# Statistical Tests

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```
# Clear all objects (from the workspace)
rm(list = ls())

# Suppress Warning messages
options(warn = -1)

# Turn off scientific notation like 1e+06
# options(scipen=999)

# Load Libs
library(GEOquery)
```

```
## Loading required package: Biobase
```

```
## Loading required package: BiocGenerics
```

```
## Loading required package: parallel
```

```
##
## Attaching package: 'BiocGenerics'
```

```
## The following objects are masked from 'package:parallel':
##
##     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##     clusterExport, clusterMap, parApply, parCapply, parLapply,
##     parLapplyLB, parRapply, parSapply, parSapplyLB
```

```
## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs
```

```
## The following objects are masked from 'package:base':
##
##     anyDuplicated, append, as.data.frame, basename, cbind, colMeans,
##     colnames, colSums, dirname, do.call, duplicated, eval, evalq,
##     Filter, Find, get, grep, grepl, intersect, is.unsorted, lapply,
##     lengths, Map, mapply, match, mget, order, paste, pmax, pmax.int,
##     pmin, pmin.int, Position, rank, rbind, Reduce, rowMeans, rownames,
##     rowSums, sapply, setdiff, sort, table, tapply, union, unique,
##     unsplit, which, which.max, which.min
```

```
## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.
```

```
## Setting options('download.file.method.GEOquery'='auto')
```

```
## Setting options('GEOquery.inmemory.gpl'=FALSE)
```

```
library(Biobase)
library(limma)
```

```
##
## Attaching package: 'limma'
```

```
## The following object is masked from 'package:BiocGenerics':
##
##     plotMA
```

