rtpcr package (version >= 1.0.7)

'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

NOTE: For ANOVA and ANCOVA analysis, each line in the input data set belongs to a separate individual (reflecting a non-repeated measure experiment).

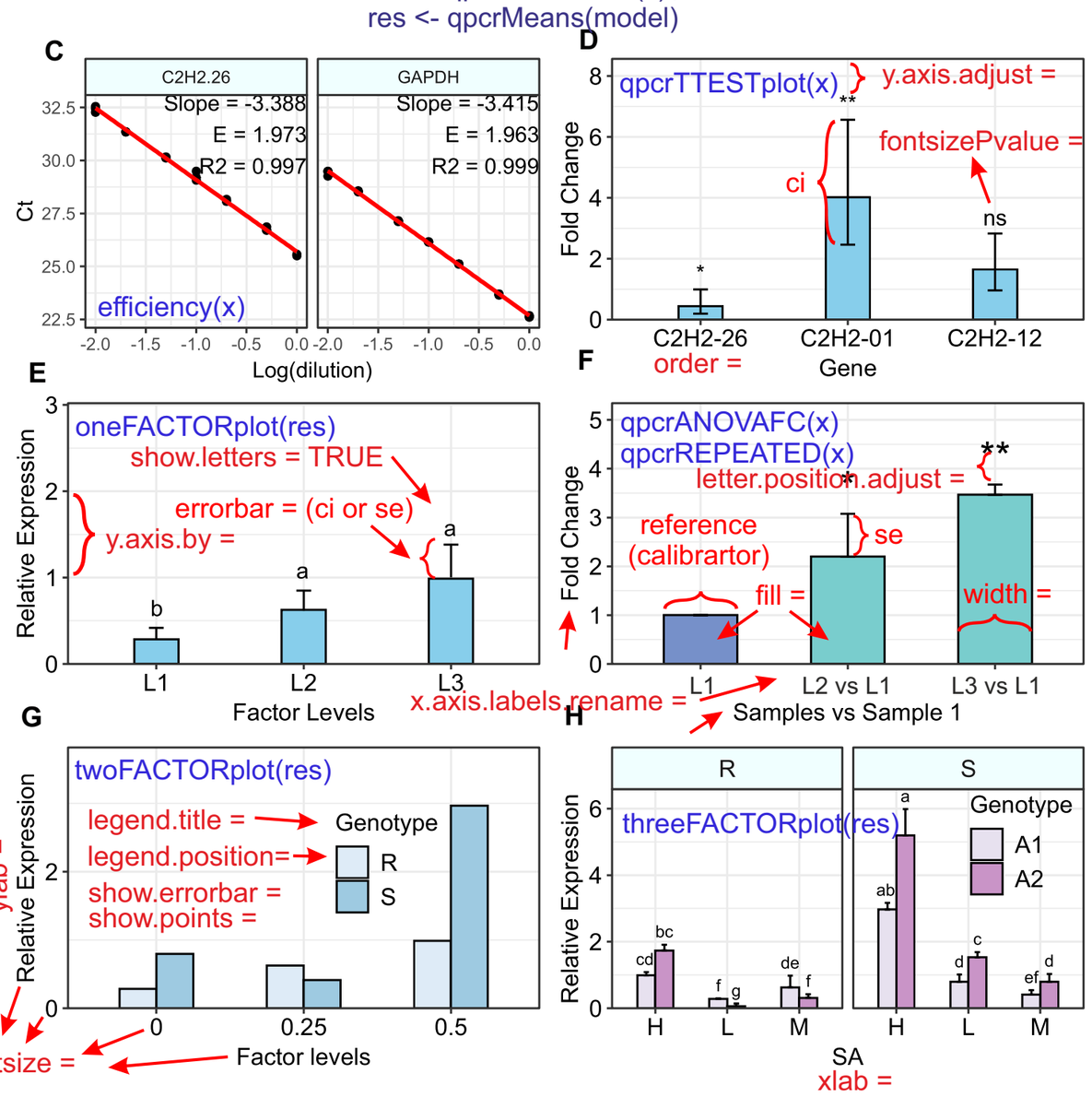
B

Repeated measure data structure

Column arrangement of the input data	Example in the package
id - time - targetE - targetCt - ref1E - ref1Ct	data_repeated_measure_1
id - time - targetE - targetCt - ref1E - ref1Ct - ref2E - ref2Ct	
id - treatment - time - targetE - targetCt - ref1E - ref1Ct	data_repeated_measure_2
id - treatment - time - targetE - targetCt - ref1E - ref1Ct - ref2E - ref2Ct	

NOTE: In the "id" column of a repeated measure data frame, a unique number is assigned to each individual, e.g. all the three number 1 indicate one individual.

```
res <- qpcrANOVARE(x)$Result
res <- qpcrMeans(model)
```



I

Output tables & objects

qpcrTTEST()
Raw data table
Fold Change statistics

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

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

qpcrREPEATED()
Raw data table
lm and ANOVA
Fold Change statistics

meanTech()
Table with mean of technical replicates

multiplot()
Producing multiple plots plate using ggplot objects

efficiency()
standard curves
Slope, Efficiency, & R2

J

qpcrREPEATED

```
       contrast     FC pvalue sig     LCL    UCL       se
1         time1 1.0000 1.0000     0.00000 0.0000 0.703589
2 time2 vs time1 1.2555 0.5540     0.43805 3.5987 0.532338
3 time3 vs time1 2.5432 0.0350   * 0.88731 7.2895 0.707415
```

qpcrANOVAFC()

```
  contrast     FC pvalue sig    LCL    UCL     se
1       D0 1.0000 1.0000     0.0000 0.0000 0.3445
2 D1 vs D0 1.0705 0.8051     0.5266 2.1762 0.2631
3 D2 vs D0 3.5967 0.0003 *** 1.7693 7.3116 0.3576
```

qpcrTTEST()

```
     Gene     dif     FC    LCL    UCL pvalue     se
1 C2H2-26  1.1933 0.4373 0.1926 0.9927 0.0488 0.4218
2 C2H2-01 -2.0067 4.0185 2.4598 6.5649 0.0014 0.2193
```

qpcrANOVARE()

```
      factor1 factor2      RE     LCL     UCL letters      se
R:0         R       0 0.28519 0.19834 0.41008       d 0.02082
R:0.25      R    0.25 0.62706 0.43609 0.90166      bc 0.43880
R:0.5       R     0.5 0.98851 0.68746 1.42140       b 0.08413
S:0         S       0 0.79554 0.55326 1.14392       b 0.21284
S:0.25      S    0.25 0.41466 0.28837 0.59625      cd 0.25403
S:0.5       S     0.5 2.96905 2.06482 4.26925       a 0.05508
```

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
```

K

```
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)

efficiency(x)

meanTech(x, groups)
```

```
qpcrANOVAFC(x,
  numberOfrefGenes,
  analysisType = "ancova",
  mainFactor.column,
  mainFactor.level.order = NULL,
  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")
```

```
qpcrANOVARE(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)

multiplot(..., cols = 1)
```

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

qpcrREPEATED(  x,
  numberOfrefGenes,
  factor,
  block = NULL,
  fill = "#BFEFFF",
  y.axis.adjust = 1,
  y.axis.by = 1,
  ylab = "Fold Change",
  xlab = "none",
  fontsizePvalue = 7,
  axis.text.x.angle = 0,
  axis.text.x.hjust = 0.5,
  x.axis.labels.rename = "none",
  letter.position.adjust = 0,
  p.adj = "none",
```

```
res <- qpcrANOVAFC(data_3factor, numberOfrefGenes = 1, mainFactor.column = 1)
qpcrMeans(res$lm_ANOVA, specs = "Conc | Type")
```