
       col = "green")
legend(4, 1, c("Raw data", "First derivative", "Second derivative"),
       pch = rep(20, 3), col = c(1, 2, 4))
```

---

plot.der   *Plot* der *objects*

Description

Plots interpolated derivatives.

Usage

```
## S4 method for signature 'der'
plot(x, what = 1:3, add = FALSE, legend = TRUE,
        plot.colors = c("black", "red", "blue"), ...)
```

Arguments

x	is a [der](#) object.
what	is a vector specifying what should be plotted. 1 means raw data, 2 means first derivative and 3 means second derivative. Any combination of mentioned values is valid, for example: c(1,2), c(1, 2, 3), c(2, 3). See Details and Examples.
add	is a "logical" argument. If TRUE, plot is added to existing plot.
legend	is a "logical" argument. If TRUE, legend is added to the plot.

plot.colors	is a vectors of colors used in plot. Must have length 3 for the original function, first, and second derivative, respectively.
...	Arguments to be passed further to the plot function, such as graphical parameters.

Author(s)

Stefan Roediger, Michal Burdukiewicz

See Also

[der](#)

Examples

```
res <- inder(AmpSim(cyc = 1:40, Cq = 25))
plot(res)
#round the result of inder
plot(rounder(res))
```

---

plot.eff	*Plot* eff *objects*

Description

Draws diagnostic plots of the amplification efficiency.

Usage

```
## S4 method for signature 'eff'
plot(x, xlab = "log10(Concentration)",
                  ylab = "Cq", main = "Efficiency Plot",
                  trend = TRUE, res.fit = "topright", CI = FALSE,
                  level = 0.95, type = "p", pch = 19,
                  er.length = 0.05, col = "black")
```

Arguments

x	is a [eff](#) object.
xlab	a title for the x axis.
ylab	a title for the y axis.
main	an overall title for the plot.
trend	Setting the option trend = TRUE shows the linear regression line in the plot.

`res.fit`	If `res.fit` sets where the results (goodness of fit, amplification efficiency, correlation) of the linear regression line in the plot should be plotted. Accepts the same location keywords as [`legend`]().
`CI`	Setting the option `CI = TRUE` shows the confidence interval lines in the plot.
`level`	Tolerance/confidence level.
`type`	is a graphical parameter setting the plot use lines, points or both (see [`plot`]()).
`pch`	is a graphical parameter used to define the symbol used in the plot.
`er.length`	`length` is a graphical parameter used to define the length of the error bar used in the plot.
`col`	`col` is a graphical parameter used to define the color of the points on the plot.

Details

The plot being a result of this function is built from three subplots.

Value

No return value, called for side effects.

Author(s)

Stefan Roediger, Michal Burdukiewicz

References

Roediger S, Boehm A, Schimke I. Surface Melting Curve Analysis with R. *The R Journal* 2013;5:37–53.

See Also

[`eff`]()

---

plot.refMFI   *Plot* refMFI *objects*

Description

Draw quick diagnostic plots of amplification reaction.

Usage

```
## S4 method for signature 'refMFI'
plot(x, CV = FALSE, type = "p", pch = 19, length = 0.05,
                      col = "black")
## S4 method for signature 'refMFI,refMFI'
plot(x, y, CV = FALSE, type = "p", pch = 19, length = 0.05,
                      col = "black")
```

Arguments

x	is a [refMFI](#) object. refMFI means referenced Mean Fluorescence Intensity (Roediger et al. 2013)
y	optionally an additional [refMFI](#) object.
CV	If CV is true the coefficient of variation (RSD, CV) is plotted. If set to FALSE the deviation as Standard Deviation or Median Absolute Deviation is plotted.
type	is a graphical parameter setting the plot use lines, points or both (see [plot](#)).
pch	is a graphical parameter used to define the symbol used in the plot.
length	length is a graphical parameter used to define the length of the error bar used in the plot.
col	col is a graphical parameter used to define the length of the error bar used in the plot.

Details

This function is a relative of the MFIerr function from the MBmca package (Roediger et al. 2013, The R Journal). The plot being a result of this function is built from three subplots. If y parameter is not absent, it is plotted in lighter color than x.

Value

No return value, called for side effects.

Author(s)

Stefan Roediger, Michal Burdukiewicz

References

Roediger S, Boehm A, Schimke I. Surface Melting Curve Analysis with R. *The R Journal* 2013;5:37–53.

See Also

[refMFI](#)

---

plotCurves	*Plot Curves in an Orthogonal Matrix*

Description

Plots many curves on one plot in table-like fashion.

Usage

```
plotCurves(x, y, cyc = 1, fluo = 2:ncol(x), one.plot = FALSE,
           nrow = ceiling(sqrt(ncol(y))), CPP = FALSE, ...)
```

Arguments

x	is the column of a data frame for the cycle or data.frame/matrix with whole data.
y	are multiple columns of fluorescence values from a `data.frame` (e.g., [, c(1:n)]).
cyc	is the index of column containing the cycle data. Used only if y is NULL.
fluo	are the columns containing the fluorescence data. Used only if y is NULL.
one.plot	`logical`, curves may be plotted on single chart (TRUE) or in matrix-like multi-plot (FALSE).
nrow	number of rows in plot. Applies only if `one.plot` is FALSE.
CPP	logical, if TRUE CPP analysis is added to a plot. Ignored if `one.plot` is TRUE.
...	additional arguments to `plot` function.

Details

`plotCurves` is a function for the quick assessment of amplification curve raw data in an orthogonal matrix.

Value

None.

Author(s)

Stefan Roediger, Michal Burdukiewicz, Konstantin A. Blagodatskikh

Examples

```
# First example
plotCurves(VIMCFX96_60[, 1], VIMCFX96_60[, 2L:16], type = "l")

# Second example
y <- VIMCFX96_60[, 2L:16]
# Introduce some missing values.
y[c(1,4,5,6,23,34), c(2,4,9,15)] <- NA
plotCurves(VIMCFX96_60[, 1], y, nrow = 4, type = "l")

# Third example
# Same as second example but the CPP option is set to TRUE.
# Noise and missing values will be removed.

y <- VIMCFX96_60[, 2L:16]
# Introduce some missing values.
y[c(1,4,5,6,23,34), c(2,4,9,15)] <- NA
plotCurves(VIMCFX96_60[, 1], y, nrow = 4, CPP = TRUE, type = "l")

# Fourth example
plotCurves(VIMCFX96_60, y = NULL, one.plot = TRUE, type = "l")
plotCurves(VIMCFX96_60, y = NULL, one.plot = FALSE, type = "l")
```

refMFI   *Class* "refMFI"

Description

An S4 class containing the output `MFIaggr` function. refMFI means referenced Mean Fluorescence Intensity (Roediger et al. 2013)

Value

A class for a `matrix` of amplifications curve data from a qPCR experiment.

Slots

`.Data`: `"matrix"` containing the "Cycle", "Location" (mean, median), "Deviation" (standard deviation, median absolute deviation), "Coefficient of Variance" (CV, RSD) sequential in the columns.

`density`: `"density"` containing results of the density analysis.

`qqnorm.data`: `"data.frame"` containing data required for Quantile-Quantile plots.

`stats`: `"numeric"` vector containing general statistics.

Methods

qqnorm signature(y = "refMFI"): plots a normal QQ plot. See `qqnorm`

qqline signature(y = "refMFI"): adds a line to QQ plot. See `qqline`

plot signature(x = "refMFI"): plots the object. See `plot.refMFI`

show signature(object = "refMFI"): prints only `.Data` slot of the object.

summary signature(object = "refMFI"): prints general statistics and allows easy access to stats slot. See `summary.refMFI`

Author(s)

Stefan Roediger, Michal Burdukiewicz

References

Roediger S, Boehm A, Schimke I. Surface Melting Curve Analysis with R. *The R Journal* 2013;5:37–53.

See Also

`MFIaggr`, `plot.refMFI`, `summary.refMFI`

Examples

```
data(VIMCFX96_60)

res <- MFIaggr(VIMCFX96_60)
#summarize analysis
summary(res)
#plot results of the analysis
plot(res)
#plot a QQ plot
qqnorm(res)
#add QQ line
qqline(res)
```

---

rounder   *Round der objects*

Description

inder calculates numeric derivatives on smoothed data which results in data points not observable in reality. The rounder function averages such result to the real values of cycle number.

Usage

```
rounder(object, cyc = 1)
```

Arguments

object	a class der object.
cyc	a column containing cycle numbers after smoothing. In case of objects created by the inder function, it is the first column.

Value

An object of the class der containing cycle number and averaged values of the fluorescence, first derivative and second derivative.

Author(s)

Stefan Roediger, Michal Burdukiewicz

See Also

inder, der

Examples