```
library(affy)
library(siggenes)
```

```
## Loading required package: multtest
```

```
## Loading required package: splines
```

```
source("./statistical_tests_lib.R")
```

---

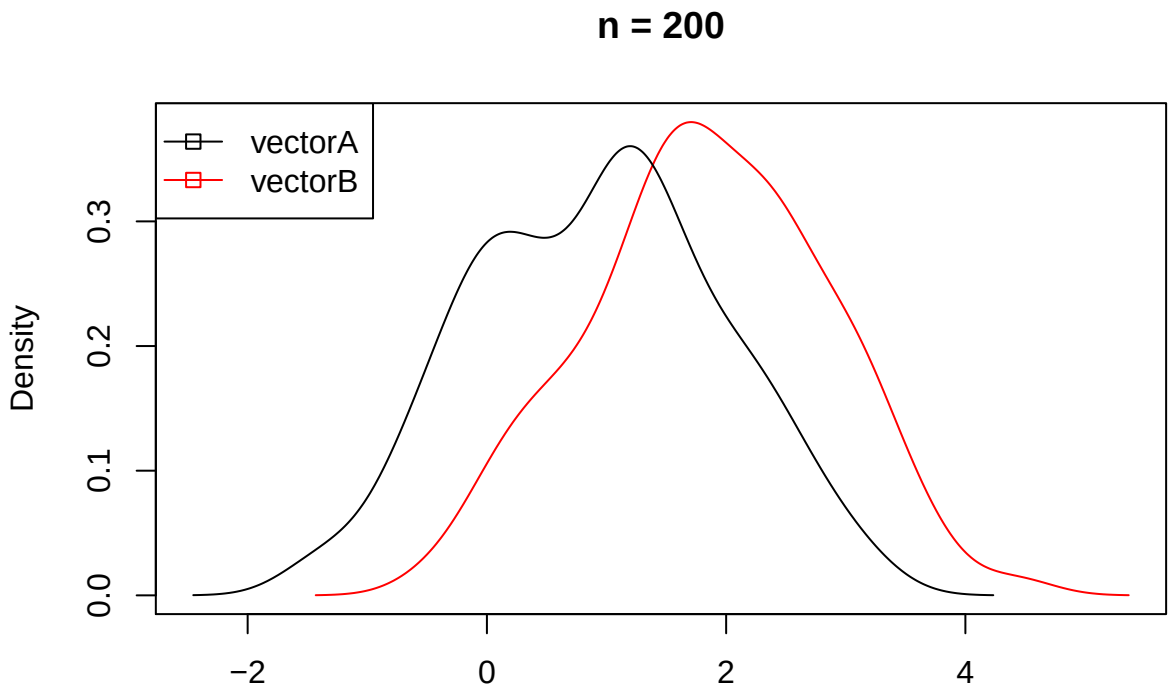
---

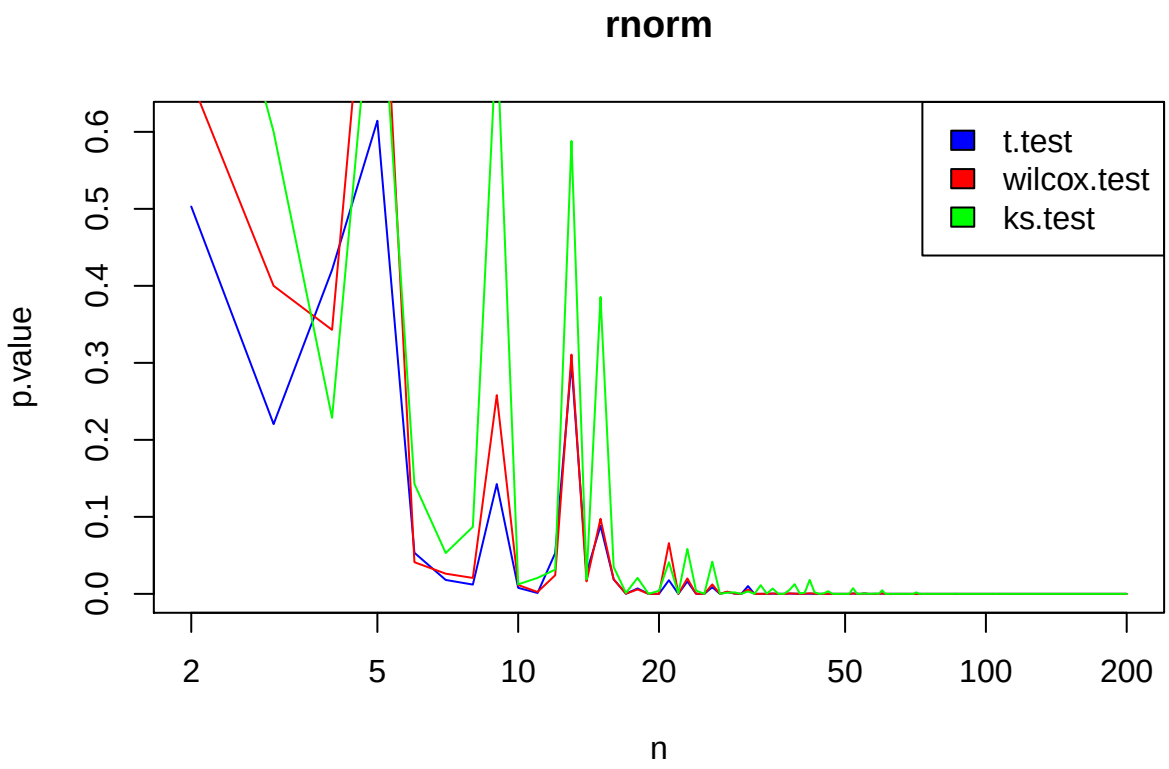
# 1) Obtener p-values con un t-test, Wilcoxon y Kolmogorov para:

## A) 2 vectores de datos con distribución normal (rnorm) y diferente media, para número de muestras n= 2. . . . . 20. Graficar los resultados y comparar.

```
getPvalues <- function(dist) {
    ns = 2:200
    ttest_pval = c()
    wtest_pval = c()
    ktest_pval = c()

    for (n in ns) {
        if (dist == "rnorm") {
            vectorA = rnorm(n=n, mean=1, sd=1)
            vectorB = rnorm(n=n, mean=2, sd=1)
        } else {
            vectorA = runif(n, -1, 1)
            vectorB = runif(n, -1, 1)
        }

        if (n == max(ns)) {
            plot.densities(vectorA, vectorB, main=paste0("n = ", n))
        }

        ttest = t.test(vectorA, vectorB)
        ttest_pval = c(ttest_pval, ttest$p.value)
        #print(ttest$p.value)

        wtest = wilcox.test(vectorA, vectorB)
        wtest_pval = c(wtest_pval, wtest$p.value)
        #print(wtest$p.value)

        ktest = ks.test(vectorA, vectorB)
        ktest_pval = c(ktest_pval, ktest$p.value)
        #print(ktest$p.value)
    }

    if (dist == "rnorm") {
        plot(
            ns, ttest_pval,
            main="rnorm",
            xlab="n",
            ylab="p.value",
            type="l",
            col="blue",
            log="x"
```

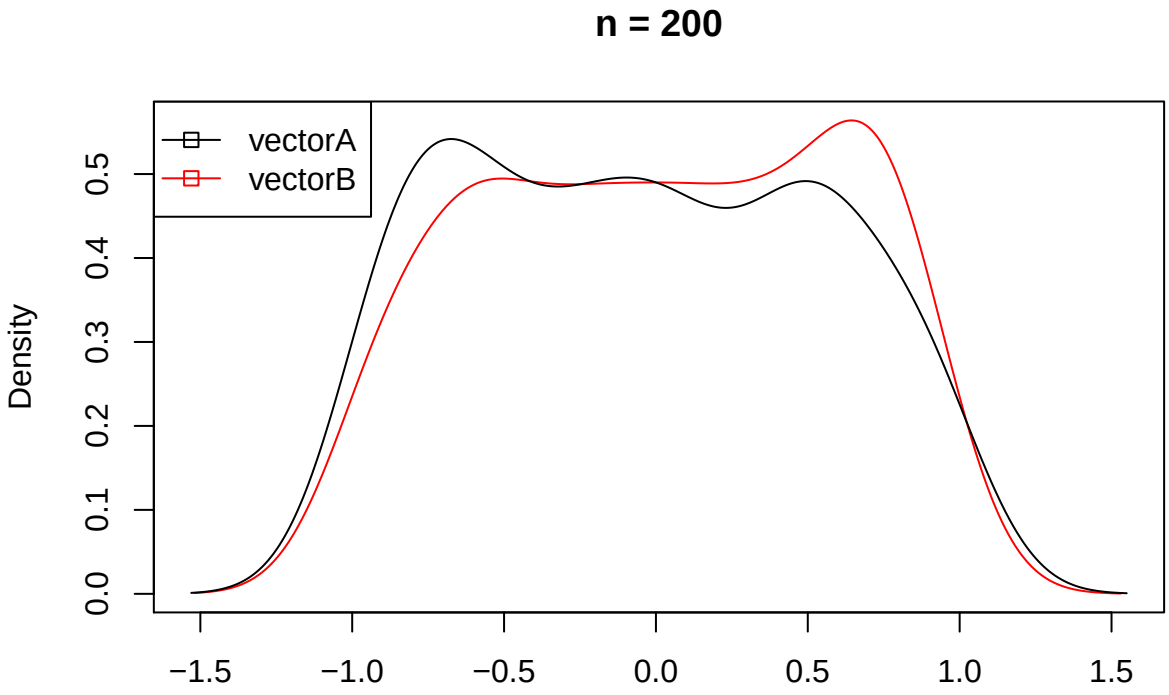
```
        )
    } else {
        plot(
            ns, ttest_pval,
            main="runif",
            xlab="n",
            ylab="p.value",
            type="l",
            col="blue"
        )
    }

    lines(
        ns, wtest_pval,
        col="red"
    )

    lines(
        ns, ktest_pval,
        col="green"
    )

    legend(
        "topright",
        c("t.test","wilcox.test","ks.test"),
        fill=c("blue","red","green")
    )
}

getPvalues("rnorm")
```

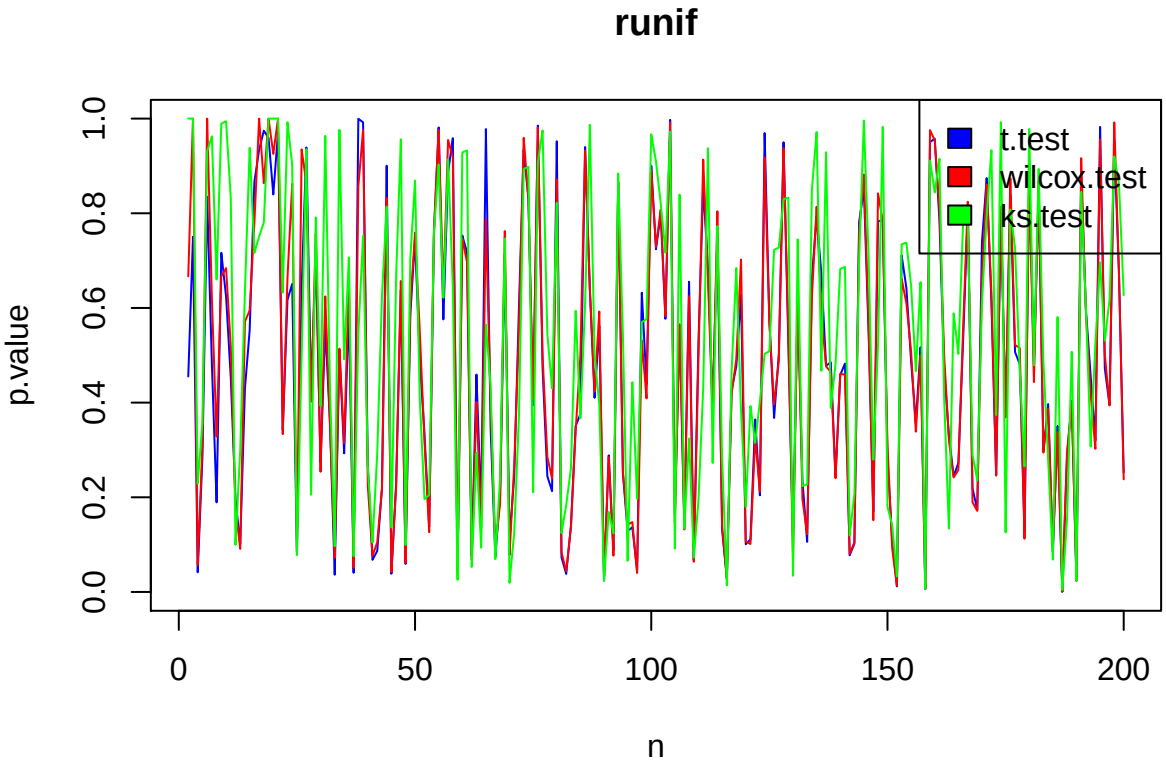





**B) Repetir (A) con 2 vectores de datos generados con la función runif.**

