Quantifying relic DNA

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Analysis of quantitative PCR data to test whether the abundance of bacterial communities is affected by relic DNA

Setup Work Environment

```
#rm(list=ls())
getwd()
setwd("~/GitHub/relicDNA/code")
require("plyr")
require("grid")
require("png")
require("car")
require("bbmle")
sem <- function(x, ...){sd(x, na.rm = TRUE)/sqrt(length(na.omit(x)))}
```

Load data and calcualte corrected copy number

```
eDNA.raw <- read.table("../data/eDNA_qPCR.txt", sep = "\t", header = T)

# Correct for dilutions and sample processing

# eDNA.raw[,7] = copies not corrected by dilution factor
# eDNA.raw[,8] = dilution factor
# eDNA.raw[,9] = volume (uL) in supernatant of phenol-chloroform extraction
# eDNA.raw[,10] = volume (ul) from supernatant of phenol-chlorofom subsampled

copies.corr <- eDNA.raw[,7] * (eDNA.raw[,8] * (eDNA.raw[,9]/eDNA.raw[,10]))

# Make new dataframe with corrected copy numbers
eDNA.corr <- data.frame(eDNA.raw, copies.corr)
```

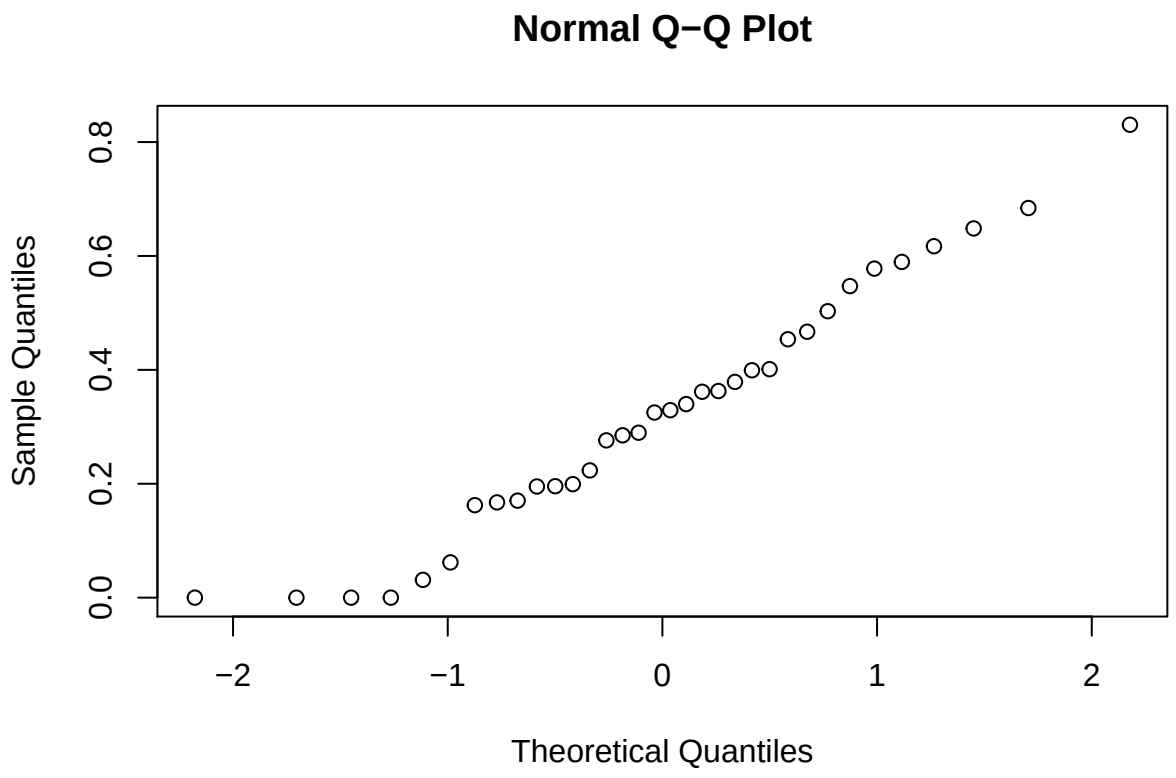
Take mean of technical replicates and sort

```
# Use `aggregate` to return means of subsamples taken for each sample
eDNA <- aggregate(eDNA.corr$copies.corr ~ eDNA.corr$sample + eDNA.corr$sample_name +
                  eDNA.corr$env + eDNA.corr$treat, eDNA.corr, mean)

# Sort by sample number
eDNA <- eDNA[order(eDNA[,1]) ,]
```

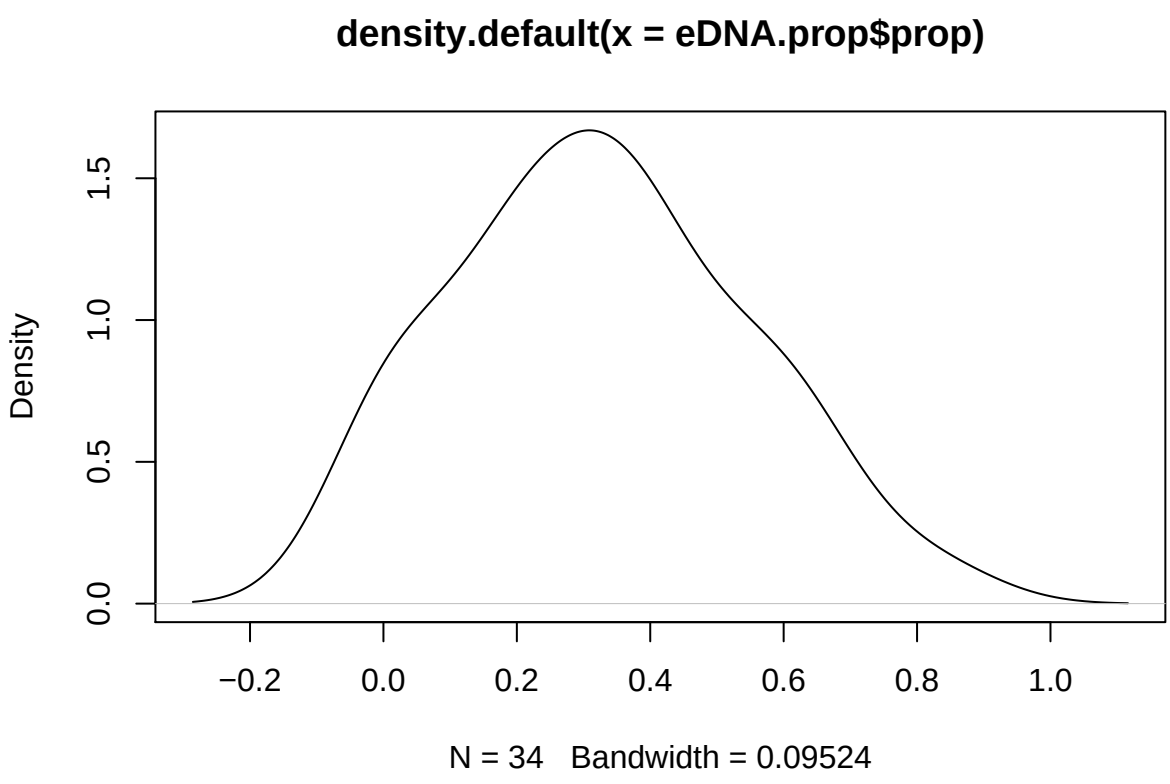
```
# Rename columns
colnames(eDNA) <- c("sample.number", "sample.name", "env", "treat", "copy.number")