```
isPCR <- AmpSim(cyc = 1:40)
res <- inder(isPCR)
rd <- rounder(res)
plot(rd)
```

RPA	*Recombinase polymerase amplification (RPA) by Lutz et al.(2009)*

Description

Real-time amplification plot of an Recombinase Polymerase Amplification (RPA) by Lutz et al.(2009) in a centrifugal microfluidic foil cartridge.

Usage

```
data("RPA")
```

Format

A data frame with 184 observations on the following 2 variables.

Reaction.Time Reaction Time [min]

RFU Relative fluorescence units [RFU]

Details

The data were digitized as described by Poisot (2011). The image for data extraction was taken from Figure 3b by Lutz et al.(2009). The amplification curve present the results of a cross-contamination test of a foil disk for a sample containing 2000 copies of the mecA gene. For further experimental and technical details refer to Lutz et al.(2009).

Source

The original data (open access under CC BY-NC-ND license) were taken from Figure 3b by Lutz et al.(2009).

References

Poisot, T. (2011). The digitize Package: Extracting Numerical Data from Scatterplots. *The R Journal* 3, 25–26.

Lutz, S., Weber, P., Focke, M., Faltin, B., Roth, G., Piepenburg, O., Armes, N., Mark, D., Zengerle, R., and von Stetten, F. (2009). Isothermal Polymerase Amplification in a Centrifugal Microfluidic Foil Cartridge. *Procedia Chemistry* 1, 529–531.

Examples

```
data(RPA)
plot(RPA, main = "RPA by Lutz et al.(2009)", xlab = "Reaction Time [min]",
     ylab = "Relative fluorescence units [RFU]", type = "l")
abline(h = 5, lty = 2, col = "grey")
```

---

smoother   *Wrapper for Several Smoothers of Amplification Data*

Description

Smoother is a wrapper for several smoothing functions including LOWESS, Moving Average, Friedman's SuperSmoother, Cubic Spline and Savitzky-Golay smoothing filter, Friedman's Super-Smoother, and Whittaker smoother for amplification curve data.

Usage

```
## S4 method for signature 'numeric,numeric'
smoother(x, y, trans = FALSE,
    bg.outliers = FALSE, method = "savgol",
    CPP = TRUE, paralell = NULL)
## S4 method for signature 'matrix,missing'
smoother(x, y, trans = FALSE,
    bg.outliers = FALSE, method = "savgol",
    CPP = TRUE, paralell = NULL)
## S4 method for signature 'data.frame,missing'
smoother(x, y, trans = FALSE,
    bg.outliers = FALSE, method = "savgol",
    CPP = TRUE, paralell = NULL)
```

Arguments

x	x is a vector containing values for smoothing or data frame/matrix with values for smoothing.
y	y values for smoothing. Used only if x is also a vector.
trans	perform a linear transformation based on the trend of the background range.
bg.outliers	logical parameter which indicates of outliers should be removed from background range.
method	a list where each element is character vector representing a smoothing method or a named list of additional arguments to a smoothing algorithm. See Examples section. The Savitzky-Golay smoothing filter is the default smoother. Use "lowess" for LOWESS smoother (locally-weighted polynomial regression, "mova" for moving average, "savgol" for Savitzky-Golay smoothing filter, "smooth" for cubic spline smooth, "spline" for standard cubic spline smooth, "supsmu" for Friedman's SuperSmoother, "whit1" for weighted Whittaker smoothing with a first order finite difference penalty, "whit2" for weighted Whittaker smoothing

	with a second order finite difference penalty or "all" for all implemented smoothing algorithms. Both upper and lower case names are accepted.
CPP	logical parameter which indicates if CPP (curve pre-processor) should be used.
paralell	should contain a cluster object, created by package `parallel` or `snow` package. If `NULL`, no parallelization is used.

Details

Amplification curve data of experimental thermo-cyclers may deliver results which are hard to interpret due to noise and scatter. For data presentation it is often useful to smooth or filter the data prior to presentation. Smoothing and filtering are different approaches with a similar outcome to preprocess an input signal in order to make it available for an analysis step. Filtering uses methods of signal processing. They take a data input and apply a function to form an output. There are linear and non-linear filters. The most common example of a linear filter is the the moving average. A moving average filter replaces sequentially data points with the average of the neighbor data points. The average is calculated from a defined span ("window") of odd count (e.g., 3, 5). The average herein may also refer to the median, the geometric or exponential mean. Smoothing in contrast uses statistical approaches. Such approaches use for example local regression models (e.g., least squares estimate) or cubic splines. Splines apply non-parametric regression by local cubic polynomials between knot points. Other examples for smoothers include Savitzky-Golay smoothing filter, Friedman's SuperSmoother, and Whittaker smoother. Several methods were integrated in the `chipPCR` package. A careful evaluation of this preprocessing step is of high importance (Spiess et al. 2014).

smoother is a wrapper for smoother functions and filters commonly used to process amplification curve data. The smoother function was enhanced by functionality of the fixNA and CPP functions. The parameter "lowess" for LOWESS smoother (locally-weighted polynomial regression) can be tuned by the parameters f and iter (see lowess for details). The parameter "mova" for moving average can be tuned by the parameter movaww. movaww is the window size used for the moving average (see filter for details). The parameter "savgol" for Savitzky-Golay smoothing filter can be tuned by the parameter p and n (see sgolayfilt for details). The parameter "smooth" for cubic spline smooth can be tuned by the parameter df.fact. A df.fact value of 1 will leave the raw data almost unaffected while a value 0.5 will smooth the curve considerably. For further details refer to the smooth.spline function. The parameter "spline" for standard cubic spline smooth has currently no additional parameter. The parameter "supsmu" for Friedman's SuperSmoother can be tuned by the parameter span. For further details refer to the supsmu function. The parameter "lambda" is used in Weighted Whittaker smoothing.

Author(s)

Stefan Roediger, Michal Burdukiewicz

References

Roediger S, Boehm A, Schimke I. Surface Melting Curve Analysis with R. *The R Journal* 2013;5:37–53.

Spiess, A.-N., Deutschmann, C., Burdukiewicz, M., Himmelreich, R., Klat, K., Schierack, P., Roediger, S., 2014. Impact of Smoothing on Parameter Estimation in Quantitative DNA Amplification Experiments. *Clinical Chemistry* clinchem.2014.230656. doi:10.1373/clinchem.2014.230656

Examples

