

rtpcr package

'rtpcr' package was developed for amplification efficiency calculation, statistical analysis and graphical display of real-time PCR data in R. By accounting for up to two reference genes and amplification efficiency values, a general calculation methodology covering both the Livak and Pfaffl methods was used. Based on the experimental conditions, the functions of the 'rtpcr' package use a t-test (for experiments with a two-level factor), analysis of variance or covariance (for cases where more than two levels or factors or a blocking factor exist) to calculate the fold change (FC) or relative expression (RE). The functions further provide standard deviations and confidence limits for means, apply statistical mean comparisons and present letter mean grouping. To facilitate function application, different data sets were used as examples and the outputs were explained. An outstanding feature of 'rtpcr' package is providing publication-ready bar plots with various controlling arguments for experiments with up to three different factors.

A

Analysis type	Column arrangement of the input data frame (x)
Amplification efficiency	Dilutions - targetCt - refCt
t-test (accepts multiple genes)	condition (control level first) - gene (ref gene(s) last)- efficiency - Ct
ANOVA or ANCOVA (Up to three factors)	factor1 - rep - targetE - targetCt - refE - refCt
	factor1 - factor2 - rep - targetE - targetCt - refE - refCt
	factor1 - factor2 - factor3 - rep - targetE - targetCt - refE - refCt
ANOVA or ANCOVA with blocking	factor1 - block - rep - targetE - targetCt - refE - refCt
	factor1 - factor2 - block - rep - targetE - targetCt - refE - refCt
	factor1 - factor2 - factor3 - block - rep - targetE - targetCt - refE - refCt
with two reference genes rep - targetE - targetCt - ref1E - ref1Ct - ref2E - ref2Ct
calculating biological replicated biologicalRep - techcicalRep - Etarget - targetCt - Eref - refCt
 biolRep - techRep - Etarget - targetCt - ref1E - ref1Ct - ref2E - ref2Ct

B

Output tables & objects

<code>efficiency()</code> standard curves Slope, Efficiency, & R2	<code>qpcrANCOVA()</code> Raw data table factorial-based lm and ANOVA table ANCOVA table Fold Change statistics
<code>qpcrTTEST()</code> Raw data table Fold Change statistics	<code>meanTech()</code> Table with mean of technical replicates
<code>qpcrANOVA()</code> Raw data table CRD-based lm and ANOVA table Relative Expression statistics	<code>multiplot()</code> Producing multiple plots plate using ggplot objects