```

Calculate proportion of degradable DNA per sample and test differences

```
# Use `ddply` to return the DNase-1 degradable proportion of 16S rRNA gene copy
eDNA.prop <- ddply(eDNA, .(sample.number, sample.name, env), summarize,
                   prop = 1 - ((copy.number[treat == "E"]) / (copy.number[treat == "C"])))

# Sort by environment
eDNA.prop <- eDNA.prop[order(eDNA.prop[,3]) ,]

# Three samples (cat feces [32], human feces [28], T7Core) have negative proportions
# Set these to zero (i.e., no eDNA)
eDNA.prop$prop <- ifelse(eDNA.prop$prop < 0, 0, eDNA.prop$prop)
write.table(eDNA.prop, "../data/relicDNA.prop.txt", sep = "\t", col.names = T, row.names = F)

# Distribution of eDNA
qqnorm(eDNA.prop$prop)
```




```
plot(density(eDNA.prop$prop))
```




```r
mean(eDNA.prop$prop)
```

```
## [1] 0.3256821
```

```r
sd(eDNA.prop$prop)
```

```
## [1] 0.2175141
```

```r
min(eDNA.prop$prop)
```

```
## [1] 0
```

```r
max(eDNA.prop$prop)
```

```
## [1] 0.8304294
```

```r
# Use glm to test whether amount of eDNA differs among environments
eDNA.prop.test <- glm(prop ~ env, data = eDNA.prop)
summary(eDNA.prop.test)
```

```
##
## Call:
## glm(formula = prop ~ env, data = eDNA.prop)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -0.35852  -0.15517  -0.01063   0.13603   0.39788
##
## Coefficients:
```

```
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.35852    0.07234   4.956 2.64e-05 ***