```
getPvalues("runif")
```



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## 2) Obtener p-values con un t-test, Wilcoxon, Kolmogorov y SAM para una base de datos GEO de su elección.

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**Dataset details**

**Title:** Severe acute respiratory syndrome expression profile

**Summary:** Expression profiling of peripheral blood mononuclear cells (PBMC) from 10 adult patients with severe acute respiratory syndrome (SARS). Results provide insight into the host immune response to the SARS coronavirus.

**Organism:** Homo sapiens

**Platform: GPL201:** [HG-Focus] Affymetrix Human HG-Focus Target Array

**Citation:**
Reghunathan R, Jayapal M, Hsu LY, Chng HH et al. *Expression profile of immune response genes in patients with Severe Acute Respiratory Syndrome.* BMC Immunol 2005 Jan 18;6:2. PMID: 15655079

**Reference Series:** GSE1739

**Sample count:** 14

**Value type:** count

**Series published:** 2005/01/18

**Dataset taken from:** GDSbrowser, GSE1739 query, and GSE1739 geo2r

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The GSE1739 was selected for the Bioinformatics class assignments and activities. The reason behind this decision is the similarities that SARS shares with the novel coronavirus COVID-19. The purpose id to work with up-to-date data that is relecant to the current crisis.

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**Let's download .soft.gz from GEO and take a look to the data**

```
# If you have network access, the more typical way to do this
# would be to use this:
gse <- getGEO("GSE1739",GSEMatrix=FALSE)
```

```
## File stored at:
## C:\Users\oskat\AppData\Local\Temp\RtmpOenEUi/GSE1739.soft.gz
## Reading file....
## Parsing....
## Found 15 entities...
## GPL201 (1 of 16 entities)
## GSM30361 (2 of 16 entities)
## GSM30362 (3 of 16 entities)
## GSM30363 (4 of 16 entities)
## GSM30364 (5 of 16 entities)
## GSM30365 (6 of 16 entities)
## GSM30366 (7 of 16 entities)
## GSM30367 (8 of 16 entities)
## GSM30368 (9 of 16 entities)
## GSM30369 (10 of 16 entities)
## GSM30370 (11 of 16 entities)
## GSM30371 (12 of 16 entities)
## GSM30372 (13 of 16 entities)
## GSM30373 (14 of 16 entities)
## GSM30374 (15 of 16 entities)
```

```
# gse <- getGEO(filename=system.file("extdata/GSE781_family.soft.gz",package="GEOquery"))

# Look at gse metadata:
head(Meta(gse))
```

```
## $contact_address
## [1] " "
##
## $contact_city
## [1] "Singapore"
##
## $contact_country
## [1] "Singapore"
##
## $contact_institute
## [1] "NUS"
##
## $contact_name
## [1] "Jayapal,,Manikandan"
##
## $`contact_zip/postal_code`
## [1] "117597"
```

```
# names of all the GSM objects contained in the GSE
names(GSMList(gse))
```

```
##  [1] "GSM30361" "GSM30362" "GSM30363" "GSM30364" "GSM30365" "GSM30366"
##  [7] "GSM30367" "GSM30368" "GSM30369" "GSM30370" "GSM30371" "GSM30372"
## [13] "GSM30373" "GSM30374"
```

