Example report of *OUKS*

This R Markdown document was provided as an example to reproduce the *OUKS* code script. Fig. 4 from article can be reproduced with the following code. Preliminarily download *“xcms after IPO MVI QC-XGB filter repeats annot+filtr LMM adj KEGG.csv”* and *“8 peaks.csv”* files into your working directory (for example: “D:/OUKS/”).

Prepare environment

First, set the folder for the working directory and load the packages.

```
setwd("D:/OUKS/)

library(data.table)
library(factoextra)

## Loading required package: ggplot2

## Welcome! Want to learn more? See two factoextra-related books at
https://goo.gl/ve3WBa

library(FactoMineR)
library(dendextend)

##
## ---------------------
## Welcome to dendextend version 1.16.0
## Type citation('dendextend') for how to cite the package.
##
## Type browseVignettes(package = 'dendextend') for the package
vignette.
## The github page is: https://github.com/talgalili/dendextend/
##
## Suggestions and bug-reports can be submitted at:
https://github.com/talgalili/dendextend/issues
## You may ask questions at stackoverflow, use the r and dendextend
tags:
##   https://stackoverflow.com/questions/tagged/dendextend
##
##  To suppress this message use:
suppressPackageStartupMessages(library(dendextend))
## ---------------------

##
## Attaching package: 'dendextend'
## The following object is masked from 'package:data.table':
##
##     set
```

```
## The following object is masked from 'package:stats':
##
##     cutree
```

```
library(rafalib)
library(pROC)
```

```
## Type 'citation("pROC")' for a citation.
```

```
##
## Attaching package: 'pROC'
```

```
## The following objects are masked from 'package:stats':
##
##     cov, smooth, var
```

```
library(parallel)
library(doParallel)
```

```
## Loading required package: foreach
```

```
## Loading required package: iterators
```

```
library(grid)
library(caret)
```

```
## Loading required package: lattice
```

```
library(dplyr)
```

```
##
## Attaching package: 'dplyr'
```

```
## The following objects are masked from 'package:data.table':
##
##     between, first, last
```

```
## The following objects are masked from 'package:stats':
##
##     filter, lag
```

```
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

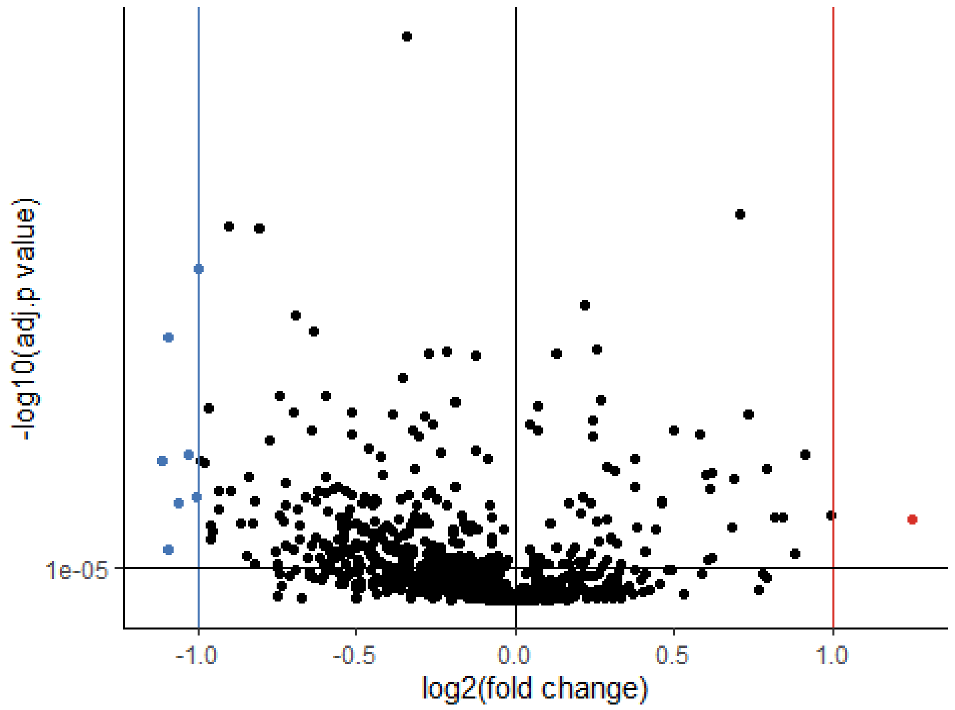
```
library(MKinfer)
library(limma)
library(ggplot2)
library(cowplot)
library(ggsci)
```

Load datasets