I

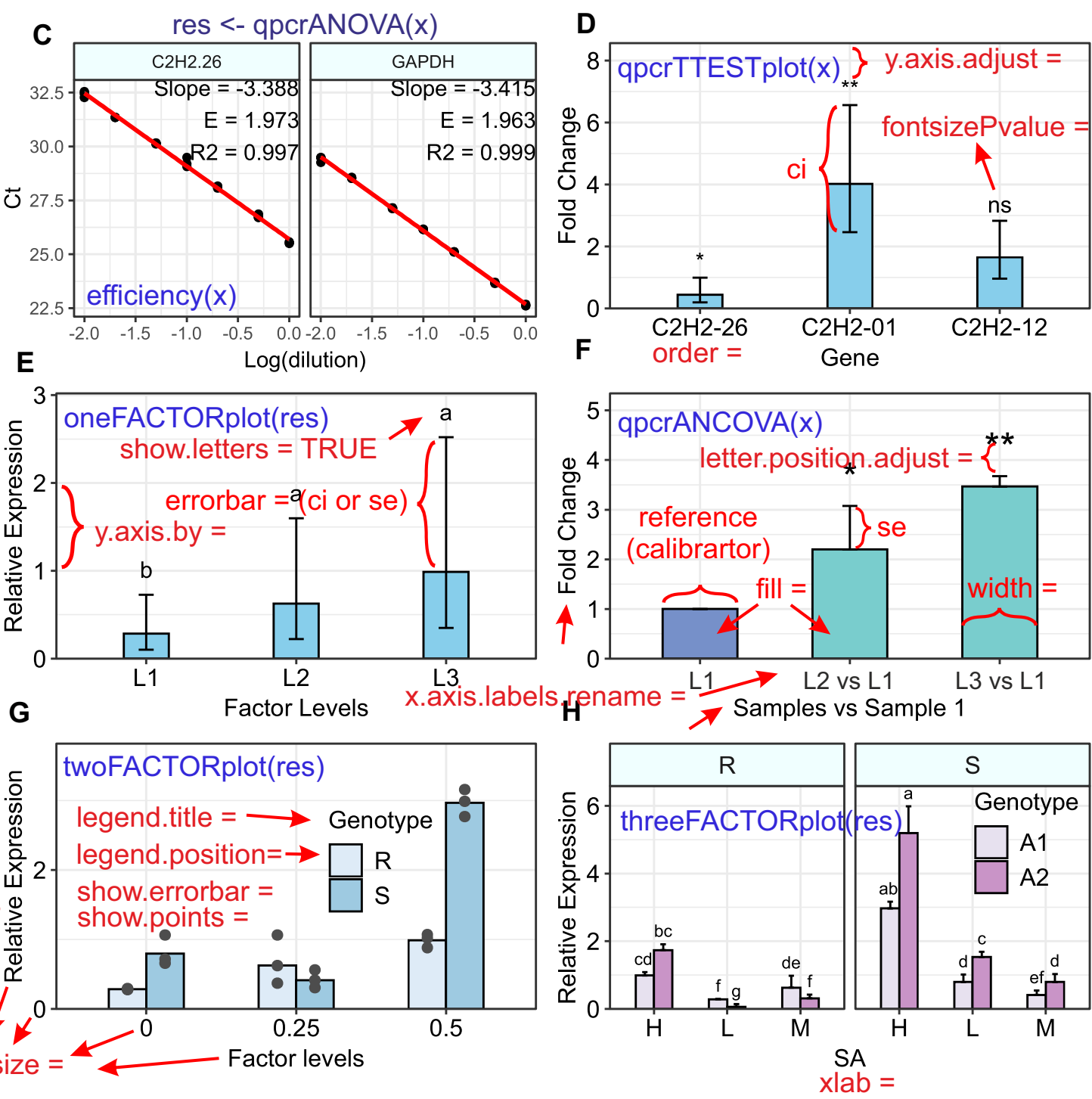
	Gene	dif	FC	LCL	UCL	pvalue
1	C2H2-26	0.3592	0.4373	0.1926	0.9927	0.0488
2	C2H2-01	-0.6041	4.0185	2.4598	6.5649	0.0014
3	C2H2-12	-0.2167	1.6472	0.9595	2.8279	0.0624

qpcrANCOVA()

	contrast	FC	pvalue	sig	LCL	UCL	sddiff
1	D7	1	1.0000		0.0000000	0.0000000	0.0000000
2	D12 vs D7	0.8903	0.8204	ns	0.2481961	3.193547	0.694117
3	D15 vs D7	0.1912	0.0028	**	0.0680464	0.537501	0.109213
4	D18 vs D7	0.0206	0.0000	***	0.0057234	0.074066	0.016105

