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#Differential abundance analysis: All time points

setwd("C:\\Princeton R script\\Publication timepoints -6h")
library(limma)
targets <- readTargets("Targets.txt")
spottypes <- readSpotTypes("Spot_Types.txt")
RG <- read.maimages(targets$FileName,source="genepix",wt.fun=wtflags(0))
RG$genes<-readGAL("august_gal2.gal")
RG$genes$Status<-controlStatus(spottypes, RG)
RG$printer<- list(ngrid.r=8, ngrid.c=4, nspot.r=11, nspot.c=24, ndups=1, npins=32, start="topleft")
library(convert)
mraw<-as(RG, "marrayRaw")
# Start of loop for diagnostic images
for (i in 1:20){
image(mraw[,i],xvar="maGb", main=RG$targets[[1]][[i]])
x11()
image(mraw[,i],xvar="maRb", main=RG$targets[[1]][[i]])
}

# Background correction
RGb<-backgroundCorrect(RG, method="normexp", offset=50)
library(convert)
mraw <- as(RGb,"marrayRaw")

# Loop for print-tip boxplots
for (i in 1:20){
x11()
title = paste("Print-tip boxplots for array", RG$targets[[1]][[i]], ": prenormalization", separator="")
boxplot(mraw[,i],xvar="maPrintTip", yvar="maM", main=title)
}

#Array boxplot to check for differences between arrays
x11()
boxplot(mraw, yvar="maM",
main="Array boxplots: pre-normalization")

# Loop for MA plots
for (i in 1:20){
title = paste("MA plot Slide ", RG$targets[[1]][[i]], separator=" ")
x11()
plotMA(RG,array=i, main=title)
}

# NormalizeWithinArrays
MA<-normalizeWithinArrays(RGb, method="robustspline")
library(convert)
mraw.norm<-as(MA, "marrayNorm")

# Loop for post-normalization plots
for (i in 1:20){
x11()

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title = paste("Print-tip boxplots for array", RG$targets[[1]][[i]], ": post robustspline normalization", separator=" ")
boxplot(mraw.norm[,i],xvar="maPrintTip", yvar="maM", main=title)
}

#Array boxplot to check for differences between arrays
xll()
boxplot(mraw.norm, yvar="maM",
main="Array boxplots time: post robustspline within normalization")
MAbet<-normalizeBetweenArrays(MA,method="Gquantile")
xll()
plotDensities(MAbet)

# Loop for MA plots
for (i in 1:20){
xll()
title = RG$targets[[1]][[i]]
plotMA(MAbet,array=i, main=)
}

# Check between slide normalization
library(convert)
mraw.norm.bet <- as(MAbet,"marrayNorm")
xll()
boxplot(mraw.norm.bet, yvar="maA",
main="Array intensity boxplots: post-Gquantile between normalization")
xll()
boxplot(mraw.norm.bet, yvar="maM",
main="Array boxplots: post-Gquantile between normalization")

#Linear model differential abundance analysis compared to relative time zero = UTt1
design<-modelMatrix(targets,ref="Reference")
design
fit<-lmFit(MAbet,design)
contrast.matrix<-makeContrasts(Tt1-UTt1, levels=design)
#Change for different comparisons e.g. Tt2 and Tt3 vs UTt1
contrast.matrix
fit2<-contrasts.fit(fit, contrast.matrix)
fit2<-eBayes(fit2)
# extract all genes for T compared to UTt1 and apply FDR
# adjustment to p-values to adjust for multiple testing
TvUTt1<-topTable(fit2, adjust="fdr", number=8448)
TvUTt1
write.table(TvUTt1,file="C:/Princeton R script/Publication timepoints -6h/top_table.txt",sep="\t",row.names=FALSE)
xll()
volcanoplot(fit2,names=fit$genes$Name)

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