```
# Results of different smoothers. A in-silico amplification was performed
# using the AmpSim function and different smoothers were applied. Optimally
# all smoothers should give the same result (which is not the case)).
# refMFI means referenced Mean Fluorescence Intensity
# (Roediger et al. 2013)
tmp <- AmpSim(cyc = 1:35, bl = 0)

plot(tmp, main = "In-silico real-time PCR\n Effect of Smoother",
     xlab = "Cycles", ylab ="refMFI", ylim = c(0,1), pch = 20,
     type = "b", lwd = 2)

legend(25, 0.8, c("Raw data", "savgol", "lowess", "mova 3", "mova 5",
  "smooth", "spline", "supsmu"), pch = 20, lwd = 2,
  col = c(1:8))

#else
tmp.smooths <- smoother(tmp, method = list("savgol",
                                           "lowess",
                                           mova = list(movaww = 3),
                                           mova = list(movaww = 5),
                                           "smooth",
                                           "spline",
                                           "supsmu"))
for (i in 1:ncol(tmp.smooths))
  lines(tmp[, 1], tmp.smooths[, i], type = "b", pch = 20, lwd = 2, col = i
+ 1)

default.par <- par(no.readonly = TRUE)
par(fig = c(0.15,0.6,0.45,0.99), new = TRUE)
plot(tmp, main = "", xlab = "Cycles", ylab ="refMFI",
     pch = 20, xlim = c(14,20), ylim = c(0,0.45))

for (i in 1:ncol(tmp.smooths))
  lines(tmp[, 1], tmp.smooths[, i], type = "b", pch = 20, lwd = 2, col = i
+ 1)


# Plot the difference of the smoothed / filtered data
# to the raw data against the cycles
# The largest error is in the transition phases between
# start and end of the detectable amplification process.
par(fig = c(0,1,0,0.65))
plot(NA, NA, type = "b", col = 2, pch = 20, xlim = c(1,35),
     ylim = c(-0.1,0.1), xlab = "Cycle",
     ylab = "delta refMFI (raw - smoothed)",
     main = "Smoothed / Filtered data")

legend(1.5, 0.1, ncol = 2, c("savgol", "lowess", "mova 3", "mova 5",
"smooth", "spline", "supsmu"), pch = 20, lwd = 2,
col = c(2:8))
```

