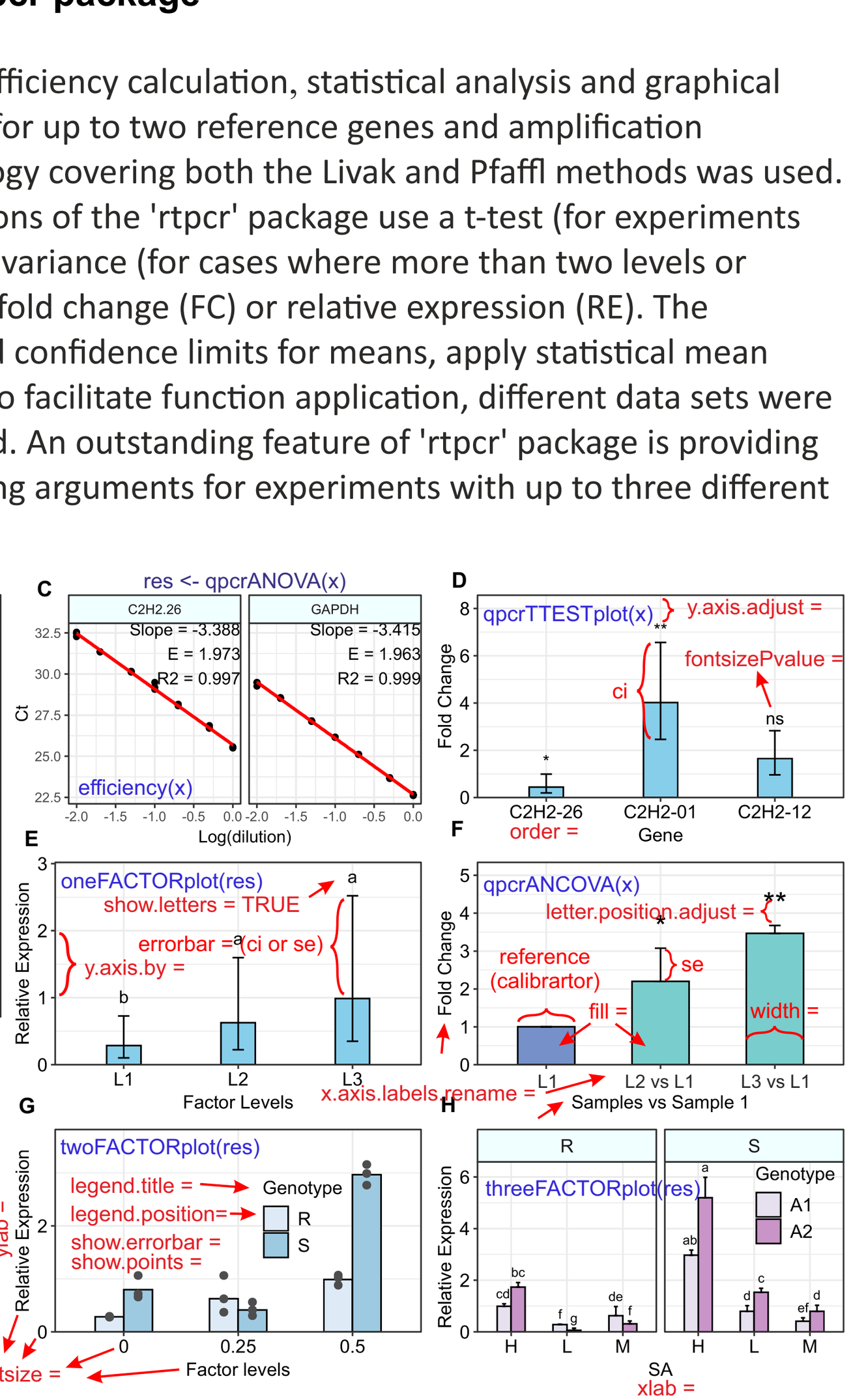
rtpcr package

'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

efficiency()
standard curves
Slope, Efficiency, & R2

qpcrTTEST()
Raw data table
Fold Change statistics

qpcrANOVA()
Raw data table
CRD-based lm and ANOVA table
Relative Expression statistics

qpcrANCOVA()
Raw data table
factorial-based lm and ANOVA table
ANCOVA table
Fold Change statistics

meanTech()
Table with mean of technical replicates

multiplot()
Producing multiple plots plate using ggplot objects

I

qpcrTTEST()

```
     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 0.000000 0.000000
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
```

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()

```
Efficiency_Analysis_Results
     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
```

J

qpcrTTESTplot(x,
order = "none",
numberOfrefGenes,
paired = FALSE,
var.equal = TRUE,
width = 0.5,
fill = "skyblue",
y.axis.adjust = 0,
y.axis.by = 2,
letter.position.adjust = 0.3,
ylab = "Average Fold Change",
xlab = "none",
fontsize = 12,
fontsizePvalue = 7,
axis.text.x.angle = 0,
axis.text.x.hjust = 0.5)

qpcrANCOVA(x,
numberOfrefGenes,
analysisType = "ancova",
mainFactor.column,
mainFactor.level.order,
block = NULL,
width = 0.5,
fill = "#BFEFFF",
y.axis.adjust = 1,
y.axis.by = 1,
letter.position.adjust = 0.1,
ylab = "Fold Change",
xlab = "none",
fontsize = 12,
fontsizePvalue = 7,
axis.text.x.angle = 0,
axis.text.x.hjust = 0.5,
x.axis.labels.rename = "none",
p.adj = "none")

qpcrANOVA(x,
numberOfrefGenes,
block = NULL,
p.adj = "none", ...)

oneFACTORplot(res,
width = 0.2,
fill = "skyblue",
y.axis.adjust = 0.5,
y.axis.by = 2,
errorbar = "std",
show.letters = TRUE,
letter.position.adjust = 0.1,
ylab = "Relative Expression",
xlab = "none",
fontsize = 12,
fontsizePvalue = 7,
axis.text.x.angle = 0,
axis.text.x.hjust = 0.5)

twoFACTORplot(res,
x.axis.factor,
group.factor,
width = 0.5,
fill = "Blues",
y.axis.adjust = 0.5,
y.axis.by = 2,
show.errorbars = TRUE,
errorbar = "std",
show.letters = TRUE,
show.points = FALSE,
letter.position.adjust = 0.1,
ylab = "Relative Expression",
xlab = "none",
legend.position = c(0.09, 0.8),
fontsize = 12,
fontsizePvalue = 7,
axis.text.x.angle = 0,
axis.text.x.hjust = 0.5)

threeFACTORplot(res,
arrangement = c(1, 2, 3),
bar.width = 0.5,
fill = "Reds",
xlab = "none",
ylab = "Relative Expression",
errorbar = "std",
y.axis.adjust = 0.5,
y.axis.by = 2,
letter.position.adjust = 0.3,
legend.title = "Legend Title",
legend.position = c(0.4, 0.8),
fontsize = 12,
fontsizePvalue = 7,
show.letters = TRUE,
axis.text.x.angle = 0,
axis.text.x.hjust = 0.5)

qpcrTTEST(x,
numberOfrefGenes,
paired = FALSE,
var.equal = FALSE)

efficiency(x)

meanTech(x, groups)

multiplot(..., cols = 1)