eDNA

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

Setup Work Environment

```
rm(list=ls())
getwd()
```

```
## [1] "/Users/mmuscarella/GitHub/eDNA/code"
```

```
setwd("~/GitHub/eDNA/code")
require("plyr")
```

```
## Loading required package: plyr
```

```
require("grid")
```

```
## Loading required package: grid
```

```
require("png")
```

```
## Loading required package: png
```

```
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 dilutioin 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/eDNA.prop.txt", sep = "\t", col.names = T, row.names = F)

# 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
## -0.33945  -0.10951  -0.04154   0.13124   0.46044
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.27373    0.07256   3.773 0.000684 ***
## envsed       0.09535    0.10577   0.901 0.374276
## envsoil     -0.02365    0.10577  -0.224 0.824573
## envwater     0.15897    0.10001   1.589 0.122107
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for gaussian family taken to be 0.04738117)
##
##     Null deviance: 1.6650  on 34  degrees of freedom
```

```
## Residual deviance: 1.4688  on 31  degrees of freedom
## AIC: -1.6555
##
## Number of Fisher Scoring iterations: 2
```

```
# 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

```
png(filename="../figures/qPCR.bar.png",
    width = 800, height = 800, res = 96*2)
plot.new()
par(lwd = 2)
bp <- barplot(eDNA.table$mean, ylim =c(0, 0.6),
              pch = 15, cex = 1.25, las = 1, cex.lab = 1.25, cex.axis = 1,
              col = "white", axis.lty = 1, lwd = 2, xlab = NA,
              ylab = "Proportion eDNA",
              names.arg = c("Gut", "Soil", "Sediment", "Water"), cex.names = 0.9)
              box(lwd = 2)
arrows(x0 = bp, y0 = eDNA.table$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()
```

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

```
graphics.off()

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

Make x-y plot with error bars by environment

```
png(filename="../figures/qPCR.nonbar.png",
    width = 800, height = 800, res = 96*2)

#plot.new()
non.bp <- plot(eDNA.table$mean, ylim = c(0, 0.6),
```

```
              xlim = c(0.5, 4.5), pch = 22, bg = "white", 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 eDNA'), side = 2,
      outer = TRUE, cex = 1.5, line = -1.5, 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)

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

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 = "white", lwd = 2, cex = 3)

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

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

```
graphics.off()

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