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#########################################################
##       Random Forest Clustering Tutorial             ##
##                                                     ##
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##                                                     ##
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ABSTRACT

This tutorial shows how to carry out RF clustering using the freely available software R
(http://cran.r-project.org/). Specifically, it shows how to analyze the tumor marker example
(motivational example) and the simulated example (ExRule) that are described in our technical report
Shi and Horvath (2005) Unsupervised Learning with Random Forest Predictors.
Technical Report:
http://www.genetics.ucla.edu/labs/horvath/publications/RFclusteringShiHorvath.pdf

For detailed description of RF clustering theory and algorithm,
please consult the following references.

REFRENCES:

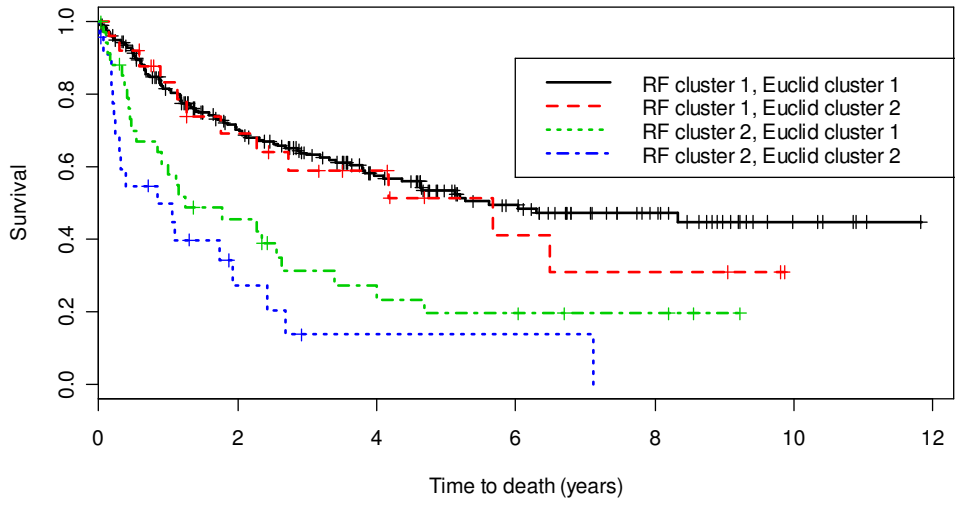
1) Shi and Horvath (2005) Unsupervised Learning with Random Forest Predictors. Technical Report.
http://www.genetics.ucla.edu/labs/horvath/publications/RFclusteringShiHorvath.pdf 1)
An OLD version with different emphasis can be found here:
Shi T, Horvath S. 2003 Using random forest similarities in unsupervised learning:
Applications to microarray data. In Atlantic Symposium on Computational Biology
and Genome Informatics (CBGI'03); The Association of Intelligent Machinery, Durham, NC
2) Breiman L. Random forests. Machine Learning 2001;45(1):5-32.
2) Breiman's random forests: http://stat-www.berkeley.edu/users/breiman/RandomForests/
4) Liaw A. and Wiener M. Classification and Regression by randomForest. R News, 2(3):18-22, Dec 2002.
5) Shi T, Seligson D, Belldegrun AS, Palotie A, Horvath S (2004)
Tumor Classification by Tissue Microarray Profiling:
Random Forest Clustering Applied to Renal Cell Carcinoma. Modern Pathology.
See also: http://www.genetics.ucla.edu/labs/horvath/kidneypaper/RCC.htm

```
######################################## DEMO CODE
#####################################################################
## Pre-requisite: install the randomForest R library (Liaw and Wiender 2002),
## which is a contributed package in R.
## To compute the RF dissimilarity, you need to specify 3 parameters:
##   1) mtry1=number of features sampled at each split
##   2) no.forests=number of forests
##   3) no.trees=number of trees in each forest.

## We will also make use of the following functions in the file "FunctionsRFclustering.txt".
## Rand= this function computes the Rand index for comparing 2 clustering results
## pamNew= our corrected version of the partitioning around medoid function pam.


## 1) To install the R software, go to http://www.R-project.org
## 2) After installing R, you need to install two additional R packages: randomForest and Hmisc
##      Open R and go to menu "Packages\Install package(s) from CRAN", then choose randomForest.
##          R will automatically install the package.
##   When asked "Delete downloaded files (y/N)? ", answer "y".
## Repeat the same for the package Hmisc.
## 3) Download the zip file containing:
##      a) R function file: "FunctionsRFclustering.txt",
##             which contains several R functions needed for RF clustering and results assessment
##      b) A test data file: "testData.csv"
##      c) The tutorial file: "RFclusteringTutorial.txt"
## 4) Unzip all the files into the same directory, for example, it is "C:\temp\RFclustering"
## 5) Open the R software by double clicking its icon.
## 6) Open the tutorial file "RFclusteringTutorial.txt" in a text editor,
##    e.g. Notepad or Microsoft Word
## 7) Copy and paste the R commands from this tutorial into the R session.
##    Comments are preceded by "#" and are automatically ignored by R.
# Start copying and pasting the following

## change the working directory in R to where the data and functions are....
setwd("C:/Documents and Settings/SHorvath/My
Documents/ADAG/TaoShi/RFclustering/RFclusterTutorial/March2005")

## load the library and ignore the warning message
source("FunctionsRFclustering.txt")

## read in the data set
## This is the data set we used in the technical report Shi and Horvath (2005)
## as the motivational example
## We will show how to generate the plots of Figure 1 in that manuscript
dat1 = read.table("testData.csv", sep=",", header=T, row.names=1)

## This is the input file for RF clustering algorithm
datRF = dat1[,1:8]
attach(datRF)

## Here is the histogram of tumor marker #1 as shown in Figure 1a
hist(datRF$Marker1, xlim=c(0,100), ylim=c(0,300), xlab="Score in %", main="Marker 1")