```
ds <- as.data.frame(fread(input = "8 peaks.csv", header=T))
rownames(ds) <- ds[,1]
ds <- ds[,-1]
colnames(ds)[1] <-"Label"
ds[,-1] <- sapply(ds[,-1], as.numeric)
ds$Label <- as.factor(ds$Label)

ds2 <- as.data.frame(fread(input = "xcms after IPO MVI QC-XGB filter
repeats annot+filtr LMM adj KEGG.csv", header=T))
ds2 <- ds2[-c(1:12),]
rownames(ds2) <- ds2[,5]
ds2 <- ds2[,-c(1,3:5)]
ds2[,-1] <- sapply(ds2[,-1], as.numeric)
ds2$Label <- as.factor(ds2$Label)
```

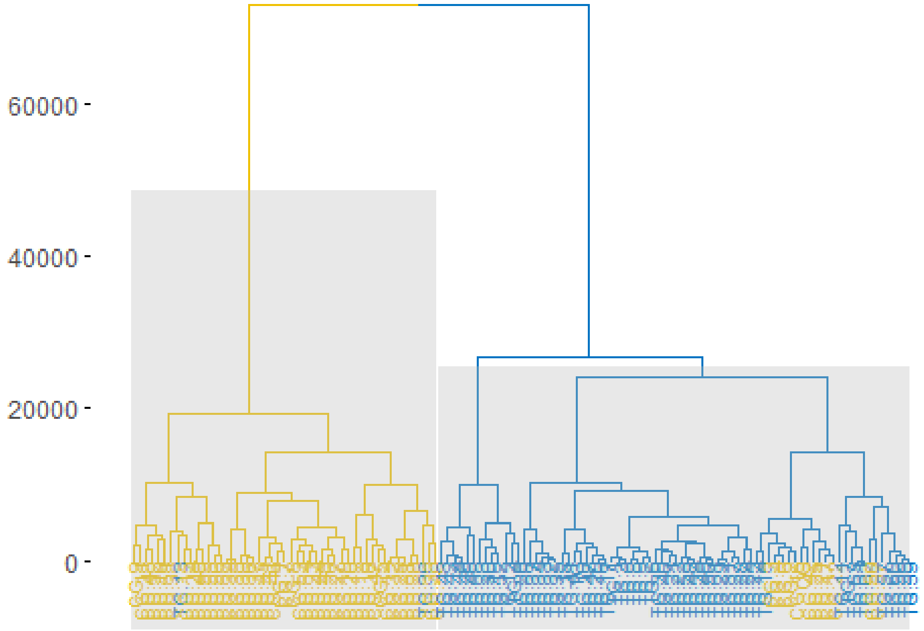
Plot volcano plot

```
ds_log <- as.data.frame(log2(ds2[,-1]))
ds_log <- cbind(Label = ds2[,1], ds_log)