```
# and get the first GSM object on the list
GSMList(gse)[[1]]
```

```
## An object of class "GSM"
## channel_count
## [1] "1"
## contact_address
## [1] " "
## contact_city
## [1] "Singapore"
## contact_country
## [1] "Singapore"
## contact_institute
## [1] "NUS"
## contact_name
## [1] "Jayapal,,Manikandan"
## contact_zip/postal_code
## [1] "117597"
## data_row_count
## [1] "8793"
## description
## [1] "PBMC normal sample RNA for Control"
## geo_accession
## [1] "GSM30361"
## last_update_date
## [1] "May 27 2005"
## molecule_ch1
## [1] "total RNA"
## organism_ch1
## [1] "Homo sapiens"
## platform_id
## [1] "GPL201"
## series_id
## [1] "GSE1739"
## source_name_ch1
## [1] "PBMC normal sample RNA"
## status
## [1] "Public on Jan 18 2005"
## submission_date
## [1] "Sep 08 2004"
## supplementary_file
## [1] "NONE"
## taxid_ch1
## [1] "9606"
## title
## [1] "N1"
## type
## [1] "RNA"
## An object of class "GEODataTable"
## ****** Column Descriptions ******
##              Column               Description
## 1            ID_REF
## 2             VALUE      raw signal intensity
## 3         ABS_CALL present, absent, marginal
## 4 DETECTION P-VALUE                   p-value
## ****** Data Table ******
##      ID_REF  VALUE ABS_CALL DETECTION P-VALUE
## 1 1007_s_at  321.8        P          0.035163
## 2   1053_at  204.8        P          0.006532
```

```
## 3    117_at  538.6        P          0.001141
## 4    121_at 1277.8        P          0.011447
## 5 1255_g_at   51.3        A          0.418069
## 8788 more rows ...
```

```
# and the names of the GPLs represented
names(GPLList(gse))
```

```
## [1] "GPL201"
```

**Let's prepare the data for further analysis**

```
# First, we need to make sure that all of the GSMs are from the same platform:
gsmplatforms <- lapply(GSMList(gse),function(x) {Meta(x)$platform_id})
head(gsmplatforms)
```

```
## $GSM30361
## [1] "GPL201"
##
## $GSM30362
## [1] "GPL201"
##
## $GSM30363
## [1] "GPL201"
##
## $GSM30364
## [1] "GPL201"
##
## $GSM30365
## [1] "GPL201"
##
## $GSM30366
## [1] "GPL201"
```

```
# If there are more GPLs, we can filter the original GSMList to include only those GSMs within
# a specific platform and use this list for further processing
gsmlist = Filter(function(gsm) {Meta(gsm)$platform_id=='GPL201'},GSMList(gse))
length(gsmlist)
```

```
## [1] 14
```

```
# So, now we would like to know what column represents the data that we would like to extract.
# Looking at the first few rows of the Table of a single GSM will likely give us an idea (and
# by the way, GEO uses a convention that the column that contains the single measurement for
# each array is called the VALUE column, which we could use if we don't know what other column
# is most relevant).

Table(gsmlist[[1]])[1:5,]
```

```
##      ID_REF  VALUE ABS_CALL DETECTION P-VALUE
## 1 1007_s_at  321.8        P          0.035163
## 2   1053_at  204.8        P          0.006532
## 3    117_at  538.6        P          0.001141
## 4    121_at 1277.8        P          0.011447
## 5 1255_g_at   51.3        A          0.418069
```

```
# and get the column descriptions
Columns(gsmlist[[1]])[1:5,]
```

```
##               Column               Description
## 1             ID_REF
## 2              VALUE      raw signal intensity
## 3          ABS_CALL present, absent, marginal
## 4  DETECTION P-VALUE                   p-value
## NA              <NA>                      <NA>
```

```
#We will indeed use the VALUE column. We then want to make a matrix of these values like so:

# get the probeset ordering
probesets <- Table(GPLList(gse)[[1]])$ID
# make the data matrix from the VALUE columns from each GSM
# being careful to match the order of the probesets in the platform
# with those in the GSMs
data.matrix <- do.call('cbind',lapply(gsmlist,function(x)
                                      {tab <- Table(x)
                                       mymatch <- match(probesets,tab$ID_REF)
                                       return(tab$VALUE[mymatch])
                                      }))
data.matrix <- apply(data.matrix,2,function(x) {as.numeric(as.character(x))})
data.matrix <- log2(data.matrix)
data.matrix[1:5,]
```