```
for (i in 1:ncol(tmp.smooths))
  lines(tmp[, 1], tmp[, 2] - tmp.smooths[, i], type = "b", pch = 20, lwd =
2, col = i + 1)

par(fig = c(0,1,0.55,1), new = TRUE)

plot(tmp, type = "b", col = 1, pch = 20, xlab = "", ylab = "RFU",
     main = "Raw data")

#different ways of using smoother
#1. single method
single.smooth <- smoother(tmp, method = list("mova"))
#single smooth, additional argument specified
single.smooth.add <- smoother(tmp, method = list(mova = list(movaww = 3)))
#3. more than one smoothing method, no additional arguments specified
double.smooth <- smoother(tmp, method = list("savgol", "mova"))
#4. more than one smoothing method, additional arguments specified
double.smooth.add <- smoother(tmp, method = list("savgol", mova =
list(movaww = 3)))
#5. all smoothing methods, no additional arguments specified
all.smooth <- smoother(tmp, method = list("all"))
par(default.par)
```

---

summary-bg   *Summary* bg *objects*

Description

Summarize bg objects and easily access results of bg function.

Usage

```
## S4 method for signature 'bg'
summary(object, print = TRUE)
```

Arguments

object   is a bg object.

print   is a logical value determining if pretty summary of the object should be printed.

Author(s)

Stefan Roediger, Michal Burdukiewicz

See Also

bg

Examples

```
res <- AmpSim(cyc = 1:40, Cq = 25)
background <- bg.max(res[, 1], res[, 2])
#just print summary
summary(background)

#assign summary to variable without printing
vals <- summary(background, print = FALSE)
print(vals)

#easily access different values
vals["FDM"]
```

---

summary-der   *Summary* der *objects*

Description

Summarize [der] objects and easily access results of [inder] function.

Usage

```
## S4 method for signature 'der'
summary(object, digits = getOption("digits") - 3, print = TRUE)
```

Arguments

object	is a [der] object.
digits	is a numeric value determining the number of decimal places. Used only for printed values. See [format].
print	is a logical value determining if pretty summary of the object should be printed.

Details

The approximate second derivative maximum (SDM) which is commonly used to quantify quantitative real-time PCR experiments. The SDM might also be useful for isothermal amplification processes. The SDM is calculated from a derived cubic spline. Similarly the first approximate approximate derivative maximum (FDM), second derivative minimum (SDm), and approximate second derivative center (SDC, geometric mean of SDM and SDm) are available. FDM, SDm and SDC values can be used to further characterize the amplification process.

Value

A named vector of four elements. The element named 2nd_der contains values of the second first derivative. The element named SDM contains approximate second derivative maximum, SDm contains approximate second derivative minimum, and SDC contains approximate second derivative center.

Author(s)

Stefan Roediger, Michal Burdukiewicz

References

Ruijter JM, Pfaffl MW, Zhao S, et al. (2013) Evaluation of qPCR curve analysis methods for reliable biomarker discovery: bias, resolution, precision, and implications. *Methods* San Diego Calif 59:32–46.

See Also

[der](#)

Examples

```
res <- AmpSim(cyc = 1:40, Cq = 25)
test.der <- inder(res)
#just print summary
summary(test.der)
#print without rounding
summary(test.der, digits = 6)

#assign summary to variable without printing and
vals <- summary(test.der, print = FALSE)
print(vals)

#easily access different values
vals["bg.start"]
```

---

summary-refMFI   *Summary* refMFI *objects*

Description

Summarize [refMFI](#) objects and access general statistics of amplification reaction. refMFI means referenced Mean Fluorescence Intensity (Roediger et al. 2013).

Usage

```
## S4 method for signature 'refMFI'
summary(object, digits = getOption("digits") - 3, print = TRUE)
```

Arguments

object	is a [refMFI](#) object.
digits	is a numeric value determining the number of decimal places. Used only for printed values. See [format](#).
print	is a logical value determining if pretty summary of the object should be printed.

Author(s)

Stefan Roediger, Michal Burdukiewicz

References

Roediger S, Boehm A, Schimke I. Surface Melting Curve Analysis with R. *The R Journal* 2013;5:37–53.

See Also

refMFI

Examples

```
res <- AmpSim(cyc = 1:50, Cq = 41)
summary(inder(res), print = TRUE)
```

---

th   *Class* "th"

Description

An S4 class containing the output `th.cyc` function.

Value

A class for calculated results (Ct value and fluorescence) from amplifications curve data of a qPCR experiment.

Slots

`.Data`: `"matrix"` is a matrix containing the threshold cycle and threshold fluorescence.

`stats`: `"summary.lm"` contains linear model used for Ct estimation.

`input`: `"matrix"` input data for linear model.

Methods

summary signature(object = "th"): prints summary of the object.

show signature(object = "th"): prints only `.Data` slot of the object.

Author(s)

Stefan Roediger, Michal Burdukiewicz

See Also

[th.cyc](th.cyc)

Examples

```
res <- th.cyc(VIMCFX96_69[, 1], VIMCFX96_69[, 3], r = 2300)
summary(res)
slot(res, "input")
```

---

th.cyc *Threshold Cycle*

Description

`th.cyc` calculates the number of cycles at which the fluorescence exceeds a defined threshold, called the threshold cycle (Ct). According to the MIQE guidelines the Ct is referred to as quantification cycle (Cq). The calculated Cq is a relative value, which depends on the template copy number, instrument, reagents, amplification efficiency and probe technology. Low Cqs correlate with high quantities template copy numbers. Real-time technologies enable the quantification of nucleic acids by calculation of specific curve parameters like the quantification point (Cq) and the amplification efficiency (AE) based on the kinetics of the amplification curve. The Cq represents the number of cycles (time for qIA) needed to reach a defined fluorescence signal level in the exponential phase of the amplification curve. The Cq can be determined from a fixed threshold value or by various analytical algorithm as described elsewhere (Bustin et al. 2009, Ruijter et al. 2013, Tellinghuisen et al. 2014).

Usage

```
th.cyc(x, y, r = 2500, auto = FALSE, linear = TRUE)
```

Arguments

x	is a vector containing the time or cycle values.
y	is a vector containing the fluorescence values.
r	a fluorescence value which defines the threshold.
auto	is logical parameter which indicates if an automatic estimation of the threshold should be used (Note: Experimental, not safe to use).
linear	is logical parameter which indicates if a linear or quadratic regression should be used for the calculation. (Note: Experimental, not safe to use).

Details

The Threshold Cycle (Ct) (Cq according to MIQE, see Bustin et al. 2009) is the cycle number at which the fluorescence exceeds significantly a point above the baseline and defined threshold in a particular samples. Thus the Ct is the cycle when sufficient numbers of amplicons have accumulated. The `th.cyc` calculates the intersection of the user defined Ct value (`r`) and a linear regression or quadratic polynomial in the range of the user defined Ct value. In contrast to other methods, `th.cyc` have no requirement to fit a "complex" non linear model to the entire data set but rather focuses on the specific area. The polynomial is calculated from four neighbor values at the fluorescence threshold.

Value

An object of class `th`.

Author(s)

Stefan Roediger, Michal Burdukiewicz

References

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Examples

```
# First example
# Raw data from the VIMCFX96_69 data set.
# Cycles
x <- VIMCFX96_69[, 1]
# Fluoresce values
y <- VIMCFX96_69[, 2]

# Plot the raw data
plot(x, y, xlab = "Cycle", ylab = "Fluo")
# Calculate the the Ct value
res <- th.cyc(x, y, r = 2300)
lines(res@input, col = 2, lwd = 2)
# Threshold fluorescence value
abline(h = res@.Data[2], col = 3)
# Calculated Ct value
abline(v = res@.Data[1], col = 4)

# Second example
# Application of the th.cyc method to determine the Cq from a continous
# amplification reaction.
plot(NA, NA, xlim = c(0,80), ylim = c(0,1200), xlab = "Time [min]",
     ylab = "Voltage [micro V]", main = "ccPCR - Raw Data")

# Threshold level "r" (50 micro Volts)
for (i in c(1,3,5,7)) {
```

```
  y.tmp <- capillaryPCR[, i + 1] - mean(capillaryPCR[1L:150, i + 1])
  Ct.tmp <- th.cyc(capillaryPCR[, i], y.tmp, r = 50, linear = FALSE)
  abline(v = Ct.tmp[1])
  text(Ct.tmp[1] * 1.1, 1200, paste(round(Ct.tmp[1], 1), "\nmin"))
  lines(capillaryPCR[, i], y.tmp, type = "b", pch = 20 - i)
  points(Ct.tmp@input, col = "red", pch = 19)
}
abline(h = 50)
legend(5,800, c("Run 1", "Run 2", "Run 3", "Control"), pch = c(19, 17, 15, 13),
       lwd = 1.5)
```

VIMCFX96_60   *Amplification Reaction Using the Bio-Rad CFX96*

Description

Data set of an amplification reaction using the Bio-Rad CFX96 thermo cycler. The samples of Vimentin were amplified in the CFX96 as replicates according to Roediger et al. (2013). The quantification was performed during the annealing step (60 degrees Celsius).

Usage