FOLD.CHANGE <- function(data) {
  ds_log_subsets <- lapply(1:length(unique(data[,1])), function(y)
filter(data[,-1], data$Label == unique(data[,1])[y]))
  mean_r_l <- lapply(1:length(ds_log_subsets), function(y)
apply(ds_log_subsets[[y]], 2, mean, na.rm = T))
  foldchange <- mean_r_l[[1]] - mean_r_l[[2]]
  fc_res <- as.data.frame(foldchange)
  return(fc_res)
}

fc_res <- FOLD.CHANGE(ds_log)
foldchange <- as.numeric(fc_res$foldchange)

mdl_mtrx <- model.matrix(~Label, ds2)
lmf <- lmFit(t(ds2[,-1]), method = "robust", design = mdl_mtrx, maxit =
1000)
efit <- eBayes(lmf)
tableTop <- topTable(efit, coef = 2, adjust = "BH",  number =
ncol(ds2), sort.by = "none")
pval <- as.numeric(tableTop$adj.P.Val)

f <- volcano(foldchange, pval, effect.low = -1.0, effect.high = 1.0,
sig.level = 0.00001,
             xlab = "log2(fold change)", ylab = "-log10(adj.p value)",
title = "") + theme_classic() +  theme(legend.position="none")
f
```



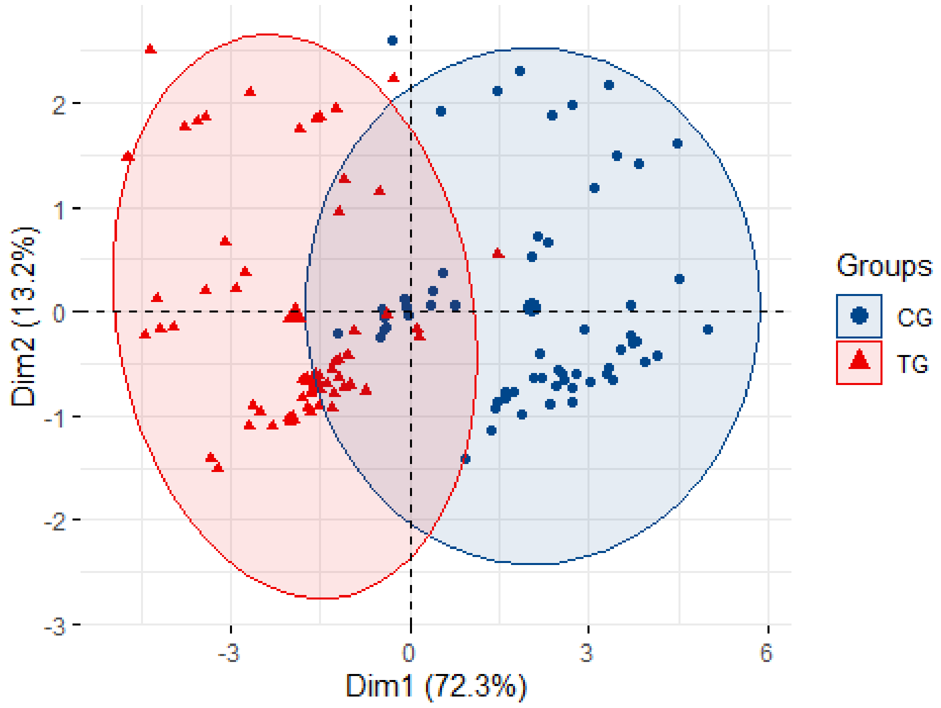
HCA plot

```
base1 <- ds
mtrx1 <- ds[,-1]
grp1 <- as.character(base1[,1])

k <- length(unique(grp1))
Cols = function(vec, ord){
  cols = pal_jco(palette = c("default"), alpha =
1)(length(unique(vec)))
  return(cols[as.fumeric(vec)[ord]])}
mtrx1_1 <- mtrx1
rownames(mtrx1_1) = make.names(grp1, unique=TRUE)
res.dist1 <- dist(mtrx1_1, method = "manhattan")
res.hc1 <- hclust(d = res.dist1, method = "ward.D2")

b <- fviz_dend(res.hc1, k = k,
                 cex = 0.55,
                 k_colors = unique(Cols(grp1,res.hc1$order)),
                 color_labels_by_k = F,
                 label_cols = Cols(grp1,res.hc1$order),
                 rect = T,
                 rect_fill = T,
                 horiz = F,
                 lwd = 0.7,
                 show_labels = T,
                 main = "",
                 ylab = "")
b
```



PCA plot

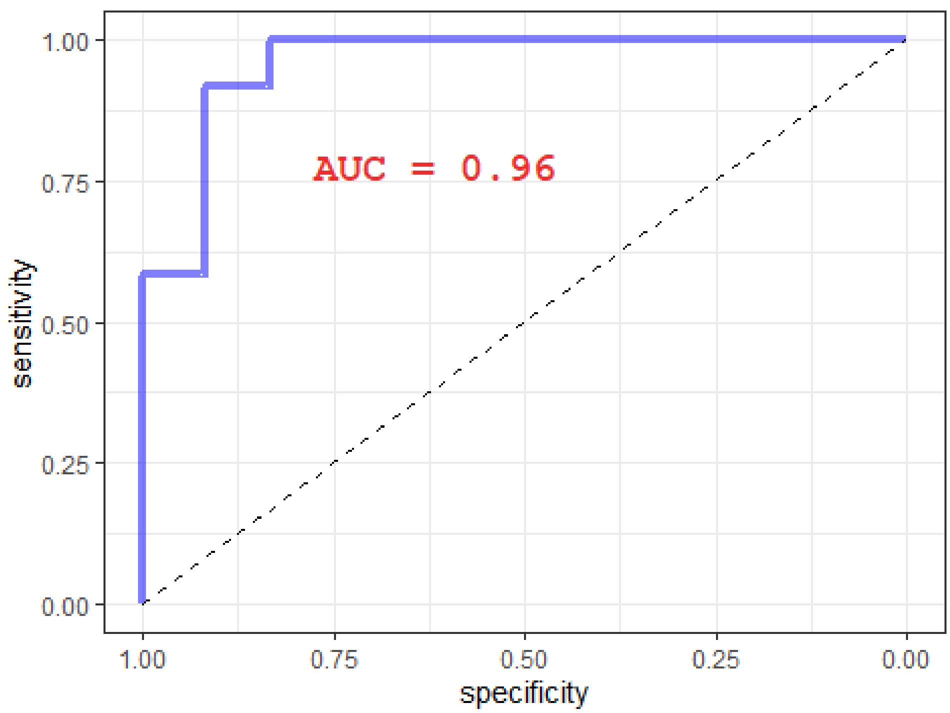
```
base1 <- ds
mtrx1 <- ds[,-1]
grp1 <- as.character(base1[,1])
palette_pca <- "lancet"

pca.ds1 <- PCA(mtrx1, scale.unit = T, graph = F)
a <- fviz_pca_ind(pca.ds1,
                    title = "",
                    geom.ind = "point",
                    col.ind = grp1,
                    palette = palette_pca,
                    addEllipses = T,
                    legend.title = "Groups")
a
```



ROC curve

```
# start parallel processing
fc <- as.numeric(detectCores(logical = T))
cl <- makePSOCKcluster(fc-1)
registerDoParallel(cl)

# cross-validation
set.seed(1234)
trainIndex <- createDataPartition(ds$Label, p = 0.8, list = F, times =
1)
dsTrain <- ds[ trainIndex,]
dsValid <- ds[-trainIndex,]
trainControl <- trainControl(method="repeatedcv", number=10,
repeats=10, classProbs = T)
metric <- "Accuracy"

# machine learning
set.seed(1234)
fit.cl <- train(Label~., data=dsTrain, method="svmRadial",
metric=metric, trControl=trainControl, tuneLength = 10)
predicted.classes <- predict(fit.cl, newdata=dsValid)
probabilities <- predict(fit.cl, newdata=dsValid, type = "prob")[,1]

