

“Calretinin is a novel candidate marker for adverse ovarian effects of early life exposure to mixtures of endocrine disruptors in the rat”

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Online Resource 1 Example of R-code used for statistical analysis of the MaxLFQ-normalized protein intensities in R statistical software (v3.31). Four statistical comparisons (control vs each of the four treatments) were performed for the proteins with normalized quantitative information in at least 75% of the samples in each comparison using the *limma* package in R

```
library(limma)

#import protein dataframe
prots.raw <- read.delim("Maxquant output.txt", row.names=1, header=TRUE) ##
dim(prots.raw)

#convert 0 values to NA
prots.raw[prots.raw ==0]<-NA

###remove columns with more than 25% NA values
prots.raw1<-prots.raw[, colMeans(is.na(prots.raw)) <= 0.25]
dim(prots.raw1)

#drop 1st column (treatment info) & log2 convert protein intensities
prots.log<-log2(prots.raw1[,-1])

##### LIMMA #####

###create phenotypic (treatment) dataframe
pheno <- data.frame(cbind(TREATMENT = c(rep("C",8), rep("X.treatment",8))), row.names=row.names(prots.log))

mod <- model.matrix(~TREATMENT, data=pheno)
fit<- lmFit(t(prots.log), mod)
fit2<-eBayes(fit)
results<- decideTests(fit2, adjust.method="none", method="global", p=0.05)
summary(results)

topTable(fit2, coef=c(2),n =10, adjust.method="BH")

# extract 2nd coefficient (treatment)
res<-topTable(fit2, coef=c(2),n =Inf, adjust.method="BH")

write.table(res, "C vs X.treatment.csv", sep=",")
```