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#===== Simulated Metabolomics Biomarkers R Code =====
#Program description: R code to perform feature selection on simulated metabolomics data in two sections. First section
#uses LASSO and Elastic Net and second one uses VIP and Student t feature selection methods. The BioMark package
#simulates data according to different study parameters (N,P,Q,delta,correlation structures). The four feature selection
#methods are used on each simulated data set and the pAROC is calculated. Output is two csv files containing the
#pAROC values according to different parameters of the simulation, one for each section.
#
#Running first half of code to generate Elastic Net and Lasso results takes about 5.6 minutes per simulation on my Dell
#with a 64-bit processor and an Intel(R) Core(TM) i7-3770OS CPU @ 3.10 GHz with 8.00 GM RAM. Running the
#second half for the Student t and VIP takes about 1hour per simulation, primarily because of the resampling to obtain p-
values for the VIP method.

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# Project name: Simulated Metabolomics Biomarkers R Code
# Project home page: https://people.ucalgary.ca/~kakopciu
# Operating system(s): Unix (including FreeBSD and Linux),Windows, MacOS
# Programming language: R 3.0 and higher
# Other requirements: R contributed packages BioMark (will load glmnet
# License: GNU GPL
# Any restrictions to use by non-academics: none

# -----Setting directory, installing packages -----#
# set working directory where source code can be found and where output will be placed
# setwd("C://Documents and Settings")

# can run program by sourcing code or by copying and pasting code directly into R
# source("C:\\Documents and Settings\\SimulationRcode.txt")
# install 2 contributed packages from CRAN
install.packages("BioMark")
#then attach library or just attach if packages already installed in a local repository
library(BioMark)

# remove ALL objects and functions to ensure only new results and variables being captured
# CAUTION – will remove everything in directory !
rm(list=ls())

#----- setting options and parameters for simulation -----#
# Setting the minimal fraction of times a variable should be in the top list to be considered as a potential biomarker.
biom.options(min.present=0.5)
#Setting the number of "top" coefficients taken into account in stability-based biomarker identification
biom.options(ntop = 10)
biom.options(lasso=list(lambda.min.ratio = 0.01, lambda = seq(0.003,0.3, 0.0025), alpha = c(0.5,1)))

seed<-set.seed(94783)
Numbermets<-c(50,200,1000) # Number of features, P
Ncontrol<-c(25,50) # Number of observations N/2 in each of the two classes(control/treated)
Numbersims<-5 #200 # Number of simulations
delta<-c(0.2,0.4,0.8) # Mean differences between groups for biomarkers
alp<-c(0.5,1) # alpha parameter used in Elastic Net
rho<- 0.5 # use this parameter for AR(1) correlation setting

startT<-Sys.time() # can use to estimate total run time for large number of simulations

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#===== ENet and LASSO =====#
mm<-matrix(ncol=6+Numbersims) #creating an empty matrix to store pAUC values

#----- independent correlation structure -----#
for(l in Ncontrol){ # Loops through different sample sizes of N
  for(i in Numbermets){ # Loops through different sizes for P
    NumberSignmets<-as.integer(c(i*0.1,i*0.2,i*0.3)) #Percentage of significant mets, Q, 10%,20%,and 30% of
#the total number of mets
    # Creating independent correlation matrix
    mycorind <- matrix(0, i, i)
    diag(mycorind) <- 1
    for( j in NumberSignmets){ # Loops through different sizes for Q
      for(k in delta){ # Loops through different values for delta
        #Creating empty vectors for pAROC values
        lassovec<-c()
        enet1<-c()
        enet2<-c()
        simdata <- gen.data(ncontrol = l, nvar = i, nbio = j,cormat = mycorind,nsimul =
Numbersims,means=rep(0,i),group.diff = k)
        for(a in alp) {
          biom.options(lasso=list(lambda.min.ratio = 0.01, lambda = seq(0.003,0.3, 0.0025), alpha = a))
          for(m in 1:Numbersims) {
            set.seed(94783+m)
            studt.coef <- get.biom(X = simdata$X[,m], Y = simdata$Y, fmethod
="lasso",ncomp=2, scale.p="none", type = "stab")
            # Checking lambdas until q mets are selected
            for(e in 1:length(studt.coef$info$lasso$lambda)){
              if(length(studt.coef$lasso[[e]]$biom.indices)-j >= 0){
                fracsele<-studt.coef$lasso[[e]]$fraction.selected
                break
              }
              else fracsele<-
studt.coef$lasso[[length(studt.coef$info$lasso$lambda)]]$fraction.selected
            }
            # Creating ROC cuves from the two methods
            true.biom <- (1:ncol(simdata$X[,m]) %in% c(1:j))
            vip.roc<-ROC(fracsele,true.biom)
            #placing the pAROC(FDR 0.2) values into the vectors
            if(a == 0.5){
              enet1<-append(enet1,AUC(vip.roc,max.mspe=0.2))
            }
            if(a == 1){
              lassovec<-append(lassovec,AUC(vip.roc,max.mspe=0.2))
            }
          }#end m loop
        }#end a loop)
        # Creating a vector with the parameters and the pAROC values
        v0<-c('enet alpha=0.5',l*2,i,j,k,"Independent",enet1)
        v1<-c('Lasso',l*2,i,j,k,"Independent",lassovec)
        # Adding the vector to the matrix
        mm<-rbind(mm,v0,v1)
      } # end k loop
    } #end j loop
  } #end i loop
} #end l loop

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#----- Low correlation -----#
for(l in Ncontrol) { #Loops through different sample sizes of N
  for(i in Numbermets) { # Loops through different sizes for P
    NumberSignmets<-as.integer(c(i*0.1,i*0.2,i*0.3)) #Percentage of significant mets, Q, 10%,20%,and 30% of
the total number of mets
    for( j in NumberSignmets) { # Loops through different sizes for Q
      for(k in delta) { # Loops through different values for delta
        #Creating empty vectors for pAROC values
        lassovec<-c()
        enet1<-c()
        enet2<-c()
        mycovlowcor <- matrix(0, i, i)
        mycovlowcor[row(mycovlowcor) <= j & col(mycovlowcor) <= j] <- 0.7
        mycovlowcor[row(mycovlowcor) > j & col(mycovlowcor) > j] <- 0.1
        diag(mycovlowcor) <- 1
        simdata <- gen.data(ncontrol = l, nvar = i, nbiom = j, cormat = mycovlowcor, nsimul =
Numbersims, means=rep(0,i), group.diff = k)
        for(a in alp){
biom.options(lasso=list(lambda.min.ratio = 0.01, lambda = seq(0.003,0.3, 0.0025), alpha = a))
          for(m in 1:Numbersims){
set.seed(94783+m)
studt.coef <- get.biom(X = simdata$X[,m], Y = simdata$Y, fmethod="lasso", ncomp=2, scale.p="none", type = "stab")
              #checking lambdas until q mets are selected
              for(e in 1:length(studt.coef$info$lasso$lambda)){
                if(length(studt.coef$lasso[[e]]$biom.indices)-j >= 0){
                  frac.sel<-studt.coef$lasso[[e]]$fraction.selected
                  break
                }
                else frac.sel<-
studt.coef$lasso[[length(studt.coef$info$lasso$lambda)]]$fraction.selected
              }
              #Creating ROC cuves from the two methods
              true.biom <- (1:ncol(simdata$X[,m]) %in% c(1:j))
              vip.roc<-ROC(frac.sel,true.biom)
              #placing the pAROC(FDR 0.2) values into the vectors
              if(a == 0.5){
                enet1<-append(enet1,AUC(vip.roc,max.ms.spec=0.2))
              }
              if(a == 1){
                lassovec<-append(lassovec,AUC(vip.roc,max.ms.spec=0.2))
              }
            } #end m loop
          } #end a loop)
        # Creating a vector with the parameters and the pAROC values
        v2<-c('enet alpha=0.5',l*2,i,j,k,"Low Correlation",enet1)
        v3<-c('Lasso',l*2,i,j,k,"Low Correlation",lassovec)
        # Adding the vector to the matrix
        mm<-rbind(mm,v2,v3)
      } # end k loop
    } #end j loop
  } #end i loop
} #end l loop