## envsed      -0.04905    0.10230  -0.479   0.6351
## envsoil     -0.18304    0.10230  -1.789   0.0837 .
## envwater     0.07403    0.09705   0.763   0.4515
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for gaussian family taken to be 0.04186231)
##
##     Null deviance: 1.5613  on 33  degrees of freedom
## Residual deviance: 1.2559  on 30  degrees of freedom
## AIC: -5.6623
##
## Number of Fisher Scoring iterations: 2
```

```
Anova(eDNA.prop.test, type = "II", test.statistic = "F")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: prop
## Error estimate based on Pearson residuals
##
##                SS Df      F  Pr(>F)
## env       0.30544  3 2.4321 0.08444 .
## Residuals 1.25587 30
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Use Anova to test whether the amount of dDNA differs among environments
eDNA.prop.lm <- lm(prop ~ env, data = eDNA.prop)
summary(eDNA.prop.lm)
```

```
##
## Call:
## lm(formula = prop ~ env, data = eDNA.prop)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.35852 -0.15517 -0.01063  0.13603  0.39788
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.35852    0.07234   4.956 2.64e-05 ***
## envsed      -0.04905    0.10230  -0.479   0.6351
## envsoil     -0.18304    0.10230  -1.789   0.0837 .
## envwater     0.07403    0.09705   0.763   0.4515
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2046 on 30 degrees of freedom
## Multiple R-squared:  0.1956, Adjusted R-squared:  0.1152
## F-statistic: 2.432 on 3 and 30 DF,  p-value: 0.08444
```

```
eDNA.anova <- Anova(eDNA.prop.lm, type = "II")
eDNA.anova
```

