

R script for statistical analysis of qPCR data, relative histone acetylation, and relative DNA methylation

```
library(multcomp)
library(FSA)
library(dplyr)
set.seed(12345)

# Input for analysis of ddCT of immune genes and epigenetic regulatory genes, and of relative histone
acetylation and relative DNA methylation
data <- read.delim("~/Input.txt", header = T)

#calculation of mean, standard deviation and standard error
data2 <- Summarize(X~gts, data = data)
data2$se <- data2$sd/sqrt(data2$n)
#one-way ANOVA for generation vs treatment vs sex
a <- aov(X~gst, data = data)

#test of normalized data: t-quantile-quantile-plots
qqt.sim <- function(m) {
  oldp <- par(mfrow = c(3, 4), mar = c(3, 3.5, 2, 0.1),
             mgp = c(1.5, 0.5, 0), tcl = -0.3)
  stdres <- rstandard(m)
  n <- length(stdres)
  df <- m$df.residual
  qqplot(x = qt(ppoints(n), df = df), y = stdres, col = "red",
        ylab = "Empirical quantiles of\nstandardized residuals",
        xlab = paste("t-quantiles with", df, "d.o.f."),
        main = "Model residuals")
  qqline(y = stdres, distribution = function(p) qt(p, df = df))
  replicate(11, {
    xx <- rt(n, df = df) #n t-distr. data with same df as empirical data
    qqplot(x = qt(ppoints(n), df = df), y = xx,
          ylab = "Empirical quantiles",
          xlab = paste("t-quantiles with", df, "d.o.f."),
          main = "Simulated t-data")
    qqline(y = xx, distribution = function(p) qt(p, df = df))
  })
  invisible(par(oldp))
}
```

```
qqt.sim(m)
```

```
#post-hoc analysis
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```
ph <- glht(a, linfct = mcp(gst = c("Cf-F0Ef=0", "Cf-F0Sf=0", "Cf-F1Ef=0", "Cf-F1Sf=0", "Cm-F0Em=0",  
    "Cm-F0Sm=0", "Cm-F1Em=0", "Cm-F1Sm=0", "F0Ef-F0Em=0",  
    "F0Ef-F0Sf=0", "F0Ef-F1Ef=0", "F0Em-F0Sm=0", "F0Em-F1Em=0",  
    "F0Sf-F0Sm=0", "F0Sf-F1Sf=0", "F0Sm-F1Sm=0", "F1Ef-F1Em=0",  
    "F1Ef-F1Sf=0", "F1Em-F1Sm=0", "F1Sf-F1Sm=0")), vcov = sandwich)
```

```
#Abbreviations:
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X = ddCT; relative histone acetylation, or relative DNA methylation;

gts = generation:treatment:sex;

F0 = parental generation;

F1 = first filial generation;

C = control;

E = *E. coli*;

S = *S. entomophila*;

f = female;

m = male