Intro to R/Bioconductor

HMS Research Computing

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What can you do with R?

- R is a statistical language
- R is free!
- Bioconductor - thousands of packages for your workflow
- biomaRt - annotate samples
- Heavy duty statistics!
- Make plots!

Course Objectives

- Learn to run R on O2
- Gain familiarity with R language
- Learn to import/export data
- Simple Statistics/Plotting
- Use case: heatmap

Notation

- mfk8@compute-a:~$

  O2 bash (your console/terminal)

- >

  In R (either personal computer or in O2 running R)

- Blue content: try it out!

R on 

R on O2

- Open a high-memory R session – better than a desktop!
- Log in to O2 with X11 enabled (important for graphics)
- Mac: Xquartz installed, in console

  ssh -XY user123@o2.hms.harvard.edu
- Linux

  ssh -XY user123@o2.hms.harvard.edu
- Windows: MobaXterm has X11 client built-in

  ssh -XY user123@o2.hms.harvard.edu

SLURM and O2

- SLURM is how we interact with the cluster
- Simple interactive session:

```
mfk8@login01:~$ srun --pty -p interactive -t 0-12:00 --mem 8G bash
```

(where 8G is memory requested)

- Graphics:

srun: add `--x11`

sbatch: add `--x11=batch`

- Parallel, BiocParallel, doMC libraries: run over multiple cores (-c up to 20 cores)
- Rmpi, SNOW, doMPI: run R scripts over multiple nodes (>20 cores)

R Versions

- mfk8@login01:~$ module spider R
- Why does it matter what version of R you run?

  Downstream packages may only work with certain versions of R.
- How to load a version of R:

  mfk8@login01:~$ module load R/version
- Unloading R

  module unload R/version
- Starting R from an interactive (not login!)

  mfk8@compute-a:~$ R

Managing your R packages on O2

- It is best to manage your own R packages to work with the version of R you select. In doing so, there are no disruptions to your workflow.
- Setting up your O2 R library (1 time, not in .bashrc)

```
mfk8@login01:~$ mkdir -p ~/R-version

mfk8@login01:~$ export R_LIBS_USER="~/R-version"

mfk8@login01:~$ echo 'R_LIBS_USER="~/R-version"'>  $HOME/.Renviron
```

- If you must manually download a package (not through Bioconductor/CRAN etc), put the package in the set up location (/home/user123/R-version)
- Accessing packages manually uploaded to your O2 R library (first time)

```
> install.packages("name-of-your-package",lib="~/R-version")
```

Installing Packages

Bioconductor

- “Bioconductor provides tools for the analysis and comprehension of high-throughput genomic data.”
- Fast, easy way to access packages.
- Source Bioconductor: tells R to look at Bioconductor repository for packages
- Top of script:

```
> source("http://bioconductor.org/biocLite.R")
> biocLite()
```

Installing packages through Bioconductor

- Available packages:

  http://www.bioconductor.org/packages/release/BiocViews.html#___Software

- Easy install of new packages:

  > biocLite("nameofpackage")
  At the top of every R session using the package, it must be called via:

  > library("nameofpackage")

- Example:
- > biocLite("biomaRt")

  > library("biomaRt")

Installing packages through CRAN

- CRAN is the *Comprehensive R Archive Network*
- First time install of a package:

  > install.packages("name-of-package")

  (asks you to select a mirror: pick something from or near the country you're in)
- At the top of every R session using the package, the package must be called via:
  > library("nameofpackage")

Installing packages through Github

- First, install devtools

> install.packages('devtools')

> library(devtools)

- Then, install_github repo/packages

> install_github("repo/package")

R documentation

- General R help on a function

  > ?name_of_function

  > help(name_of_function)

- Learn how to use your package (vignettes open in your web browser)
  > browseVignettes(package = "name_of_package")

- > ?t.test

Setting your R “working directory”

- Simplifies paths
- What is your current WD?

  > getwd()
- Setting your WD:

  > setwd(“path”)
- An O2 example:

  > setwd(“/home/mfk8/MyDataDirectory”)
- A Mac example:

  > setwd(“/Users/mfk8/MyDataDirectory”)
- A Windows example (note forward slashes):

  > setwd(“C:/Users/mfk8/My Documents/MyDataDirectory”)

R Basics:

- To “print” in R
  Just type a variable or object’s name, R will display as much as it can
- “Commenting” in R

  # means what appears afterwards is not passed as an argument
- Keep a file of your commands – anything from Sublime to Notepad++ to Google Drive notepad: have syntax highlighting
- You can copy-paste multiple times, this overwrites.
- Watch quotation marks (often introduced by Microsoft Word/Powerpoint): R gets fussy with certain smart quotes/fonts

Data Objects

How R Thinks: Variables

- Assign variables with a <- (traditional) or = (new way)
- A variable can be overwritten so be careful with naming
- Names can be UPPER/lowercase/./_ mixes, but can't start with a number

```
> myX <-  5

> myX

[1] 5
```

Data Type: Vectors

- Basic way to store data
- c stands for “concatenate”: put these together as a vector

```
> myvector <- c(3,5,7)

> myvector

[1] 3 5 7
```

Vectors Types

- atomic: all the same data type
- numeric:

  > mynumeric <- c(3,5,7)

- character:

  > mycharacter <- c(“bob”, “nancy”, “jose”)

  #note quotation marks “ “ (single or double fine, try to be consistent)

- logical:

  > mylogical <- c(TRUE, FALSE, TRUE)

Changing Your Vector Type

- General workflow:

  > variable <- as.type(variable)

  > myvector <- as.character(myvector)

  > myvector

  [1] “3”, “5”, “7” #see how these are now in “ “ like a character vector
- Applicable to changing between other types
- Where this comes in handy: when R says you are trying to do an operation on your variable that is one type of vector, when it has to be another type. Use wisely.

Data Type: Lists

- Like vectors with mixed data types (numeric, character, logical)
- mylist <- c(3, “TP53”, FALSE)
- “unlist”-ing a list tries to coerce the data to an atomic vector of all the same type (lowest common denominator, usually a character)

Data Type: Factors

- Makes a vector nominal (able to be ordered by integers)
- Create a variable “gender” with 2 "male" entries and 4 "female" entries

  > gender <- c(rep("male", 2), rep("female", 4))

  > gender <- factor(gender) # stores gender as 2 2’s and 4 1’s and associates

  > gender

  [1] male male female female female female

  Levels: female male
- Now 1=female, 2=male internally (alphabetically)
- R now treats gender as a nominal variable

Data Type: Matrices

- Data must be all the same type (numeric, character, logical)
- Columns must have the same length
- Creation:

```
> mymatrix <- matrix(c(1,2,3,4,5,6), nrow=3, ncol=2)
```

- Indexed by [row,column]

```
> mymatrix[1,1] #returns item in row 1, column 1
> mymatrix[1,] #returns all of row 1
> mymatrix[,1] #returns all of column 1
```

Data Type : Data Frames

- Subset of matrices allowing mixed types (numeric, character, logical): lists!
- > mydataframe<-as.data.frame(mymatrix)
- You can give columns names so you can index by them

  > names(mydataframe) <- c("column1name", "column2name")

  You can use unique identifiers as rownames (no repeats!)

  > row.names(mydataframe)<-mydataframe[,1]

Dataframes: Indexing/Converting

Can use matrix or $ notation

> mydataframe$column1name #works on column1

> mydataframe[,1] #works on column1

> mydataframe[“rowname1”,] #works on rowname1

> mydataframe[1,] #works on row 1

> mydataframe[-1,] #excludes row 1

- To make a data frame into a matrix for certain operations:

  > mymatrix <- as.matrix(mydataframe) #turns data into all the same type (lowest common denominator is usually character)

Dataframes: indexing shortcut

- “attach” makes names of lists/dataframes accessible without $

```
> attach(mydataframe)
```

- “detach” when finished

```
> detach(mydataframe)
```

Adding and joining rows/columns

- "rbind" to add a row or another df/matrix to a pre-existing dataframe/maxtrix

  > mymatrix <- rbind(mymatrix, newrow)

  > mymatrix <- rbind(mymatrix, matrixtwo)

- "cbind" to add a column or another df/matrix to a pre-existing dataframe/matrix

  > mymatrix <- cbind(mymatrix, newcol)

  > mymatrix <- cbind(mymatrix, matrixtwo)

Useful functions:

> class(object) #gives object class

> mode(object) #gives object type

> length(vector) #gives length

> dim(object) #gives matrix/dataframe dimensions

> nrow(object) #gives number of rows

> ncol(object) #gives number of columns

> str(object) #gives object structure

> head(object) #gives first 6 rows

> tail(object) #gives last 6 rows

> summary() #quick statistics

Missing Values

- Placeholders
- NA: Not Available
- NaN: Not a Number
- is.na(x) is a logical test for NA/NaN
- is.nan(x) is a logical test for only NaN
- x[!is.na(x)] subsets and excludes NAs

Doing Math

Simple Arithmetic

```
> 18 + 22 #addition
> 18 – 12 #subtraction
> 18 * 2 #multiplication
> 18 / 2 #division
> 18 %/% 4 #integer part of quotient
> 18 %% 4 #modulo (remainder)
> 18 ^ 2 #exponent
```

Built-in math functions

```
> log(10) #natural log (base e)
> exp(2.302585) #antilog (e raised to power)
> log10(100) #log base 10
> sqrt(88) #square root
> factorial(8) #factorial
> choose(12, 8) #combinations (binomial coefficients)
> round(log(10), digits=3) #round to specified digits
> runif(5) #number of random numbers between 0-1
> rnorm(5) #random numbers from uniform normal distribution
> abs(18 / -12) #absolute value
```

Built-in math functions 2

> max(object) # max

> min(object) #min

> sum(object) #sum

> mean(object) #mean

> median(object) #median

> range(object) #range

> var(object) #variance

> sd(object) #standard deviation

> length(object) #number of values

Series Shortcuts

- Series: colon or “seq”

```
> 10:1
> seq(from, to, by)
> seq(1, 10, 2) # gives odd numbers
```

- Repeat

```
> rep(what, times)
> rep(10, 10)
```

Logical Operations: < > =

- Test of condition: returns logical TRUE/FALSE

```
> test1<- c(1,2,3)
> test1 > 2
[1] FALSE FALSE  TRUE
> test1 >= 2
[1] FALSE  TRUE  TRUE
> which(test1 >= 2)
[1] 2 3
> test1[test1 >=2] #subsetting data based on equality condition
> any(test1 >=5)  #FALSE
> all(test1 >=5) #FALSE
```

Control Structures

“for” loops

- Way to iterate over data
- R sometimes breaks with complicated “for” “if” “else” loops
- > myvector <- c(1,3,5)
- > j <- NULL #initialize it, good practice
- > newvector <- NULL #initialize it, good practice
- > for (i in myvector) {

```
    j <- i + 20
    newvector <- c(newvector, j)
}
> newvector
```

Functions

- Way to pack up commands into a repeatable format

```
> five = 5
> three = 3
> myfunction <- function(x,y) {
      z = x + y
      return(z) #what output is seen outside of function
}
> myfunction(five,three)
[1] 8
> myanswer <- myfunction(five, three); myanswer
[1] 8
```

Lambda-like functions

- “Anonymous” in-place functions
- Call function on some variable, and use that variable in the calculations

```
> (function (x) x^2)
> plot(function (x) x^2)
```

Apply

- Returns an object based on applying a function to a dataframe or matrix or list
- Format: apply (to_what, how, function)
- “1” is to apply over rows, “2” is to apply over columns

```
> mymatrix <- apply(mymatrix, 1, function)

> mymatrix <- apply(mymatrix, 2, function)

> apply(mymatrix, 1, sum) #row sums

> apply(mymatrix, 2, sum)  #column sums
```

Variations on apply

- sapply: (to_what, function) returns a vector

```
> sapply(1:3, function(x) x^2)
[1] 1 4 9
```

- lapply: (to_what, function): returns a list

```
> lapply(1:3, function(x) x^2)
[[1]]
[1] 1

[[2]]
[1] 4

[[3]]
[1] 9
```

More applies

- mapply (multivariate): pass more arguments

```
> mapply(function, arguments, MoreArgs=X)
```

- by: factors

```
> by(data, factor, function)
```

- replicate: repeat

```
> replicate(repetitions, function(data))
```

- Also tapply, eapply, rapply, vapply
- Useful packages: plyr, dplyr

Importing Data

Importing Data: text file

- You can specify how your data is separated (comma separated: “,” tab: “\t” space: ” “), and if the first row is a “header” row containing the column names)

- mydata <- read.table(file=”PathToFile/filename.csv”, header=TRUE, sep=”,”)
- add “row.names=1 to make column 1 the rownames (only if these are unique identifiers!)
- stringAsFactors=FALSE converts all to characters

Importing Data from MS Excel

- Read in the first worksheet from the workbook myexcel.xlsx
- First row contains variable (column) names

  > library(xlsx) #install the first time from CRAN

  > mydata <- read.xlsx("c:/myexcel.xlsx", 1)
- Read in the worksheet named mysheet

  > mydata <- read.xlsx("c:/myexcel.xlsx", sheetName = "mysheet")

Importing Data from SPSS

- In SPSS: save SPSS dataset in transport format

  get file='c:\mydata.sav'.

  export outfile='c:\mydata.por'.

- in R

  > library(Hmisc) #install the first time from CRAN

  > mydata <- spss.get("c:/mydata.por", use.value.labels=TRUE)

  # last option converts value labels to R factors

Importing Data from SAS

- In SAS: save SAS dataset in transport format

  libname out xport 'c:/mydata.xpt';

  data out.mydata;

  set sasuser.mydata;

  run;

- In R

  > library(Hmisc) #install the first time from CRAN

  > mydata <- sasxport.get("c:/mydata.xpt")

character variables are converted to R factors

Importing Data from STATA

- In R: input Systat file

  > library(foreign) #install the first time from CRAN

  > mydata <- read.systat("c:/mydata.dta")

Exporting Data

- Easy way to export a variable (vector, dataframe, matrix, etc):

  > write.table(nameofvariable, file="path/nameoffile.tsv", sep="\t") #sep="," or " " etc

- Add

  row.names=FALSE #turn off row names

  col.names=FALSE #turn off column names

  quote=FALSE #turn off character string quoting

Saving your workspace

- Save and pick up where you leave off – saves variables

  > save.image()

  > save(object list, file=”mysaves.RData”)
- Loading workspaces

  > load(“mysaves.RData”)

Class Example

Class Example

- Download class data and R script to a folder from http://hmsrc.me/rclassfiles

Set your working directory to the folder where your data is

- > setwd("pathtofolder/note/forward/slashes")
- A Mac example:

  > setwd("/Users/mfk8/Downloads")

- A Windows example (note forward slashes):

  > setwd("C:/Users/mfk8/Downloads")

Class Sample – Import Data

- Import Rcoursetestdata1.csv as data frame, with headers and row names

  > mydf <- read.table("Rcoursetestdata1.csv", header=TRUE, row.names=1, sep=",")

  > head(mydf)

	TNBC1	TNBC2	TNBC3	Normal1	Normal2	Normal3
ENSG00000008988	15258	15077	144720	12095	43544	46883
ENSG00000009307	14660	20767	8678	13774	23030	18917
ENSG00000019582	50866	55775	15089	6696	13754	86319
ENSG00000026025	21174	47966	26682	6068	21126	12728
ENSG00000034510	25645	31574	56403	29590	25216	37199
ENSG00000044574	23910	27200	13757	13364	10852	12378

Class Sample - Continued

- Get basic statistics on mydf

  > summary(mydf)

```
TNBC1          TNBC2          TNBC3          Normal1
Min.   :    0  Min.   :   65  Min.   :   31  Min.   :   22
1st Qu.:  7888  1st Qu.:  9538  1st Qu.:  9324  1st Qu.: 5074
Median : 13034  Median : 16568  Median : 19108  Median :10869
Mean   : 18596  Mean   : 26036  Mean   : 25646  Mean   :14746
3rd Qu.: 23850  3rd Qu.: 28194  3rd Qu.: 30389  3rd Qu.:18866
Max.   :103007  Max.   :351603  Max.   :272582  Max.   :89837
   Normal2         Normal3
Min.   :  208   Min.   :   15
1st Qu.:  7124  1st Qu.:  8944
Median : 14005  Median : 17710
Mean   : 19425  Mean   : 25481
3rd Qu.: 21576  3rd Qu.: 32191
Max.   :212582  Max.   :244692
```

Class Sample – Transposing Data

- Need your data to read the other way? Turn it into a matrix, and transpose!

  > mymatrix <- as.matrix(mydf)

  > myTmatrix<- t(mymatrix) #t = transpose

  > myTdf <- as.data.frame(myTmatrix) #makes a data frame again

  > myTdf

	ENSG00000008988	ENSG00000009307	ENSG00000019582	ENSG00000026025
TNBC1	15258	14660	50866	21174
TNBC2	15077	20767	55775	47966
TNBC3	144720	8678	15089	26682
Normal1	12095	13774	6696	6068
Normal2	43544	23030	13754	21126
Normal3	46883	18917	86319	12728

Class Example – Data Ops Cont'd

- Get basic statistics on transposed data frame

  > summary(myTdf)

```
   ENSG00000008988  ENSG00000009307 ENSG00000019582 ENSG00000026025
Min.   : 12095   Min.   : 8678   Min.   : 6696   Min.   : 6068
1st Qu.: 15122   1st Qu.:13996   1st Qu.:14088   1st Qu.:14828
Median : 29401   Median :16789   Median :32978   Median :21150
Mean   : 46263   Mean   :16638   Mean   :38083   Mean   :22624
3rd Qu.: 46048   3rd Qu.:20305   3rd Qu.:54548   3rd Qu.:25305
Max.   :144720   Max.   :23030   Max.   :86319   Max.   :47966
```

Class Example: Simple t-test

- Do a t-test on TNBC and Normal from mydf

  > t.test(mydf[,1:3], mydf[,4:6])

```
 Welch Two Sample t-test
data:  mydf[, 1:3] and mydf[, 4:6]
t = 2.3053, df = 1168.9, p-value = 0.02132
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
  527.5122 6556.6411
sample estimates:
mean of x mean of y
 23426.08  19884.01
```

Class Example: Simple Wilcoxon

- wilcox.test(mymatrix[,1:3], mymatrix[,4:6])

Wilcoxon rank sum test with continuity correction

data: mymatrix[, 1:3] and mymatrix[, 4:6]

W = 199820, p-value = 0.0009604

alternative hypothesis: true location shift is not equal to 0

Class Example: Linear Modelling

- model<-lm(y ~ x1 + x2 …, data=data)

```
> mymodel  <- lm(TNBC1 ~ Normal1, data=mydf)
> anova(mymodel)
Analysis of Variance Table


Response: TNBC1
           Df     Sum Sq    Mean Sq F value    Pr(>F)
Normal1     1 5.4597e+09 5459744483  20.215 1.177e-05 ***
Residuals 198 5.3477e+10  270084874
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Class Sample: Practical Plotting

Saving plots

- Use “File->”save as” or,
- Open up a plot file (png, pdf, jpeg, tiff, bmp)

  > png(file=“nameyourfile.png”)
- Make your plot

  > plot()
- Turn plot off

  > dev.off()

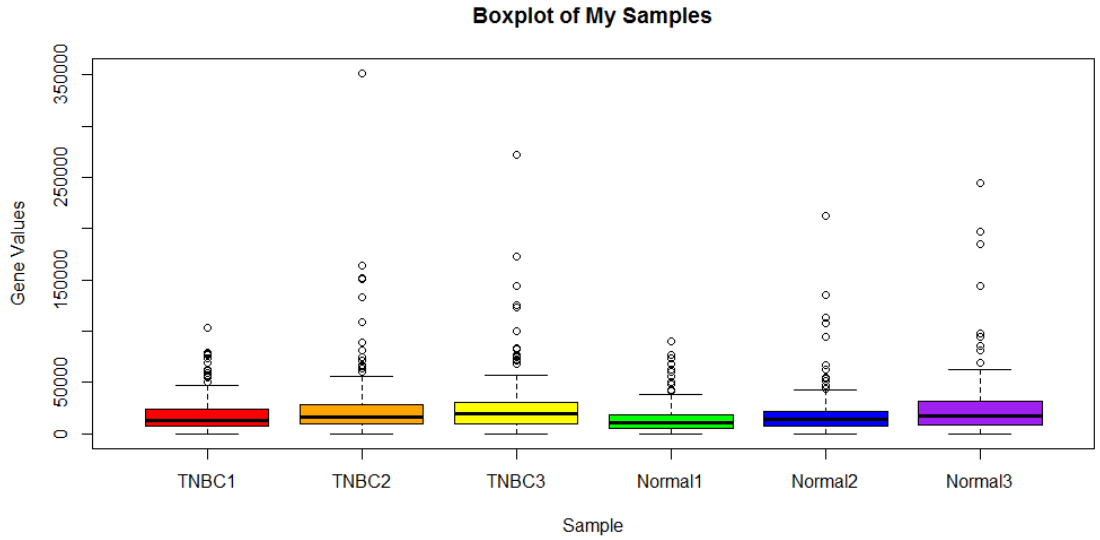
Plotting Options

- main = “Title” #title
- xlab= “x label” #x-axis label
- ylab=“y label” #y-axis label
- xlim(N,N) #x-axis start, stop
- ylim(N,N) #y-axis start, stop
- col =c(“color1”, “color2”) #vector with colors
- lty = c(N, N) #line type
- lwd= c(N, N) #line width
- cex = N #size of text and symbols
- pch = N #plot point symbol type
- par(mfrow(x,y)) #multiple figures in one plot

Class Example – Boxplot of Samples

- Boxplot of Samples

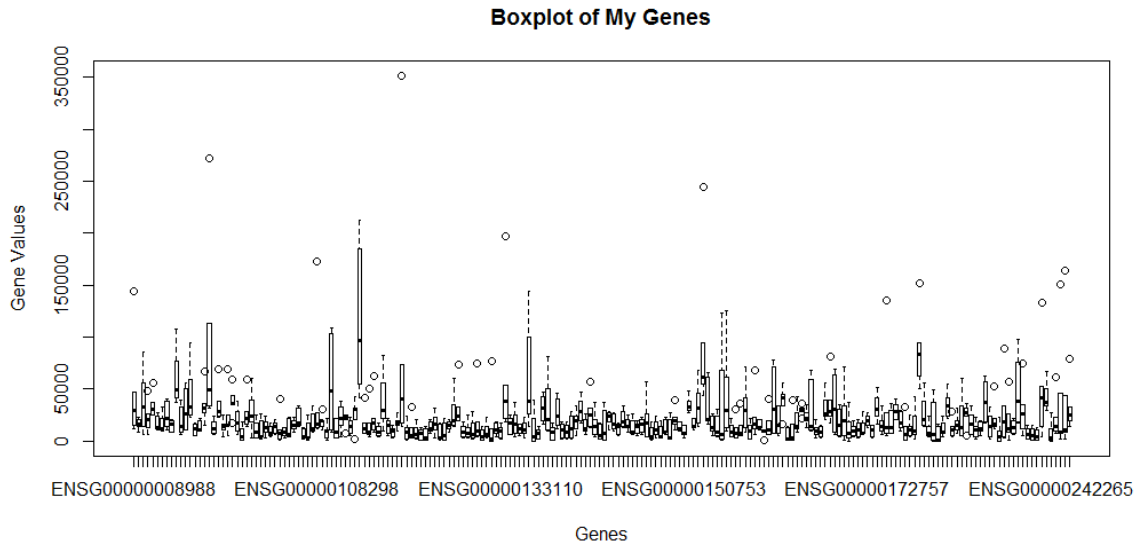
  > boxplot(mydf, main="Boxplot of My Samples", xlab="Sample", ylab="Gene Values")



Class Example – Boxplot of Genes

- Boxplot of Genes

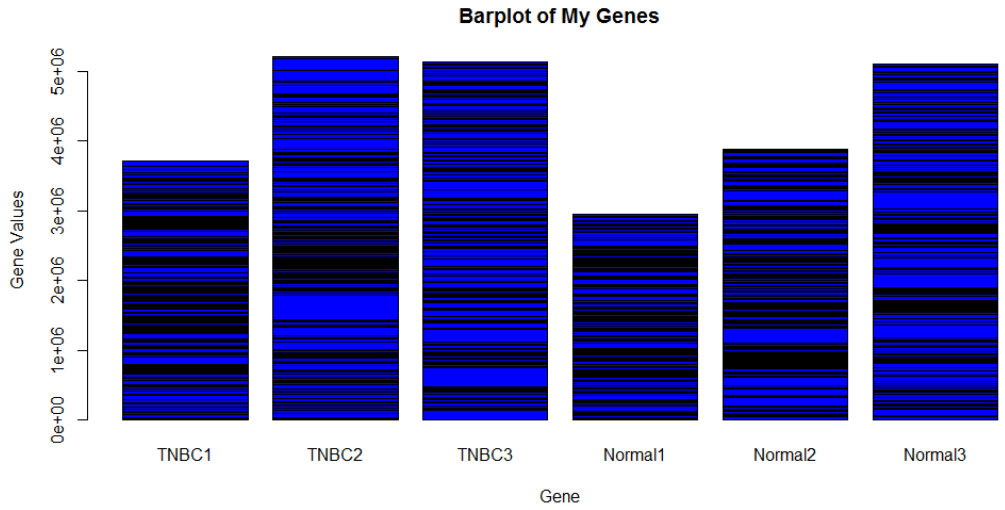
  > boxplot(myTdf, main="Boxplot of My Genes", xlab="Gene", ylab="Gene Values")



Class Example – Barplot of Genes

- Barplot of Genes #barplot needs a matrix

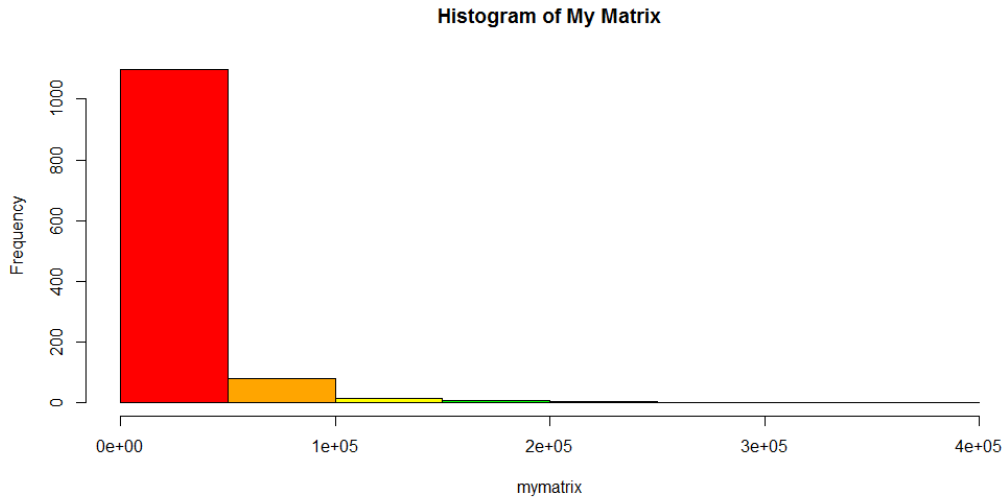
  > barplot(myTmatrix, main="Barplot of My Genes", xlab="Gene", ylab="Sample Values")



Class Example – Histogram of Values

- Plot a histogram of the frequency of values in mymatrix

  >hist(mymatrix)



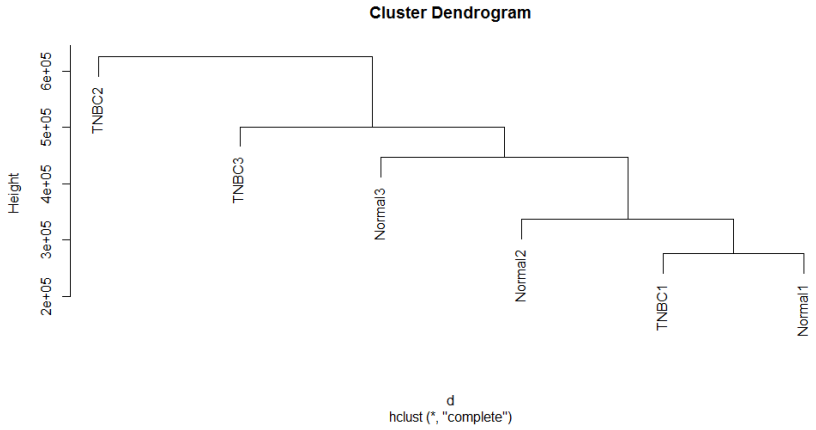
Class Example - HCL

- Do hierarchical clustering of samples

  > d <- dist(myTmatrix) #takes matrix as input, calculates distance

  > hc <- hclust(d) #performs HCL on distance matrix

  > plot(hc)



Class Sample – Line Graphs

- #start a line plot with ENSG00000008988 "b" means both points and lines

  > plot(myTdf$ENSG00000008988, type="b", col="green", ylim=c(10000,150000), main="Gene Values Over Samples", xlab="Sample", ylab="Gene Values")

- #add a new line for ENSG00000009307 "lines" adds a line to the current plot

  > lines(myTdf$ENSG00000009307, type="b", col = "blue")

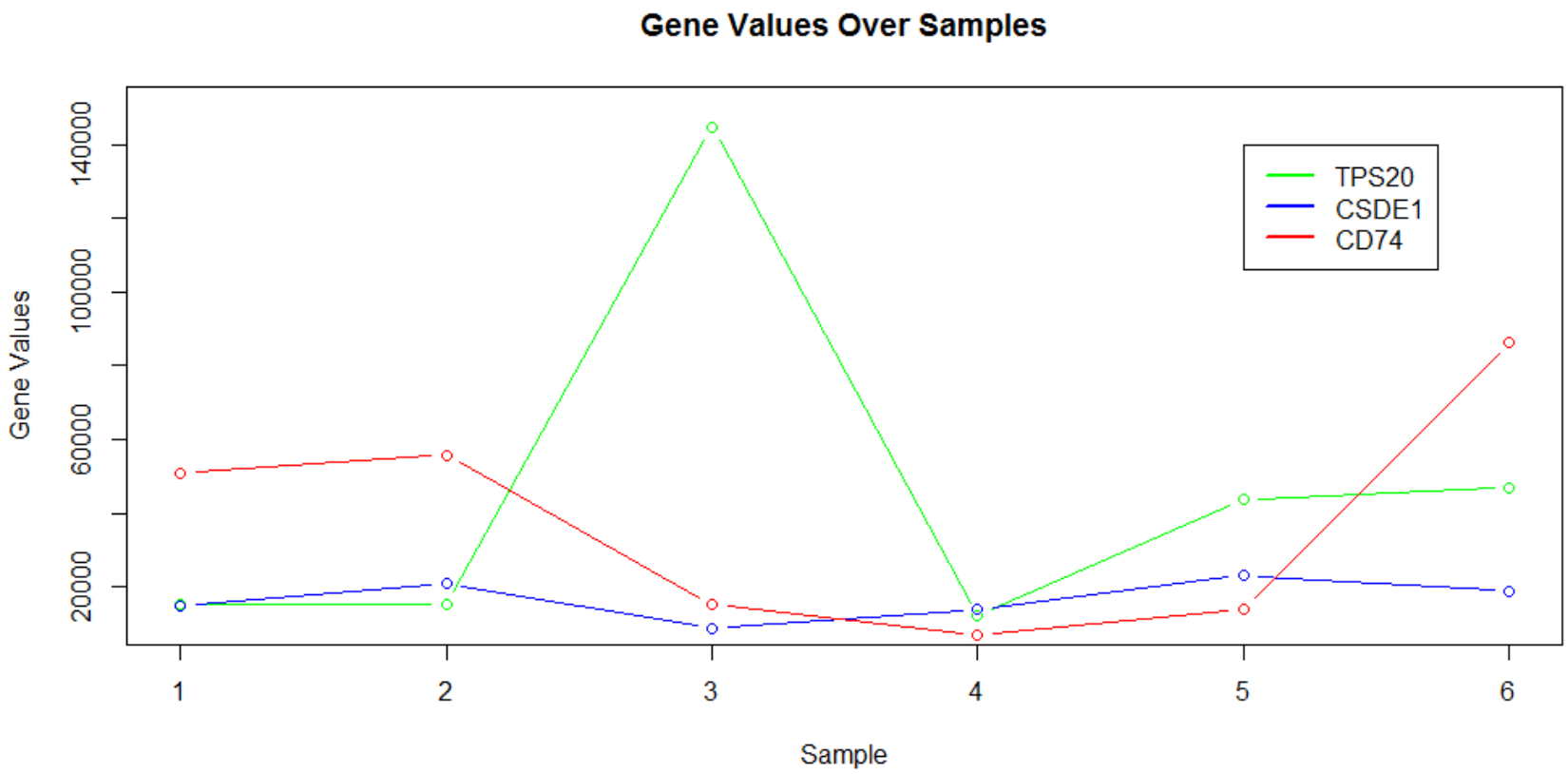
- #add a new line for ENSG00000019582

  > lines(myTdf$ENSG00000019582, type="b", col="red")

Class Sample – Line Graphs Legend

```
> legend(5, 140000, #positions x, y
c("TPS20", "CSDE1", "CD74"), #line names
lty=c(1,1), #specifies lines
lwd=c(2.5,2.5), #specifies line width
col=c("green", "blue", "red") #add colors
) #ends legend
```

Class Sample – Line Graphs



Class Example – Count Data

- Import count data

```
> mytable<-read.table("Rcoursetestdata2.csv", header=T, row.names=1, sep=",")
> mytable
```

	neversmoke	smoke	pastsmoke	gender
1	0	1	0	male
2	0	1	0	female
3	0	0	0	male
4	1	0	0	female
5	0	0	1	male
6	0	1	0	female
7	0	0	1	male
8	0	0	1	female
9	1	0	0	male
10	0	0	1	female

Class Example – Count Data Cont'd

- Get summary statistic on mytable

> summary(mytable)

```
> summary(mytable)
  neversmoke      smoke       pastsmoke      gender
 Min.   :0.0   Min.   :0.00   Min.   :0.0   female:5
 1st Qu.:0.0   1st Qu.:0.00   1st Qu.:0.0   male  :5
 Median :0.0   Median :0.00   Median :0.0
 Mean   :0.2   Mean   :0.30   Mean   :0.4
 3rd Qu.:0.0   3rd Qu.:0.75   3rd Qu.:1.0
 Max.   :1.0   Max.   :1.00   Max.   :1.0
>
```

Class Example – Tables Cont'd

- Take a subset of a table to do statistics on

```
> genderVsmoke <- table(mytable$gender, mytable$smoke)
> genderVsmoke
        0 1
female 3 2
male   4 1
```

Class Example – Tables Cont'd

- summary(genderVsmoke) does a Chi Square Test of Independence

  > summary(genderVsmoke)

Number of cases in table: 10

Number of factors: 2

Test for independence of all factors:

Chisq = 0.4762, df = 1, p-value = 0.4902

Chi-squared approximation may be incorrect

Class Example – Tables Cont'd

- Get the frequencies of a table via prop.table

  > propgenderVsmoke <- prop.table(genderVsmoke)

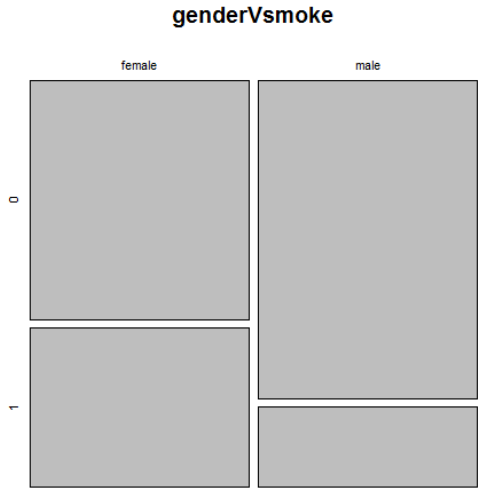
  > propgenderVsmoke

	0	1
female	0.3	0.2
male	0.4	0.1

Class Example – Plotting Tables

- Do a mosaicplot of table genderVsmoke

  > mosaicplot(genderVsmoke)



Bonus Plot: Heatmap

- Try installing packages “gplots” and “RColorBrewer”

  > install.packages(“gplots”, “RColorBrewer”)
- Call the libraries

  > library(“gplots”)

  > library(“RColorBrewer”)
- Check out the code!

Useful R packages

- "RColorBrewer" and "Viridis" – define colors and pallets
- "gplots" and "ggplots2" – great for plotting
- "genefilter" – useful to apply filters over matrices
- "plyr" and "dplyr" – advanced matrix/df operations
- "edgeR" and "DESeq2" – RNAseq differential analysis alternatives using count data as input
- "biomaRt" – cross-annotate samples

RStudio

- Feature-rich GUI for R, works on top of version of R installed
- RMarkdown, Rshinyapps
- