library(ropls); library (dplyr)

design <- read.table("Exudatedesign.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

Metab.0 <- read.table("Metab.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F, quote="")

anno <- Metab.0

Metab <- Metab.0

Metab <- Metab[, -c(1, 2, 3)]

head (Metab); dim(Metab)

remove <- rownames (Metab [apply(Metab < 50000, 1, any), ])

Metab <- Metab [-which(rownames(Metab) %in% remove), ]

dim(Metab)

library(caret); library(mixOmics)

Myplsda <- plsda(t(Metab), design$Trt, ncomp = 10)

Mycol <- c("cyan", "chartreuse")

Mypch <- c(16, 16 )

plotIndiv(Myplsda, col = Mycol, pch= Mypch , ellipse = TRUE, ellipse.level = 0.95, legend= T,

legend.position= "right", cex=4, xlim = c(-22, 22), abline = T )

#######################################################################################

design$Trt <- as.factor(design$Trt)

Metab.data <- list()

Metab.data$dataMatrix <- t(Metab)

Metab.data$sampleMetadata <- design

sacurine.plsda <- opls(x = Metab.data$dataMatrix, y = Metab.data$sampleMetadata [, 'Trt'] ,

orthoI = NA, crossvalI = 4 , permI=200) #, permI=200

vipVn <- getVipVn(sacurine.plsda)

vipVn\_select <- vipVn[vipVn > 1]

head(vipVn\_select)

vipVn <- as.data.frame(vipVn)

library (readr); library(edgeR); library(phyloseq); library (ggplot2); library(ggpubr); library (ggrepel)

############Define enriched OTUs#########

edgeR\_enrich <- DGEList(counts= Metab,

group= design$Trt)

edgeR\_enrich <- calcNormFactors(edgeR\_enrich)

otu\_norm\_enrich <- **cpm**(edgeR\_enrich, normalized.lib.sizes=T, log=F)

model\_mat\_enrich <- model.matrix(~Trt, data= design)

dge\_enrich <- estimateGLMRobustDisp(edgeR\_enrich, design=model\_mat\_enrich)

fit\_enrich <- glmFit(dge\_enrich, design=model\_mat\_enrich)

lrt\_enrich <- glmLRT(fit\_enrich, coef=2)

tt\_enrich <- **topTags**(lrt\_enrich, n=Inf, p.value=1)

head(tt\_enrich$table)

I\_enrich <- tt\_enrich$table[tt\_enrich$table$logFC < 0 & tt\_enrich$table$ PValue < 0.05,] ; dim (I\_enrich)

M\_enrich <- tt\_enrich$table[tt\_enrich$table$logFC > 0 & tt\_enrich$table$ PValue < 0.05,] ; dim (M\_enrich)

tt\_enrich <- as.data.frame(tt\_enrich)

tt\_enrich$VIP <- vipVn[rownames(tt\_enrich), ]

tt\_enrich <- tt\_enrich [order(tt\_enrich $VIP, decreasing = TRUE), ]

tt\_enrich.all <- tt\_enrich

tt\_enrich <- tt\_enrich[tt\_enrich$PValue<0.01, ]

tt\_enrich <- tt\_enrich[tt\_enrich$logCPM > 5, ]

tt\_enrich <- tt\_enrich[abs(tt\_enrich$logFC) > 0.5, ]

tt\_enrich <- tt\_enrich[abs(tt\_enrich$VIP) > 1.5, ]

tt\_enrich$anno <- anno[rownames(tt\_enrich),]$Compound

dim (tt\_enrich)

range (tt\_enrich$logFC)

**ggdotchart**(tt\_enrich, x ="anno", y = "VIP", color = "logFC", # Color by groups

sorting = "descending", # Sort value in descending order

add = "segments", # Add segments from y = 0 to dots

rotate = TRUE, dot.size = 4, ggtheme = theme\_pubr() ) + # ggplot2 theme

scale\_color\_gradient2(name="logFC", low = "dodgerblue2", mid = "white", high = "red3", limit = c(-2.1, 2.1) ) +

scale\_y\_continuous(limits=c(0, 2.25), breaks=seq(0, 2.25, 0.25) )