```
data(VIMCFX96_60)
```

Format

A data frame with 40 observations on the following 97 variables. The first column ("Cycle") contains the number of cycles and consecutive columns contain the replicates ("A1" to "H12").

Source

Stefan Roediger, Claudia Deutschmann (BTU Cottbus - Senftenberg)

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Examples

```
data(VIMCFX96_60)
data(VIMCFX96_69)

T60 <- rowMeans(VIMCFX96_60[, 2:ncol(VIMCFX96_60)])
T69 <- rowMeans(VIMCFX96_69[, 2:ncol(VIMCFX96_69)])
```

```
plot(1:length(T60), T60, main = "Fluorescence at different
      temperatures\nQuantification in CFX96 (Bio-Rad)", xlab = "Cycle",
      ylab = "Cycle dependent fluorescence", pch = 15, type = "b")
lines(1:length(T69), T69, pch = 19, type = "b", col = 2)
legend(1, 4500, c("Annealing (60 deg C)", "Elongation (69 deg C)"),
pch = c(15, 19), col = c(1,2))
```

VIMCFX96_69   *Amplification Reaction Using the Bio-Rad CFX96*

Description

Data set of an amplification reaction using the Bio-Rad CFX96 thermo cycler. The samples of
Vimentin were amplified in the CFX96 as replicates according to Roediger et al. (2013). The
quantification was performed during the elongation step (69 degrees Celsius).

Usage

```
data(VIMCFX96_69)
```

Format

A data frame with 40 observations on the following 97 variables. The first column ("Cycle") con-
tains the number of cycles and consecutive columns contain the replicates ("A1" to "H12").

Source

Stefan Roediger, Claudia Deutschmann (BTU Cottbus - Senftenberg)

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time De-
tection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J.
Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W.
Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74,
2013.

Examples

```
data(VIMCFX96_60)
data(VIMCFX96_69)

T60 <- rowMeans(VIMCFX96_60[, 2:ncol(VIMCFX96_60)])
T69 <- rowMeans(VIMCFX96_69[, 2:ncol(VIMCFX96_69)])

plot(1:length(T60), T60, main = "Fluorescence at different
      temperatures\nQuantification in CFX96 (Bio-Rad)", xlab = "Cycle",
      ylab = "Cycle dependent fluorescence", pch = 15, type = "b")
lines(1:length(T69), T69, pch = 19, type = "b", col = 2)
```

```
legend(1, 4500, c("Annealing (60 deg C)", "Elongation (69 deg C)"),
pch = c(15, 19), col = c(1,2))
```

VIMCFX96_meltcurve   *Melting Curve Measured with the Bio-Rad CFX96*

Description

Data set of a melting curve using the Bio-Rad CFX96 thermo cycler. The samples of Vimentin were measured in the CFX96 as replicates according to Roediger et al. (2013). The quantification was performed during the gradient from 55 to 95 degrees Celsius with a resolution of 0.5 degrees Celsius per step.

Usage

```
data(VIMCFX96_meltcurve)
```

Format

A data frame with 81 observations on the following 97 variables. The first column ("Temperature") contains the temperature and consecutive columns contain the replicates ("A1" to "H12").

Source

Stefan Roediger, Claudia Deutschmann (BTU Cottbus - Senftenberg)

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Examples

```
data(VIMCFX96_meltcurve)
tmp <- VIMCFX96_meltcurve

plot(NA, NA, xlim = c(55,95), ylim = c(2000, 7000), xlab = "Temperature
    (degrees Celsius)",
    ylab = "RFU", main = "Melting curve in CFX96 (Bio-Rad)")
apply(tmp[, 2:ncol(tmp)], 2,
      function(x) lines(tmp[1:nrow(tmp),1],x))

Fmean <- rowMeans(tmp[, 2:ncol(tmp)])
lines(tmp[1:nrow(tmp),1], Fmean, col = "red", lwd = 3)

legend(55, 4000, c("Raw", "Mean"), pch = c(19,19), col = c(1,2))
```

VIMiQ5_595 *Amplification Reaction Using the Bio-Rad iQ5*

Description

Data set of an amplification reaction using the Bio-Rad iQ5 thermo cycler. The samples of Vimentin were amplified in the iQ5 as replicates according to Roediger et al. (2013). The quantification was performed during the annealing step (59.5 degrees Celsius).

Usage

```
data(VIMiQ5_595)
```

Format

A data frame with 40 observations on the following 97 variables. The first column ("Cycles") contains the number of cycles and consecutive columns contain the replicates ("A01" to "H12").

Source

Stefan Roediger, Claudia Deutschmann (BTU Cottbus - Senftenberg)

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Examples

```
T595 <- rowMeans(VIMiQ5_595[, 2:ncol(VIMiQ5_595)])
T685 <- rowMeans(VIMiQ5_685[, 2:ncol(VIMiQ5_685)])

plot(1:length(T595), T595, main = "Fluorescence at different
    temperatures\nQuantification in iQ5 (Bio-Rad)", xlab = "Cycle",
    ylab = "Cycle dependent fluorescence", pch = 15, type = "b")
lines(1:length(T685), T685, pch = 19, type = "b", col = 2)
legend(1, 10000, c("Annealing (59.5 deg C)", "Elongation (68.5 deg C)"),
pch = c(15, 19), col = c(1,2))
```

VIMiQ5_685	*Amplification Reaction Using the Bio-Rad iQ5*

Description

Data set of an amplification reaction using the Bio-Rad iQ5 thermo cycler. The samples of Vimentin were amplified in the iQ5 as replicates according to Roediger et al. (2013). The quantification was performed during the elongation step (68.5 degrees Celsius).

Usage

```
data(VIMiQ5_685)
```

Format

A data frame with 40 observations on the following 97 variables. The first column ("Cycles") contains the number of cycles and consecutive columns contain the replicates ("A01" to "H12").

Source

Stefan Roediger, Claudia Deutschmann (BTU Cottbus - Senftenberg)

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Examples

```
T595 <- rowMeans(VIMiQ5_595[, 2:ncol(VIMiQ5_595)])
T685 <- rowMeans(VIMiQ5_685[, 2:ncol(VIMiQ5_685)])

plot(1:length(T595), T595, main = "Fluorescence at different
      temperatures\nQuantification in iQ5 (Bio-Rad)", xlab = "Cycle",
      ylab = "Cycle dependent fluorescence", pch = 15, type = "b")
lines(1:length(T685), T685, pch = 19, type = "b", col = 2)
legend(1, 10000, c("Annealing (59.5 deg C)", "Elongation (68.5 deg C)"),
      pch = c(15, 19), col = c(1,2))
```

VIMiQ5_melt   *Melting Curve Measured with the Bio-Rad iQ5*

Description

Data set of a melting curve using the Bio-Rad iQ5 thermo cycler. The samples of Vimentin were measured in the CFX96 as replicates according to Roediger et al. (2013). The quantification was performed during the gradient from 55 to 95 degrees Celsius with a resolution of 0.5 degrees Celsius per step.

Usage

```
data(VIMiQ5_melt)
```

Format

A data frame with 81 observations on the following 97 variables. The first column ("T") contains the temperature and consecutive columns contain the replicates ("A01" to "H12").

Source

Stefan Roediger, Claudia Deutschmann (BTU Cottbus - Senftenberg)

References

A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. S. Roediger, P. Schierack, A. Boehm, J. Nitschke, I. Berger, U. Froemmel, C. Schmidt, M. Ruhland, I. Schimke, D. Roggenbuck, W. Lehmann and C. Schroeder. *Advances in Biochemical Bioengineering/Biotechnology*. 133:33–74, 2013.

Examples

```
data(VIMiQ5_melt)
tmp <- VIMiQ5_melt

plot(NA, NA, xlim = c(55,95), ylim = c(0, 40000),
    xlab = "Temperature (degrees Celsius)",ylab = "RFU",
    main = "Melting curve in iQ5 (Bio-Rad)")
apply(tmp[, 2:ncol(tmp)], 2,
      function(x) lines(tmp[1:nrow(tmp),1],x))

Fmean <- rowMeans(tmp[, 2:ncol(tmp)])
lines(tmp[1:nrow(tmp),1], Fmean, col = "red", lwd = 3)

legend(55, 4000, c("Raw", "Mean"), pch = c(19,19), col = c(1,2))
```

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