```
##       GSM30361 GSM30362  GSM30363  GSM30364 GSM30365 GSM30366  GSM30367
## [1,]  8.330021 8.006186  8.370687  8.518850 7.680887 7.806711  8.033974
## [2,]  7.678072 8.197217  7.764208  8.032321 7.753551 8.053111  5.809929
## [3,]  9.073070 8.519636  9.047124  8.500244 9.132114 8.513333  8.106432
## [4,] 10.319446 9.781688 10.010948 10.016948 9.627351 9.988827 10.186981
## [5,]  5.680887 5.459432  5.375039  4.722466 5.741467 5.189825  4.478972
##       GSM30368  GSM30369 GSM30370  GSM30371  GSM30372 GSM30373 GSM30374
## [1,]  8.292322  7.997179 7.879583  7.622052  7.624978 8.186857 7.050937
## [2,]  7.668885  7.488644 7.089583  7.566054  7.309249 6.072535 7.260214
## [3,] 10.018617  9.144403 9.201389 10.172177 10.452653 8.000000 8.724855
## [4,] 10.215654 10.021813 9.954778  9.737585 10.087728 9.471472 9.545737
## [5,]  6.203593  5.672425 5.409391  6.002252  5.057450 5.133399 6.213347
```

```
#Correr SAM
data.matrix_sam <- sam(data.matrix, c(rep(1,dim(data.matrix)[2])), method = "d.stat", gene.names = colnar
```

```
## We're doing 16384 complete permutations
## and randomly select 100 of them.
```

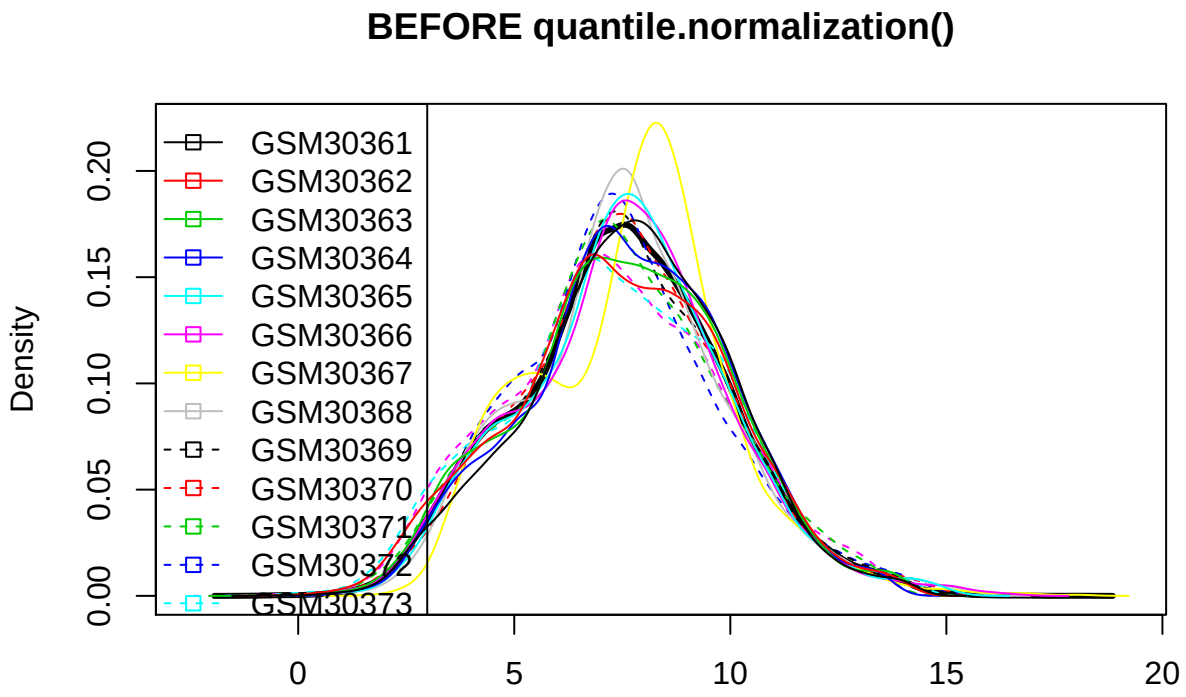
```
#Vector con p-values
sam_pval = data.matrix_sam@p.value
length(sam_pval)
```

