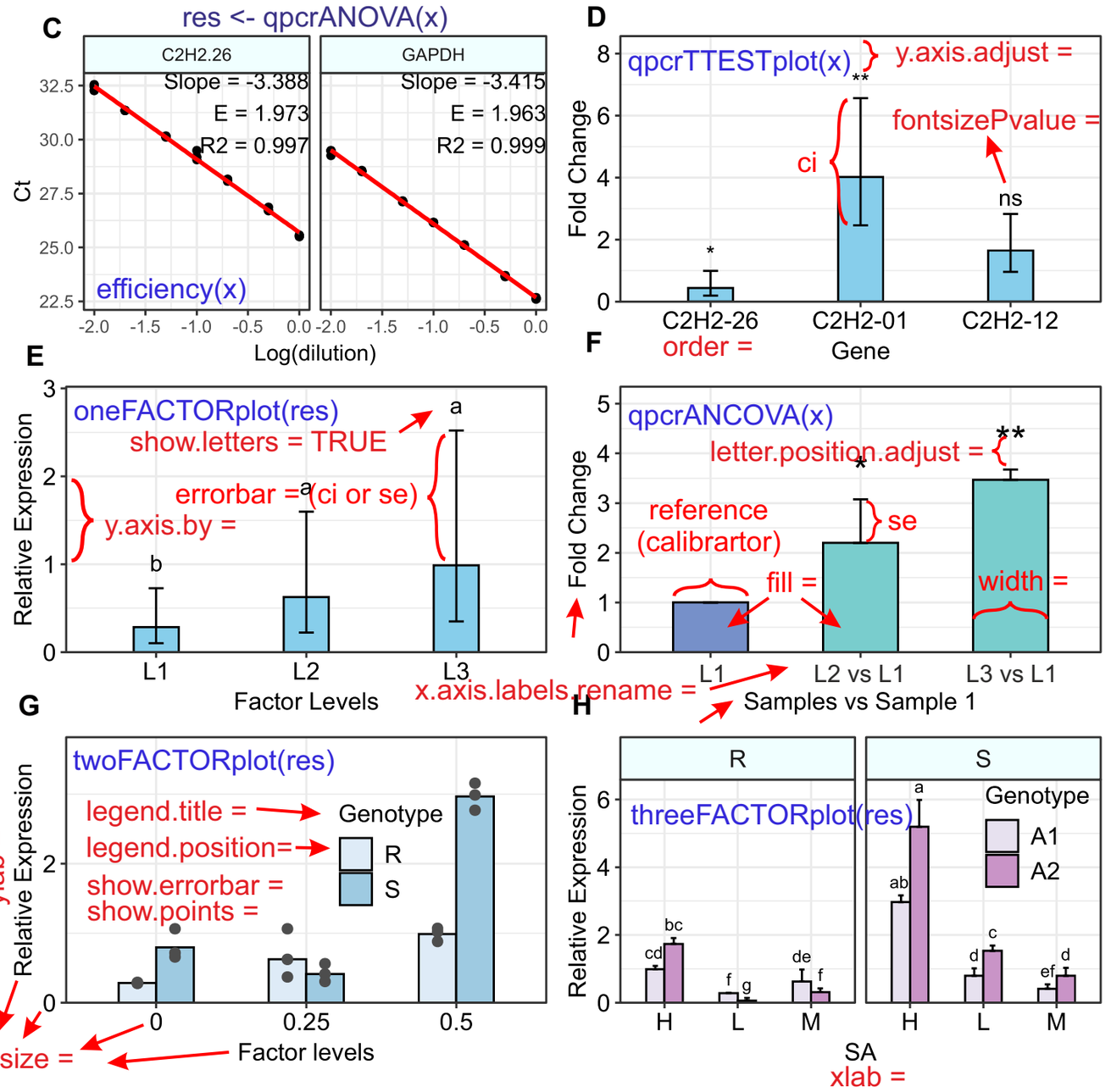
rtpcr package

'rtpcr' package was developed for amplification efficiency calculation, statistical analysis and graphical display of real-time PCR data in R.

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)