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#----- AR(1) structure -----#

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for(l in Ncontrol) { # Loops through different sample sizes of N
  for(i in Numbermets){# Loops through different sizes for P
    NumberSignmets<-as.integer(c(i*0.1,i*0.2,i*0.3)) #Percentage of significant mets, Q, 10%,20%,and 30% of
the total number of mets
    for( j in NumberSignmets) { # Loops through different sizes for Q
      for(k in delta) { # Loops through different values for delta
        #creating empty vectors for pAROC values
        lassovec<-c()
        enet1<-c()
        enet2<-c()
        #Creating AR(1) correlation matrix
        mycovAR <- rho^(as.matrix(dist(1:i)))
        diag(mycovAR) <- 1
        simdata <- gen.data(ncontrol = l, nvar = i, nbiom = j,cormat = mycovAR,nsimul =
Numbersims,means=rep(0,i),group.diff = k)
        for(a in alp){
          biom.options(lasso=list(lambda.min.ratio = 0.01, lambda = seq(0.003,0.3, 0.0025), alpha = a))
          for(m in 1:Numbersims){
            set.seed(94783+m)
            studt.coef <- get.biom(X = simdata$X[,m], Y = simdata$Y, fmethod ="lasso",ncomp=2, scale.p="none", type = "stab")
            # Checking lambdas until q mets are selected
            for(e in 1:length(studt.coef$info$lasso$lambda)){
              if(length(studt.coef$lasso[[e]]$biom.indices)-j >= 0){
                fracsel<-studt.coef$lasso[[e]]$fraction.selected
                break
              }
              else fracsel<-
studt.coef$lasso[[length(studt.coef$info$lasso$lambda)]]$fraction.selected
            }
            # Creating ROC cuves from the two methods
            true.biom <- (1:ncol(simdata$X[,m]) %in% c(1:j))
            vip.roc<-ROC(fracsel,true.biom)
            # Placing the pAROC(FDR 0.2) values into the vectors
            if(a == 0.5){
              enet1<-append(enet1,AUC(vip.roc,max.mspect=0.2))
            }
            if(a == 1){
              lassovec<-append(lassovec,AUC(vip.roc,max.mspect=0.2))
            }
          } #end m loop
        } #end a loop)
        # Creating a vector with the parameters and the pAROC values
        v4<-c('enet alpha=0.5',l*2,i,j,k,"AR(1)",enet1)
        v5<-c('Lasso',l*2,i,j,k,"AR(1)",lassovec)
        # Adding the vector to the matrix
        mm<-rbind(mm,v4,v5)
      } # end k loop
    } #end j loop
  } #end i loop
} #end l loop

#----- create output .csv file for ENet and LASSO Results-----
ff<-data.frame(mm[-1,])
# Naming the columns. After sigma, each column represents the pAROC values estimated in each simulation
names<-c('method','n','p','q','delta','sigma',rep(1:Numbersims))
colnames(ff)<-names

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# Writing output to csv file
write.csv(ff,"lassoelasticNet.csv")
Sys.time() - startT

#===== VIP and Student t ===== #
startT<-Sys.time()
# remove storage matrices with same names as in previous part
rm("ff")
rm("mm")
mm<-matrix(ncol=6+Numbersims)#creating an empty matrix

