

MOB qPCR

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Overview

The following code takes output from the qPCR instrument and calculates gene copy abundance for methane monooxygenase (pmoA) gene

Set working directory

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

```
## [1] "/Users/lennonj/GitHub/radiolyticCH4/code/caves/qPCR"
```

```
setwd("~/GitHub/radiolyticCH4")
```

Read MOB data

```
mob <- read.delim("~/GitHub/radiolyticCH4/data/caves/qPCR/20150817_VN_661_qPCR.txt",  
  stringsAsFactors = FALSE)
```

Change copy numbers to their correct values

```
# calculate the number of pmoA genes present in a 2.5 ng/uL standard  
cpn <- 2.5*10-(9)*((202+661-189)*(660))-(1)*6.022*10(23)  
  
mob[57,4] <- cpn # The copy numbers entered into RealPlex were incorrect.  
mob[58,4] <- cpn*10-(1) # These steps use the 189 - 661 primer pair to calculate  
mob[59,4] <- cpn*10-(2) # the number of gene copies present in solution  
mob[60,4] <- cpn*10-(3)  
mob[61,4] <- cpn*10-(4)  
mob[62,4] <- cpn*10-(5)  
mob[63,4] <- cpn*10-(6)  
mob[64,4] <- cpn*10-(7)  
  
mob[,3] <- as.numeric(mob[,3]) # change data type of column 3
```

```
## Warning: NAs introduced by coercion
```

```
mob[,4] <- as.numeric(mob[,4]) # change data type of column 4
```

```
## Warning: NAs introduced by coercion
```

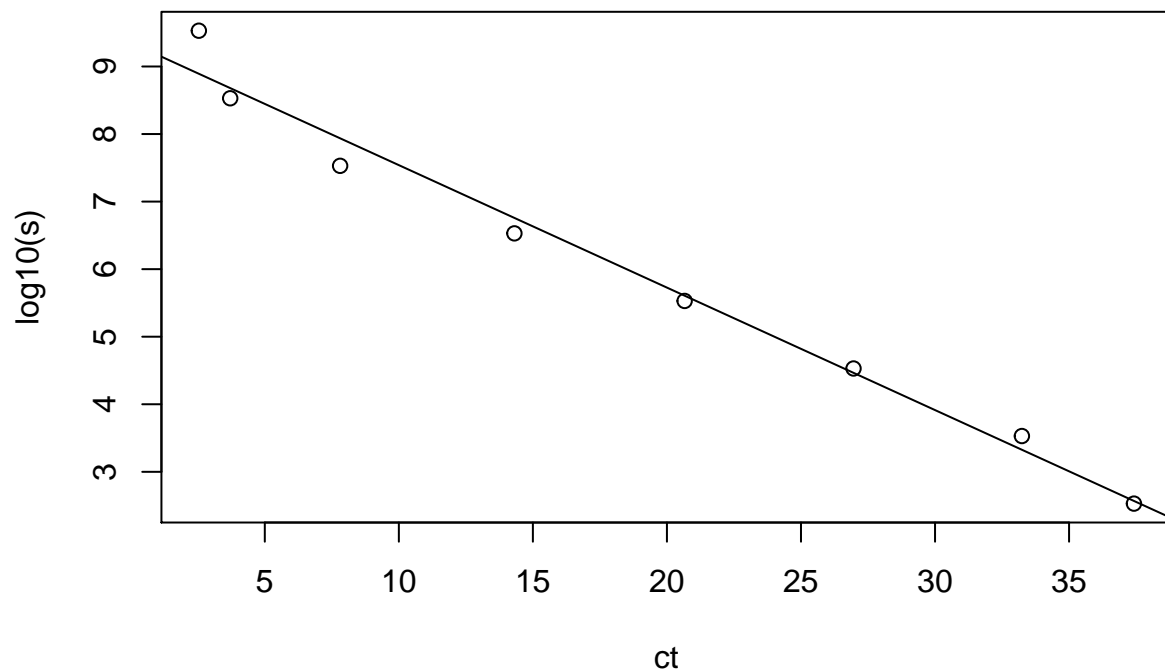
```
ct <- mob[57:64,3] # flourescent values of the standards
```

```
s <- mob[57:64,4] # copy numbers of standards
```

```
plot(ct,log10(s)) # plots copy number against standard flouresence for a visual check
```

```
reg <- lm(log10(s) ~ ct) # linear regression of log10(s) vs ct
```

```
abline(reg)
```



```
reg_sum <- summary(reg) # summary of analysis
```

qPCR statistics

```
mob.ef <- -1+10^(-reg$coefficients[2]) # efficiency
```

```
mob.r2 <- summary(lm(log10(s)~ct, mob))$r.squared # r^2 of the standard curve
```

Loop to calculate MOB gene copy number from standard curve coefficients

```
for (i in 1:51) {  
  mob[i,4] <- (10^(reg$coefficients[1] + reg$coefficients[2]*mob[i,3]))  
}  
  
write.table(mob, file = "~/GitHub/radiolyticCH4/data/caves/qPCR/mob.661.out.txt")
```