J

<code>qpcrTTESTplot(x,</code> <code>order = "none",</code> <code>numberOfrefGenes,</code> <code>paired = FALSE,</code> <code>var.equal = TRUE,</code> <code>width = 0.5,</code> <code>fill = "skyblue",</code> <code>y.axis.adjust = 0,</code> <code>y.axis.by = 2,</code> <code>letter.position.adjust = 0.3,</code> <code>ylab = "Average Fold Change",</code> <code>xlab = "none",</code> <code>fontsize = 12,</code> <code>fontsizePvalue = 7,</code> <code>axis.text.x.angle = 0,</code> <code>axis.text.x.hjust = 0.5)</code>	<code>qpcrANCOVA(x,</code> <code>numberOfrefGenes,</code> <code>analysisType = "ancova",</code> <code>mainFactor.column,</code> <code>mainFactor.level.order,</code> <code>block = NULL,</code> <code>width = 0.5,</code> <code>fill = "#BFEFFF",</code> <code>y.axis.adjust = 1,</code> <code>y.axis.by = 1,</code> <code>letter.position.adjust = 0.1,</code> <code>ylab = "Fold Change",</code> <code>xlab = "none",</code> <code>fontsize = 12,</code> <code>fontsizePvalue = 7,</code> <code>axis.text.x.angle = 0,</code> <code>axis.text.x.hjust = 0.5,</code> <code>x.axis.labels.rename = "none",</code> <code>p.adj = "none")</code>	<code>qpcrANOVA(x,</code> <code>numberOfrefGenes,</code> <code>block = NULL,</code> <code>p.adj = "none", ...)</code> <code>oneFACTORplot(res,</code> <code>width = 0.2,</code> <code>fill = "skyblue",</code> <code>y.axis.adjust = 0.5,</code> <code>y.axis.by = 2,</code> <code>errorbar = "std",</code> <code>show.letters = TRUE,</code> <code>letter.position.adjust = 0.1,</code> <code>ylab = "Relative Expression",</code> <code>xlab = "none",</code> <code>fontsize = 12,</code> <code>fontsizePvalue = 7,</code> <code>axis.text.x.angle = 0,</code> <code>axis.text.x.hjust = 0.5)</code>	<code>twoFACTORplot(res,</code> <code>x.axis.factor,</code> <code>group.factor,</code> <code>width = 0.5,</code> <code>fill = "Blues",</code> <code>y.axis.adjust = 0.5,</code> <code>y.axis.by = 2,</code> <code>show.errorbars = TRUE,</code> <code>errorbar = "std",</code> <code>show.letters = TRUE,</code> <code>show.points = FALSE,</code> <code>letter.position.adjust = 0.1,</code> <code>ylab = "Relative Expression",</code> <code>xlab = "none",</code> <code>legend.position = c(0.09, 0.8),</code> <code>fontsize = 12,</code> <code>fontsizePvalue = 7,</code> <code>axis.text.x.angle = 0,</code> <code>axis.text.x.hjust = 0.5)</code>	<code>threeFACTORplot(res,</code> <code>arrangement = c(1, 2, 3),</code> <code>bar.width = 0.5,</code> <code>fill = "Reds",</code> <code>xlab = "none",</code> <code>ylab = "Relative Expression",</code> <code>errorbar = "std",</code> <code>y.axis.adjust = 0.5,</code> <code>y.axis.by = 2,</code> <code>letter.position.adjust = 0.3,</code> <code>legend.title = "Legend Title",</code> <code>legend.position = c(0.4, 0.8),</code> <code>fontsize = 12,</code> <code>fontsizePvalue = 7,</code> <code>show.letters = TRUE,</code> <code>axis.text.x.angle = 0,</code> <code>axis.text.x.hjust = 0.5)</code>	<code>qpcrTTEST(x,</code> <code>numberOfrefGenes,</code> <code>paired = FALSE,</code> <code>var.equal = FALSE)</code> <code>efficiency(x)</code> <code>meanTech(x, groups)</code> <code>multiplot(..., cols = 1)</code>
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For details about how to prepare data and how to use functions, refer to the rtpcr package examples.

qpcrANOVA()

	factor1	factor2	RE	LCL	UCL	letters	std
R:0	R	0	0.2852	0.4101	0.1983	d	0.0072
R:0.25	R	0.25	0.6271	0.9017	0.4361	bc	0.3508
R:0.5	R	0.5	0.9885	1.4214	0.6875	b	0.0979
S:0	S	0	0.7955	1.1439	0.5533	b	0.2190
S:0.25	S	0.25	0.4147	0.5962	0.2884	cd	0.1289
S:0.5	S	0.5	2.9690	4.2692	2.0648	a	0.1955

efficiency()

	Gene	Slope	E	R2
1	C2H2.26	-3.388	1.973	0.997
2	GAPDH	-3.415	1.963	0.999

\$Slope_of_differences
[1] 0.0264574