#----- independent correlation structure -----#
for(l in Ncontrol) { # Loops through different sample sizes of N
  for(i in Numbermets) { # Loops through different sizes for P
    NumberSignmets<-as.integer(c(i*0.1,i*0.2,i*0.3)) # Percentage of significant mets, Q, 10%,20%,and 30% of
the total number of mets
    # Creating independent correlation matrix
    mycorind <- matrix(0, i, i)
    diag(mycorind) <- 1
    for( j in NumberSignmets){# Loops through different sizes for Q
      for(k in delta){# Loops through different values for delta
        # Creating empty vectors for pAROC values
        studtvec<-c()
        vipvec<-c()
        studtHCvec<-c()
        vipHCvec<-c()
        studtHCadjvec<-c()
        vipHCadjvec<-c()
        for(m in 1:Numbersims){# Loops through all simulated datasets
          set.seed(94783+m)
          # Simulating metabolomics data with different parameters in each loop
          simdata <- gen.data(ncontrol = l, nvar = i, nbio = j,cormat = mycorind,
            nsimul = Numbersims,means=rep(0,i),group.diff = k)
          # Using Student T and VIP methods on the simulated data
          studt.coef <- get.biom(X = simdata$X[,m], Y = simdata$Y, fmethod
=c("studentt","vip"),ncomp=2, scale.p="none", type = "stab")
          # Creating a vector of true/false, where true represents the true biomarkers
          true.biom <- (1:ncol(simdata$X[,m]) %in% c(1:j))

# now get pvalues and adjusted pvalues for Student t and vip using HC
studt.HC <- get.biom(X = simdata$X[,m], Y = simdata$Y, fmethod =c("studentt","vip"),ncomp=2, scale.p="none", type
= "HC")

          # Creating ROC cuves from the two methods
          vip.roc<-ROC(studt.coef$vip$`2`$fraction.selected,true.biom)
          stab.roc <- ROC(studt.coef$studentt[[1]]$fraction.selected,true.biom)

          vipHC.roc<-ROC( (1/(studt.HC$ vip [[1]]$pvals)), true.biom)
          vipHCadj.roc<-ROC( (1/(p.adjust(studt.HC$ vip [[1]]$pvals, method="fdr"))),true.biom)
          studtHC.roc <- ROC((1/(studt.HC$ studentt [[1]]$pvals)),true.biom)
          studtHCadj.roc <- ROC((1/(p.adjust(studt.HC$ studentt [[1]]$pvals, method="fdr"))),true.biom)
          # Placing the pAROC(FDR 0.2) values into the vectors
          studtvec<-append(studtvec,AUC(stab.roc, max.mspect = .2))
          vipvec<-append(vipvec,AUC(vip.roc, max.mspect = .2))
          studtHCvec<-append(studtHCvec, AUC(studtHC.roc, max.mspect = .2))
          vipHCvec<-append(vipHCvec, AUC(vipHC.roc, max.mspect = .2))
          studtHCadjvec<-append(studtHCadjvec,AUC(studtHCadj.roc, max.mspect = .2))
        }
      }
    }
  }
}

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vipHCadjvec<-append(vipHCadjvec,AUC(vipHCadj.roc, max.mspect = .2))
} #end m loop

# Creating a vector with the parameters and the pAROC values
va2<-c('Student T',l*2,i,j,k,"Independent",studtvec)
va3<-c('vip',l*2,i,j,k,"Independent",vipvec)
vb1<-c('Student T HC',l*2,i,j,k,"Independent",studtHCvec)
vb2<-c('Student T HC adj',l*2,i,j,k,"Independent",studtHCadjvec)
vb3<-c('vip HC',l*2,i,j,k,"Independent",vipHCvec)
vb4<-c('vip HC adj',l*2,i,j,k,"Independent",vipHCadjvec)
# Adding the vector to the matrix
mm<-rbind(mm,va2,va3,vb1,vb2,vb3,vb4)
} # end k loop
} #end j loop
} #end i loop
} #end l loop