## Calculating RF distance between samples based on the 8 marker measurements
## This will take quite long time depends how many tree and repetitions you choose
## We suggest to use relatively large number of forests with large number of trees

no.forests=25 # for the final version,you would want to increase this number to say 50 or 100
no.trees=3000 # this could also be increased to say 4000
# Since we are mainly interested in the Addcl1 RF dissimilarity we set addcl1=T,addcl2=F
# imp=T specificies that we are also interested in the importance measures.
distRF = RFdist(datRF, mtry1=3, no.trees, no.forests, addcl1=T,addcl2=F,imp=T, oob.prox1=T)
```

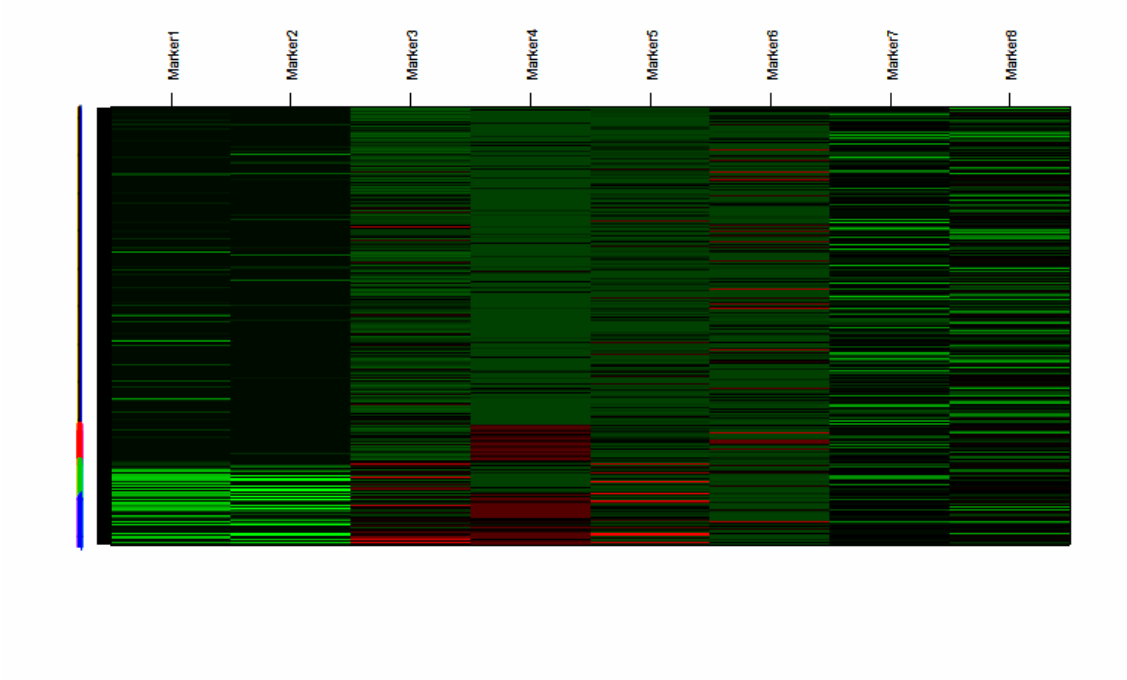
```
## PAM clustering based on the Addcl1 RF dissimilarity
no.clusters = 2
labelRF = pamNew(distRF$cl1, no.clusters)

## PAM clustering based on Euclidean distance
labelEuclid = pamNew(dist(datRF), no.clusters)

## Due to the randomness of RF procedure, the exact distance measure will vary a bit
## Therefore, we also include our RF clustering result in our data
labelRF = dat1$labelRF

## Check the agreement between RF cluster and Euclidean distance cluster
fisher.test(table(labelRF, labelEuclid))  ## Fisher's exact p value
```

```
                    Selected Output
                            Fisher's Exact Test for Count Data
                    data:  table(labelRF, labelEuclid)
                    p-value = 1.216e-15
```

```
## Define a new clustering label based on labelRF and labelEuclid
## labelNew=1, if labelRF=1 and labelEuclid=1
## labelNew=2, if labelRF=1 and labelEuclid=2
## labelNew=3, if labelRF=2 and labelEuclid=1
## labelNew=4, if labelRF=2 and labelEuclid=2
labelNew = ifelse(labelRF==1&labelEuclid==1, 1,
            ifelse(labelRF==1&labelEuclid==2, 2,
            ifelse(labelRF==2&labelEuclid==1, 3, 4)))

## check survival difference as in Figure 1b
## variables "time" and "event" in dat1 are survival time and cencering indicator, respectively

## NOTE: the RF clusters are more meaningful with respect to survival time.
fit1 = survfit(Surv(time, event)~labelNew, data=dat1, conf.type="log-log")
plot(fit1, conf.int=F,col= unique(labelNew), lty=1:4, xlab="Time to death
(years)",ylab="Survival", lwd=2)
legend(6,0.9, c("RF cluster 1, Euclid cluster 1", "RF cluster 1, Euclid cluster 2",
     "RF cluster 2, Euclid cluster 1","RF cluster 2, Euclid cluster 2"), lty=1:4, col=1:4, lwd=2)
#Output: Kaplan Meier plot: Proportion of Surviving Patients versus Time
```

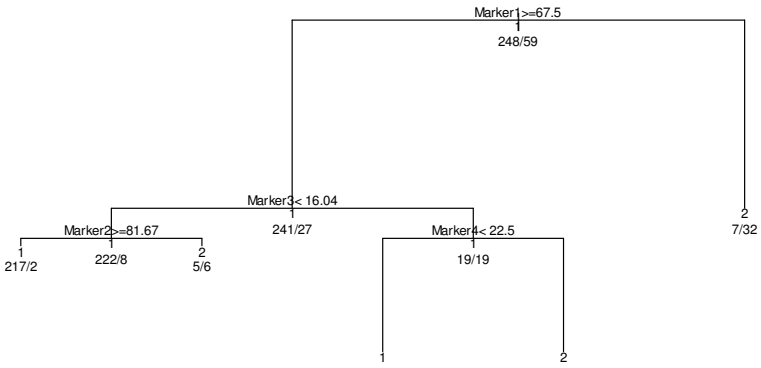


```
## Figure 1c (need library 'sma')
library(sma)
colorh = labelNew
order1 = order(labelNew)
par(mfrow=c(1,1))
plot.mat( scale(datRF[order1,]), rlabels=colorh[order1], rcols=colorh[order1],
clabels=dimnames(datRF)[[2]], ccols=1, margin=0.5)
# Heatmap plot: rows correspond to observations (tumor samples) columns to tumor markers
# the rows are sorted according to the color label defined by the K-M plot above
```




```
# Messages:
# In the green and blue samples (i.e. RF cluster 2) markers 1 and 2 are under-expressed (green)
# while in red and blue samples, marker 4 is over-expressed (red)
```

```
## Using rpart tree the dissect the relationship between RF clusters and markers expression value
## need library 'rpart'
library(rpart)
rp1 = rpart(factor(labelRF)~., datRF)
plot(rp1); text(rp1, all=T, use.n=T, cex=0.7)
```




```
summary(rp1)

Selected Output
Call:
rpart(formula = factor(labelRF) ~ ., data = datRF)
  n= 307

          CP nsplit rel error    xerror       xstd
1 0.42372881      0 1.0000000 1.0000000 0.11701207
2 0.12711864      1 0.5762712 0.6610169 0.09889595
3 0.01694915      3 0.3220339 0.4406780 0.08268346
4 0.01000000      4 0.3050847 0.4745763 0.08549878

Node number 1: 307 observations,    complexity param=0.4237288
  predicted class=1  expected loss=0.1921824
    class counts:   248    59
   probabilities: 0.808 0.192
  left son=2 (268 obs) right son=3 (39 obs)
  Primary splits:
      Marker1 < 67.5     to the right, improve=35.27559, (0 missing)
      Marker2 < 92.5     to the right, improve=30.47311, (0 missing)
      Marker3 < 16.04165 to the left,  improve=27.02816, (0 missing)
      Marker4 < 23.33335 to the left,  improve=22.40886, (0 missing)
      Marker5 < 41.66665 to the left,  improve=13.10948, (0 missing)
  Surrogate splits:
      Marker2 < 30.5952  to the right, agree=0.906, adj=0.256, (0 split)
      Marker3 < 31.25    to the left,  agree=0.883, adj=0.077, (0 split)

Node number 2: 268 observations,    complexity param=0.1271186