# ROC curve
res.roc <- roc(dsValid$Label, probabilities, levels =
levels(dsValid$Label))
```

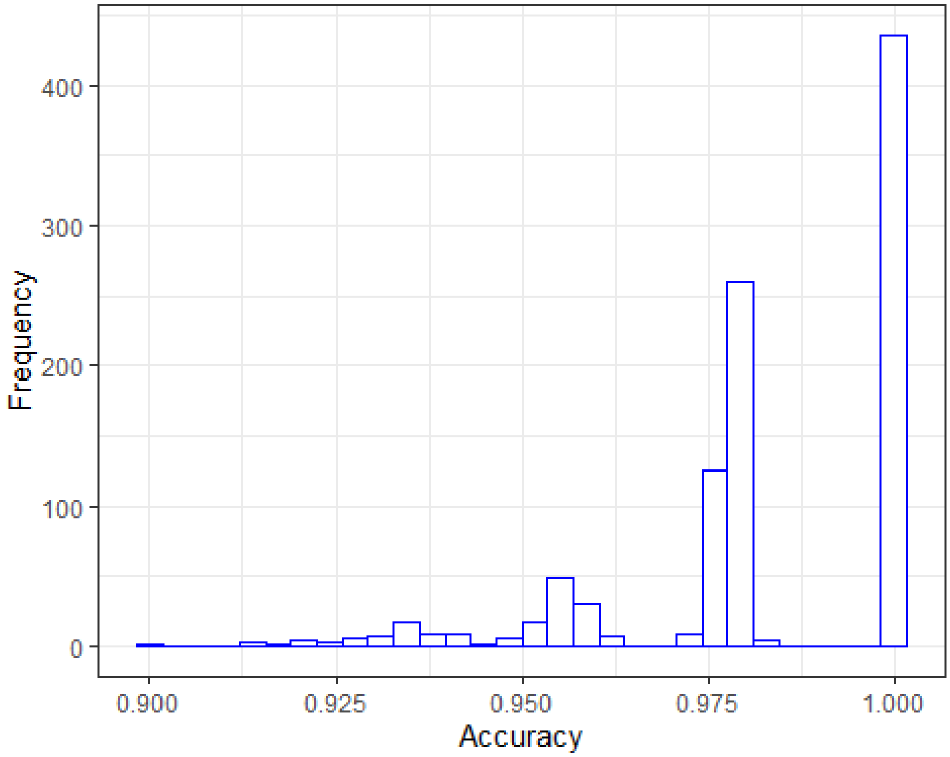
```
## Setting direction: controls > cases

auroc <- round(as.numeric(auc(res.roc)),2)
grob <- grobTree(textGrob(paste0("AUC = ", auroc), x=0.25,  y=0.75,
hjust=0,
                          gp=gpar(col="firebrick2", fontsize=15,
fontface=11)))
c <- ggroc(res.roc, alpha = 0.5, colour = "blue1", linetype = 1, size =
1.5) +theme_bw() + ggtitle("") +
  geom_segment(aes(x = 1, xend = 0, y = 0, yend = 1), color="black",
linetype="dashed")  + annotation_custom(grob)
c
```



bootstrap histogram

```
# start parallel processing
fc <- as.numeric(detectCores(logical = T))
cl <- makePSOCKcluster(fc-1)
registerDoParallel(cl)

# bootstrap
set.seed(1234)
trainControl <-trainControl(method="boot", number=1000)
metric <- "Accuracy"

# machine learning
set.seed(1234)
```

```r
fit.cl <- train(Label~., data=ds, method="svmRadial", metric=metric,
trControl=trainControl)
results <- resamples(list(svm=fit.cl, svm1=fit.cl), trControl =
trainControl, metric=metric)

# histogram
d <-ggplot(results$values, aes(x=results$values[,2])) +
  geom_histogram(colour="blue", fill="white") + theme_bw() +
xlab("Accuracy") + ylab("Frequency")
d

## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```


Frequency
Accuracy