```
## Anova Table (Type II tests)
##
## Response: prop
##            Sum Sq Df F value  Pr(>F)
## env       0.30544  3  2.4321 0.08444 .
## Residuals 1.25587 30
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(aov(eDNA.prop.lm), "env")
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = eDNA.prop.lm)
##
## $env
##                     diff          lwr        upr     p adj
## sed-feces   -0.04905302 -0.327221734 0.22911570 0.9630471
## soil-feces  -0.18304416 -0.461212879 0.09512455 0.2981258
## water-feces  0.07403322 -0.189860799 0.33792723 0.8704386
## soil-sed    -0.13399114 -0.412159862 0.14417757 0.5640774
## water-sed    0.12308623 -0.140807782 0.38698025 0.5896087
## water-soil   0.25707738 -0.006816637 0.52097139 0.0583852
```

```
# Calculate means, sem, and sample size by enviroment
eDNA.mean <- aggregate(eDNA.prop$prop ~ eDNA.prop$env, eDNA.prop, mean)
eDNA.n <- aggregate(eDNA.prop$prop ~ eDNA.prop$env, eDNA.prop, length)
eDNA.sem <- aggregate(eDNA.prop$prop ~ eDNA.prop$env, eDNA.prop, sem)

# Make table of proportion eDNA by environment
eDNA.table <- data.frame(eDNA.mean, eDNA.sem[ ,2], eDNA.n[ ,2])
colnames(eDNA.table) <- c("env", "mean", "sem", "n")
eDNA.table <- eDNA.table[order(eDNA.table[,2]), ]
```

```
# # Make bar plot with error bars by environment
# ```{r, eval=F}
# png(filename="../figures/qPCR.bar.png",
#     width = 800, height = 800, res = 96*2)
#
# bp <- barplot(eDNA.table$mean, ylim =c(0, 0.6),
#               pch = 15, cex = 1.25, las = 1, cex.lab = 1.25, cex.axis = 1,
#               col = "gray90", axis.lty = 1, lwd = 2, xlab = NA,
#               ylab = "Proportion eDNA",
#               names.arg = c("Soil", "Sediment", "Gut", "Water"), cex.names = 0.9)
#               box(lwd = 2)
# arrows(x0 = bp, y0 = eDNA.table$mean, y1 = eDNA.table$mean - eDNA.table$sem,
#        angle = 90,  length = 0.1, lwd = 2)
# arrows(x0 = bp, y0 = eDNA.table$mean, y1 = eDNA.table$mean + eDNA.table$sem,
#        angle = 90, length=0.1, lwd = 2)
#
# # Close Plot Device
# dev.off()
# graphics.off()
#
```

```
# # Show Plot
# img <- readPNG("../figures/qPCR.bar.png")
# grid.raster(img)
```

```
# Make x-y plot with error bars by environment
# ```{r}
# png(filename="../figures/Figure2-Prop_eDNA.png",
#     width = 800, height = 800, res = 96*2)
#
# par(mar = c(3, 5, 1, 1))
# non.bp <- plot(eDNA.table$mean, ylim = c(0, 0.6),
#                xlim = c(0.5, 4.5), pch = 22, bg = "gray90", lwd = 2,
#                cex = 3, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 1.5,
#                las = 1, ylab = "", xlab = "")
# box(lwd = 2)
#
# mtext(expression('Proportion Relic DNA'), side = 2,
#       outer = FALSE, cex = 1.5, line = 3, adj = 0.5)
#
# # Major Axes
# axis(side = 2, lwd.ticks = 2, cex.axis = 1.25, las = 1,
#      labels = c(0.0, 0.2, 0.4, 0.6), at = c(0.0, 0.2, 0.4, 0.6))
#
# axis(side = 4, lwd.ticks = 2, cex.axis = 1.5, las = 1,
#      at=c(0.0, 0.2, 0.4, 0.6), labels = F, tck = -0.02)
#
# axis(side = 1, lwd.ticks = 2, cex.axis = 0.9, las = 1,
#      labels = c("Soil", "Sediment", "Gut", "Water"), at = c(1, 2, 3, 4))
#
# axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
#      at = c(1, 2, 3, 4), labels = F, tck = -0.02)
#
# axis(side = 1, labels = F, lwd.ticks = 2, tck = 0.02, at = c(1, 2, 3, 4))
# axis(side = 2, labels = F, lwd.ticks = 2, tck = 0.02, at = c(0, 0.2, 0.4, 0.6))
# axis(side = 3, labels = F, lwd.ticks = 2, tck = 0.02, at = c(1, 2, 3, 4))
# axis(side = 4, labels = F, lwd.ticks = 2, tck = 0.02, at = c(0, 0.2, 0.4, 0.6))
#
# arrows(x0 = c(1, 2, 3, 4), y0 = eDNA.table$mean,
#        y1 = eDNA.table$mean - eDNA.table$sem, angle = 90,
#        length = 0.1, lwd = 2)
#
# arrows(x0 = c(1,2,3,4), y0 = eDNA.table$mean,
#        y1 = eDNA.table$mean + eDNA.table$sem, angle = 90,
#        length=0.1, lwd = 2)
#
# points(x = c(1:4), eDNA.table$mean,
#       pch = 22, bg = "gray90", lwd = 2, cex = 3)
#
# # Close Plot Device
# dev.off()
# graphics.off()
#
# # Show Plot
# img <- readPNG("../figures/Figure2-Prop_eDNA.png")
```