```
## [1] 8793
```

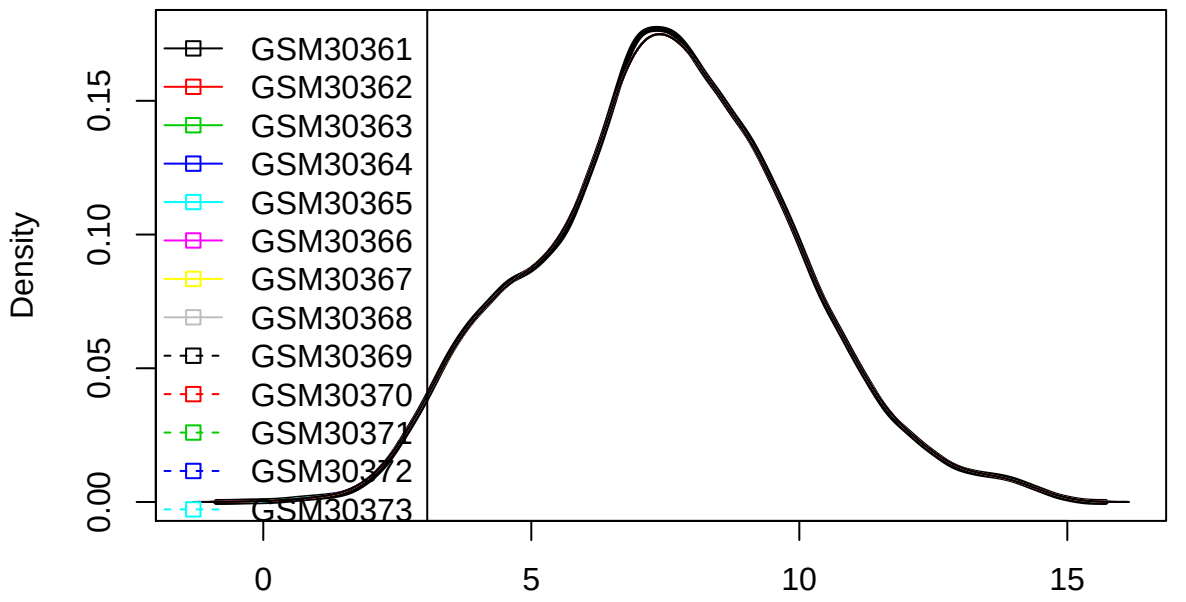
```
#Mostrar resultados de SAM con cierto valor de delta
data.matrix_sam_sum <- summary(data.matrix_sam, 1.9)

#Acceder a matrix de Genes significativos
dim(data.matrix_sam_sum@mat.sig)
```

```
## [1] 4282    6
```

```
plot.densities(data.matrix, main="BEFORE quantile.normalization()")
```




```
data.matrix_qn <- quantile.normalization(data.matrix)
plot.densities(data.matrix_qn, main="AFTER quantile.normalization()")
```




```
pval = de.test(
    x=data.matrix,
    classes=c(rep(1,5),rep(0,5)),
```

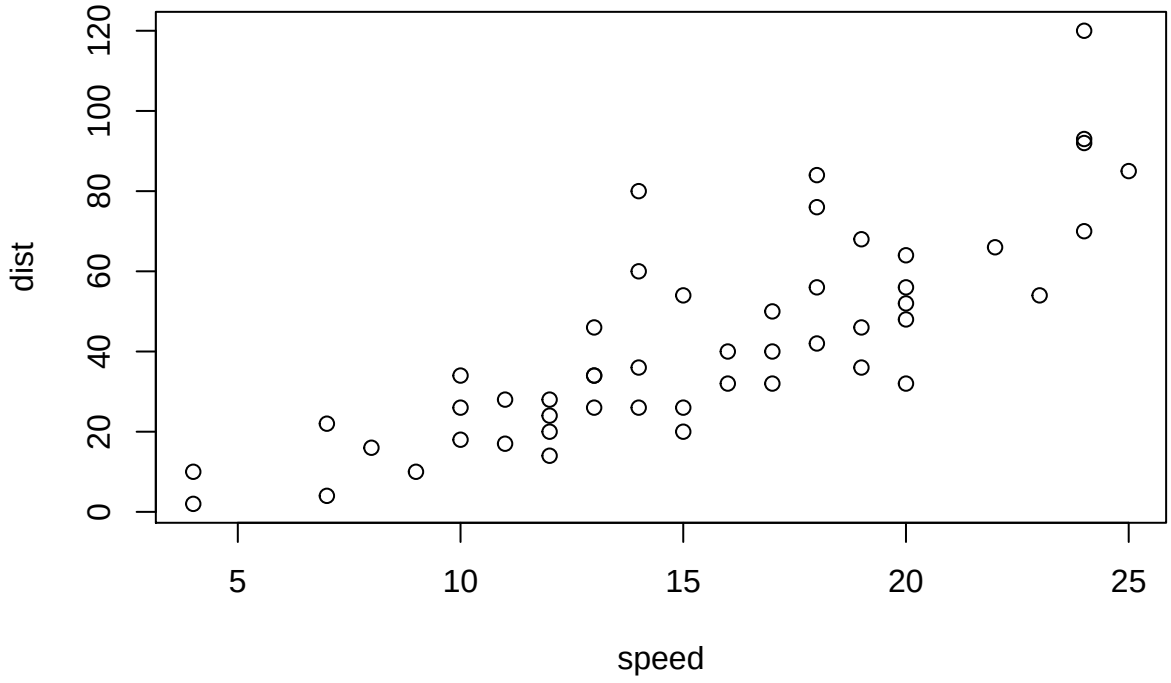
```
    test=c("ttest", "kolmogorov", "wilcoxon")
)

ttest_pval = pval$ttest
wtest_pval = pval$kolmogorov
ktest_pval = pval$wilcoxon

pval_qn = de.test(
    x=data.matrix_qn,
    classes=c(rep(1,5),rep(0,5)),
    test=c("ttest", "kolmogorov", "wilcoxon")
)

ttest_pval_qn = pval_qn$ttest
wtest_pval_qn = pval_qn$kolmogorov
ktest_pval_qn = pval_qn$wilcoxon
```