  predicted class=1  expected loss=0.1007463
    class counts:   241    27
   probabilities: 0.899 0.101
  left son=4 (230 obs) right son=5 (38 obs)
  Primary splits:
      Marker3 < 16.04165 to the left,  improve=14.116220, (0 missing)
      Marker2 < 81.66665 to the right, improve=10.177620, (0 missing)
      Marker5 < 39.58335 to the left,  improve=10.158150, (0 missing)
      Marker4 < 23.33335 to the left,  improve= 9.135023, (0 missing)
      Marker8 < 94.14285 to the left,  improve= 3.628743, (0 missing)
  Surrogate splits:
      Marker2 < 43.125   to the right, agree=0.866, adj=0.053, (0 split)
      Marker5 < 55       to the left,  agree=0.866, adj=0.053, (0 split)

ETC
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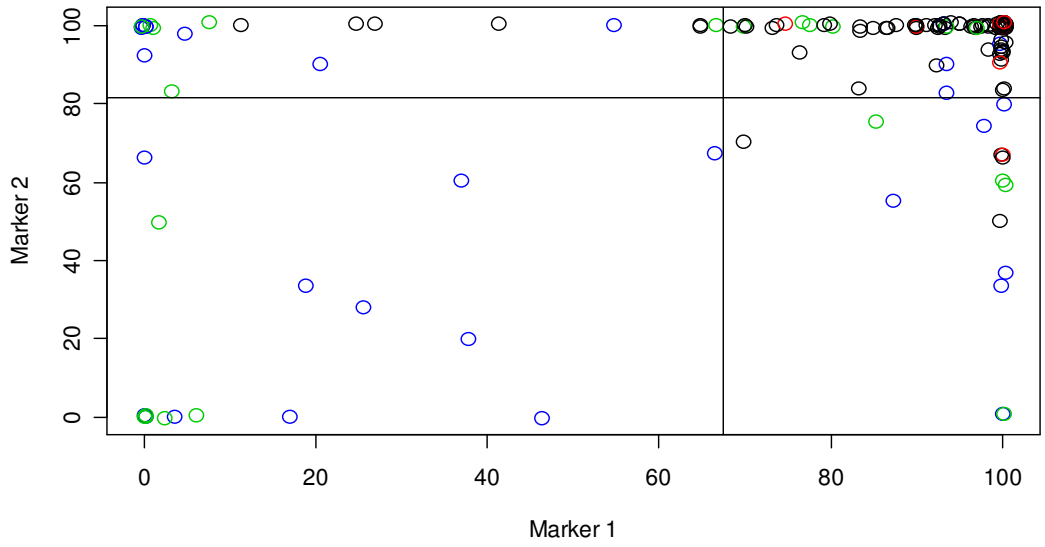
```
## This suggests to construct a rule based on first two splits

rule1 = 2-I(datRF$Marker1>=67.5 & datRF$Marker3<16.04)
table(rule1,labelRF) # 11.4 percent disagreement
           labelRF
      rule1 1   2
          1 222   8
          2  26  51
## Another rule using the sencond highest ranking split for the split number 2 (Marker2>=81.67)
## leads to better agreement between the rule and RF clusters
rule1 = 2.0-I(datRF$Marker1>=67.5 & datRF$Marker2>=81.67)
table(rule1,labelRF) # 9.4 percent disagreement
           labelRF
      rule1 1   2
          1 222   8
          2  26  51


## This means that we can only use two markers to explain the RF clusters (Figure 1d)
plot(jitter(Marker1,5),jitter(Marker2,5), col=labelNew, cex=1.5, xlab="Marker 1", ylab="Marker 2")
abline(h=81.67); abline(v=67.5)
```



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##############################################################################################
###################################Simulated Example   ######################################
## The following codes show how to arrive at the plots for our simulation example 'ExRule'.

## Here is how we simulate the data for the example 'ExRule'
## There are 2 signal variables.
## For observations in cluster 1 and cluster 2, the 2 signal variables X1 and X2 have random
## uniform distributions on the intervals U[0.8, 1] and U[0, 0.8], respectively.
## Thus cluster 1 observations can be predicted using the
## threshold rule X1 > 0.8 and X2 > 0.8.