#----- Low correlation -----#
for(l in Ncontrol){# Loops through different sample sizes
  for(i in Numbermets){# Loops through different sizes for p
    NumberSignmets<-as.integer(c(i*0.1,i*0.2,i*0.3))# Percentage of significant mets, 10%,20%,and 30% of
the total number of mets
    for(j in NumberSignmets){# Loops through different sizes for q
      for(k in delta){# Loops through different values for delta
        # Creating empty vectors for pAROC values
        studtvec<-c()
        vipvec<-c()
        studtHCvec<-c()
        vipHCvec<-c()
        studtHCadjvec<-c()
        vipHCadjvec<-c()
        # Creating low correlation matrix
        mycovlowcor <- matrix(0, i, i)
        mycovlowcor[row(mycovlowcor) <= j & col(mycovlowcor) <= j] <- 0.7
        mycovlowcor[row(mycovlowcor) > j & col(mycovlowcor) > j] <- 0.1
        diag(mycovlowcor) <- 1
        for(m in 1:Numbersims){#loops through all simulated datasets
          set.seed(94783+m)
          #Simulating metabolomics data with different parameters in each loop
          simdata <- gen.data(ncontrol = l, nvar = i, nbio = j,cormat = mycovlowcor, nsimul =
Numbersims,means=rep(0,i),group.diff = k)
          # Using Student T and VIP methods on the simulated data
          studt.coef <- get.biom(X = simdata$X[,m], Y = simdata$Y, fmethod
=c("studentt","vip"),ncomp=2,scale.p="none", type = "stab" )
          # Creating a vector of true/false, where true represents the true biomarkers
          true.biom <- (1:ncol(simdata$X[,m]) %in% c(1:j))
          # Creating ROC cuves from the two methods
          vip.roc<-ROC(studt.coef$vip$`2`$fraction.selected,true.biom)
          stab.roc <- ROC(studt.coef$studentt[[1]]$fraction.selected, true.biom)

# now get pvalues and adjusted pvalues for student t and vip using HC
          studt.HC <- get.biom(X = simdata$X[,m], Y = simdata$Y, fmethod =c("studentt","vip")
,ncomp=2, scale.p="none", type = "HC")
          # Creating ROC cuves from the two methods
          vipHC.roc<-ROC( (1/(studt.HC$ vip [[1]]$pvals)), true.biom)
          vipHCadj.roc<-ROC( (1/(p.adjust(studt.HC$ vip [[1]]$pvals, method="fdr"))), true.biom)

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studtHC.roc <- ROC((1/(studt.HC$ studentt [[1]]$pvals)),true.biom)
studtHCadj.roc <- ROC((1/(p.adjust(studt.HC$ studentt [[1]]$pvals, method="fdr"))),true.biom)

# Placing the pAROC(FDR 0.2) values into the vectors
studtvec<-append(studtvec,AUC(stab.roc, max.mspect = .2))
vipvec<-append(vipvec,AUC(vip.roc, max.mspect = .2))

studtHCvec<-append(studtHCvec, AUC(studtHC.roc, max.mspect = .2))
vipHCvec<-append(vipHCvec, AUC(vipHC.roc, max.mspect = .2))
studtHCadjvec<-append(studtHCadjvec,AUC(studtHCadj.roc, max.mspect = .2))
vipHCadjvec<-append(vipHCadjvec,AUC(vipHCadj.roc, max.mspect = .2))
} #end m loop
# Creating a vector with the parameters and the pAROC values
va4<-c('Student T',l*2,i,j,k,"Low correlation",studtvec)
va5<-c('vip',l*2,i,j,k,"Low correlation",vipvec)
vb5<-c('Student T HC',l*2,i,j,k,"Low correlation",studtHCvec)
vb6<-c('Student T HC adj',l*2,i,j,k,"Low correlation",studtHCadjvec)
vb7<-c('vip HC',l*2,i,j,k,"Low correlation",vipHCvec)
vb8<-c('vip HC adj',l*2,i,j,k,"Low correlation",vipHCadjvec)
# Adding the vector to the matrix
mm<-rbind(mm,va4,va5,vb5,vb6,vb7,vb8)
} #end k loop
} #end j loop
} #end i loop
} #end l loop
# ----- AR(1) structure -----#
for(l in Ncontrol){# Loops through different sample sizes
  for(i in Numbermets){# Loops through different sizes for p
    NumberSignmets<-as.integer(c(i*0.1,i*0.2,i*0.3))# Percentage of significant mets, 10%,20%,and 30% of the
    total number of mets
    for(j in NumberSignmets){# Loops through different sizes for q
      for(k in delta){# Loops through different values for delta
        # Creating empty vectors for pAROC values
        studtvec<-c()
        vipvec<-c()
        studtHCvec<-c()
        vipHCvec<-c()
        studtHCadjvec<-c()
        vipHCadjvec<-c()
        # Creating AR(1) correlation matrix
        mycovAR <- rho^(as.matrix(dist(1:i)))
        diag(mycovAR) <- 1
        for(m in 1:Numbersims){# Loops through all simulated datasets
          # Simulating metabolomics data with different parameters in each loop
          simdata <- gen.data(ncontrol = l, nvar = i, nbio = j,cormat = mycovAR, nsimul
          = Numbersims,means=rep(0,i),group.diff = k)
          #Using Student T and VIP methods on the simulated data
          studt.coef <- get.biom(X = simdata$X[,m], Y = simdata$Y, fmethod
          =c("studentt","vip"),ncomp=2, scale.p="none", type = "stab" )
          # Creating a vector of true/false, where true represents the true biomarkers
          true.biom <- (1:ncol(simdata$X[,m]) %in% c(1:j))
          # Creating ROC cuves from the two methods
          studt.roc <- ROC(studt.coef$studentt[[1]]$fraction.selected, true.biom)
          vip.roc<-ROC(studt.coef$vip$`2`$fraction.selected,true.biom)

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# now get pvalues and adjusted pvalues for student t and vip using HC
studt.HC <- get.biom(X = simdata$X[,m], Y = simdata$Y, fmethod
=c("studentt", "vip"), ncomp=2, scale.p="none", type = "HC")
# Creating ROC cuves from the two methods
vipHC.roc<-ROC( (1/(studt.HC$ vip [[1]]$pvals)), true.biom)
vipHCadj.roc<-ROC( (1/(p.adjust(studt.HC$ vip [[1]]$pvals, method="fdr"))),
, true.biom)

studtHC.roc <- ROC((1/(studt.HC$ studentt [[1]]$pvals)), true.biom)
studtHCadj.roc <- ROC((1/(p.adjust(studt.HC$ studentt [[1]]$pvals,
method="fdr"))), true.biom)

# Placing the pAROC(FDR 0.2) values into the vectors
studtvec<-append(studtvec,AUC(stab.roc, max.mspect = .2))
vipvec<-append(vipvec,AUC(vip.roc, max.mspect = .2))
studtHCvec<-append(studtHCvec, AUC(studtHC.roc, max.mspect = .2))
vipHCvec<-append(vipHCvec, AUC(vipHC.roc, max.mspect = .2))
studtHCadjvec<-append(studtHCadjvec,AUC(studtHCadj.roc, max.mspect = .2))
vipHCadjvec<-append(vipHCadjvec,AUC(vipHCadj.roc, max.mspect = .2))
} #end m loop
# Creating a vector with the parameters and the pAROC values
va6<-c('Student T',l*2,i,j,k,"AR(1)",studtvec)
va7<-c('vip',l*2,i,j,k,"AR(1)",vipvec)
vb9<-c('Student T HC',l*2,i,j,k,"AR(1)",studtHCvec)
vb10<-c('Student T HC adj',l*2,i,j,k,"AR(1)",studtHCadjvec)
vb11<-c('vip HC',l*2,i,j,k,"AR(1)",vipHCvec)
vb12<-c('vip HC adj',l*2,i,j,k,"AR(1)",vipHCadjvec)
# Adding the vector to the matrix
mm<-rbind(mm,va6,va7,vb9,vb10,vb11,vb12)
} #end k loop
} #end j loop
} #end i loop
} #end l loop

#----- create output .csv file -----#
#Changing the matrix into a dataframe
ff<-data.frame(mm[-1,])
#Naming the columns. After sigma, each column represents the pAROC values estimated in each simulation
names<-c('method','n','p','q','delta','sigma',rep(1:Numbersims))
colnames(ff)<-names
#Writing output to csv file
write.csv(ff,"studentT_VIP.csv")
Sys.time() - startT

#===== END PROGRAM =====#

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