```
# grid.raster(img)
```

Make x-y plot with error bars by environment with individual data points

```
# Relic DNA data for plotting
relic.soil <- eDNA.prop[ which(eDNA.prop$env == "soil"),]
relic.sed <- eDNA.prop[ which(eDNA.prop$env == "sed"),]
relic.feces <- eDNA.prop[ which(eDNA.prop$env == "feces"),]
relic.water <- eDNA.prop[ which(eDNA.prop$env == "water"),]

# Relic DNA table
relic.mean <- aggregate(eDNA.prop$prop ~ env, eDNA.prop, mean)
relic.sem <- aggregate(eDNA.prop$prop ~ env, eDNA.prop, sem)
relic.95.LL <- aggregate(prop ~ env, eDNA.prop,
          FUN = function(x) t.test(x)$conf.int[1])
relic.95.UL <- aggregate(prop ~ env, eDNA.prop,
          FUN = function(x) t.test(x)$conf.int[2])
relic.table <- data.frame(relic.mean[1], relic.mean[2], relic.sem[2],
          relic.95.LL[2], relic.95.UL[2])
colnames(relic.table) <- c("env", "mean", "sem", "LCI", "UCI")
relic.table <- relic.table[order(relic.table$mean),]

png(filename="../figures/Figure2-Prop.relic.png",
    width = 800, height = 800, res = 96*2)

par(mar = c(4, 5, 1, 1))

non.bp.rich <- plot(jitter(rep(1, length(relic.soil$prop)), amount = 0.1), relic.soil$prop,
      ylim = c(-0.1, 1), xlim = c(0.5, 4.5), pch = 21, col = "lightgrey", bg = "lightgrey", lwd = 2,
      cex = 1.7, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 1.5,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
points(jitter(rep(2, length(relic.sed$prop)), amount = 0.1), relic.sed$prop, pch = 21,
       bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)
points(jitter(rep(3, length(relic.feces$prop)), amount = 0.1), relic.feces$prop, pch = 21,
       bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)
points(jitter(rep(4, length(relic.water$prop)), amount = 0.1),relic.water$prop, pch = 21,
       bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)

# rect(xleft, ybottom, xright, ytop)
rect(0.8, (mean(relic.soil$prop) + 0.025), 1.2, (mean(relic.soil$prop)- 0.025), col = "NA", border = "bl

rect(1.8, (mean(relic.sed$prop) + 0.025), 2.2, (mean(relic.sed$prop)- 0.025), col = "NA", border = "bla

rect(2.8, (mean(relic.feces$prop) + 0.025), 3.2, (mean(relic.feces$prop) - 0.025), col = "NA", border =

rect(3.8, (mean(relic.water$prop) + 0.025), 4.2, (mean(relic.water$prop) - 0.025), col = "NA", border =

box(lwd = 2)
```

```
mtext(expression('Proportion Relic DNA'), side = 2,
      outer = FALSE, cex = 1.5, line = 3.6, adj = 0.5)

# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1.25, las = 1,
     labels = c("0.0", "0.25", "0.50", "0.75", "1.00"), at = c(0.0, 0.25, 0.5, 0.75, 1.0))

axis(side = 4, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at=c(0.0, 0.25, 0.5, 0.75, 1.0), labels = F, tck = -0.02)

axis(side = 1, lwd.ticks = 2, cex.axis = 0.9, las = 1,
     labels = c("Soil", "Sediment", "Gut", "Water"), at = c(1, 2, 3, 4))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at = c(1, 2, 3, 4), labels = F, tck = -0.02)

arrows(x0 = c(1,2,3,4), y0 = relic.table$mean, y1 = relic.table$LCI, angle = 90,
       length = 0.1, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = relic.table$mean, y1 = relic.table$UCI, angle = 90,
       length=0.1, lwd = 2)

# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```
graphics.off()

# Show Plot
img <- readPNG("../figures/Figure2-Prop.relic.png")
grid.raster(img)
```