## We simulate 150 cluster 1 observations and 150 cluster 2 observations.
## Noise variable X3 is simulated to follow a binary (Bernoulli) distribution with hit probability
## =0.5, which is independent of all other variables.
## Noise variables X4, ... ,X10 are simulated by randomly permuting variable X1,
## i.e. they follow the same distribution of X1 but are independent of all other variables.
nobs = 300
ratio1 = 0.5
X1 = c(runif(nobs*ratio1, min=0.8, max=1), runif((1-ratio1)*nobs,min=0,max=0.8) )
X2 = c(runif(nobs*ratio1, min=0.8, max=1), runif((1-ratio1)*nobs,min=0,max=0.8) )
X3 = sample(rep(c(0,1),c(nobs/2,nobs/2) ))
data1 =
data.frame(X1,X2,X3=X3,X4=sample(X1),X5=sample(X1),X6=sample(X1),X7=sample(X1),X8=sample(X1),X9=sa
mple(X1),X10=sample(X1))

## Generate RF dissimilarity
distRF2 = RFdist(data1, mtry1=3, 1000, 7, addcl1=T,addcl2=T,imp=T, oob.prox1=T)

## Classical Multidimensional scaling based on RF or Euclidean distances
cmd1 = cmdscale(as.dist(distRF2$cl1),2)
cmd2 = cmdscale(as.dist(distRF2$cl2),2)
cmdEuclid = cmdscale(dist(data1),2)
## Isotonic Multidimensional scaling based on RF or Euclidean distances
iso1 = isoMDS(as.dist(distRF2$cl1),k=2)$points
iso2 = isoMDS(as.dist(distRF2$cl2),k=2)$points
isoEuclid = isoMDS(dist(data1),k=2)$points

## Color the points based on X1 and X3
color1 = ifelse( X1>=0.8 & X3==0, "black",
          ifelse( X1>=0.8 & X3==1, "red",
          ifelse( X1<0.8  & X3==1, "blue","green")))
```
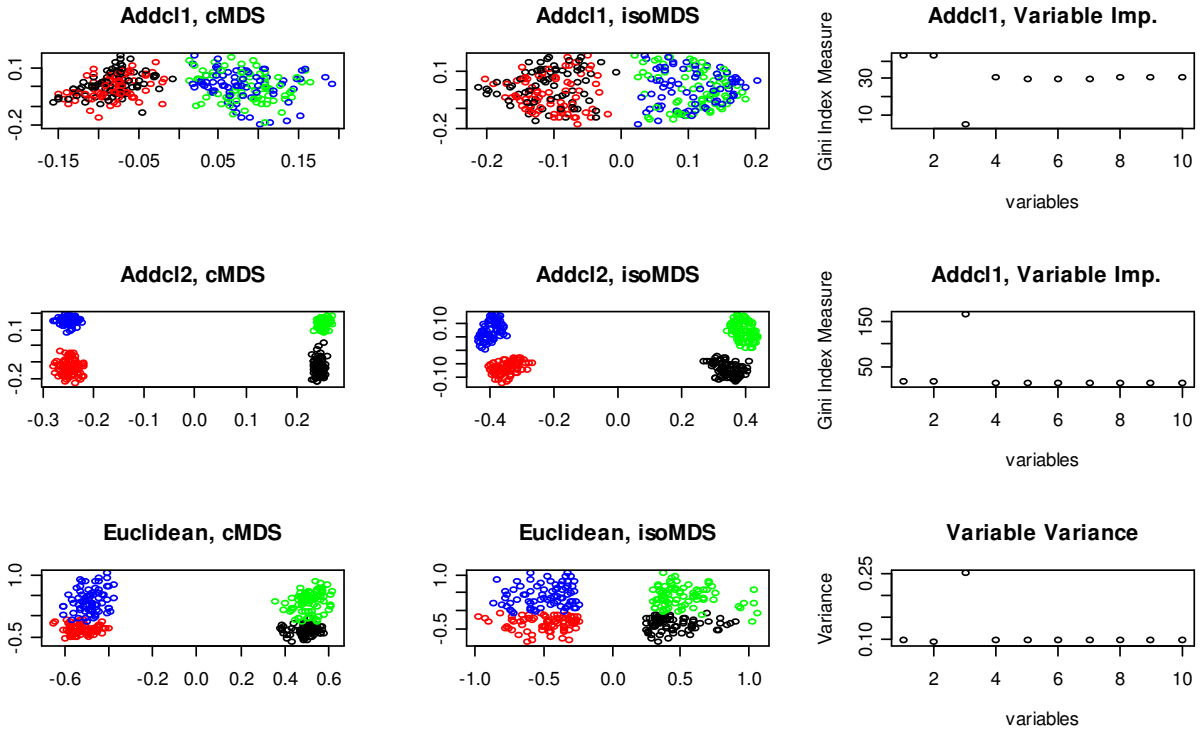

```
## Figure 3
par(mfrow=c(3,3))
plot(cmd1,col=color1,xlab=NA, ylab=NA, main="Addcl1, cMDS" )
plot(iso1,col=color1,xlab=NA, ylab=NA, main="Addcl1, isoMDS")
plot(distRF2$imp1[,4]  ,xlab="variables",ylab="Gini Index Measure", main="Addcl1, Variable Imp.")
plot(cmd2,col=color1,xlab=NA, ylab=NA, main="Addcl2, cMDS")
plot(iso2,col=color1,xlab=NA, ylab=NA,main="Addcl2, isoMDS")
plot(distRF2$imp2[,4] ,xlab="variables",ylab="Gini Index Measure", main=" Addcl1, Variable Imp.")
plot(cmdEuclid,col=color1,xlab=NA, ylab=NA, main="Euclidean, cMDS" )
plot(isoEuclid,col=color1,xlab=NA, ylab=NA, main="Euclidean, isoMDS")
plot(apply(data1,2,var) ,xlab="variables",ylab="Variance", main="Variable Variance")
```




```
#THE END
```