```
# go through the necessary steps to make a compliant ExpressionSet
rownames(data.matrix) <- probesets
colnames(data.matrix) <- names(gsmlist)
pdata <- data.frame(samples=names(gsmlist))
rownames(pdata) <- names(gsmlist)
pheno <- as(pdata,"AnnotatedDataFrame")
eset2 <- new('ExpressionSet',exprs=data.matrix,phenoData=pheno)
eset2
```

```
## ExpressionSet (storageMode: lockedEnvironment)
## assayData: 8793 features, 14 samples
##   element names: exprs
## protocolData: none
## phenoData
##   sampleNames: GSM30361 GSM30362 ... GSM30374 (14 total)
##   varLabels: samples
##   varMetadata: labelDescription
## featureData: none
## experimentData: use 'experimentData(object)'
## Annotation:
```

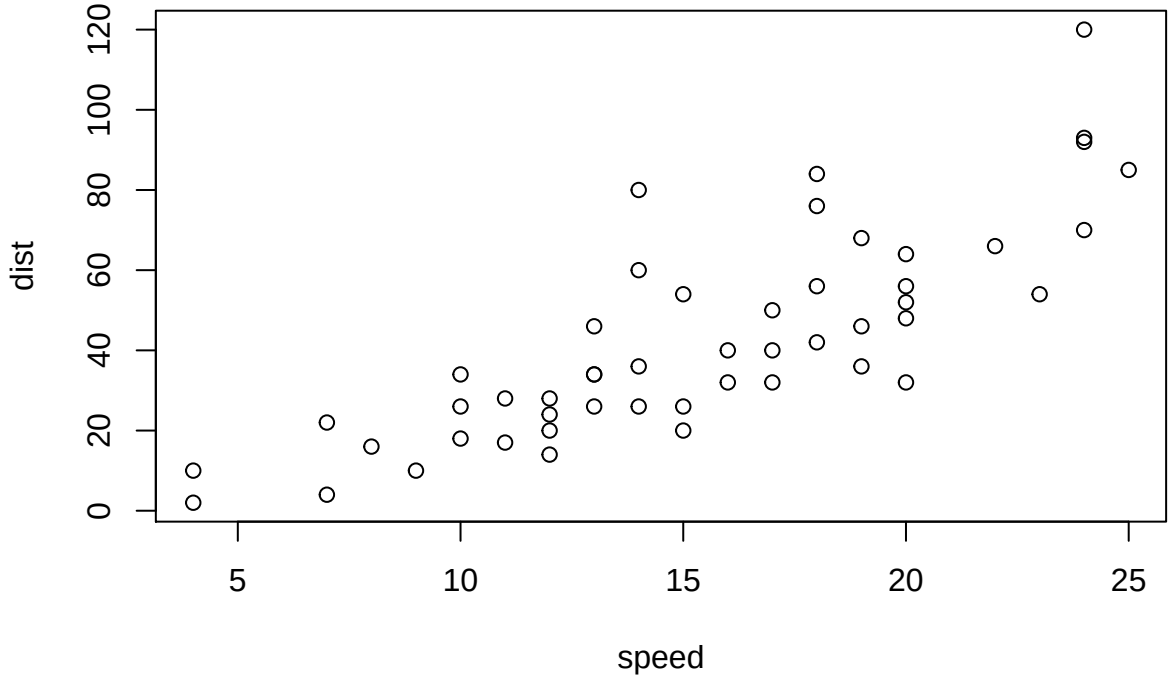
### A) Verifique si necesita normalizar los datos con quantile normalization. Obtenga p-values antes y después de normalizar.

```
plot(cars)
```




**B) Compare los p-values y grafique. Comente sobre la correlación entre las distintas pruebas estadísticas y la cantidad de genes significativos.**

```
plot(cars)
```



**C) Usando el GPL de los datos de GEO, dar una significancia biológica de los genes significativos.**

## 3) Repetir el ejercicio (2) pero con una base de datos de GEO con menos o más muestras, según sea el caso.

```
# Enable Warning messages
options(warn = 0)
```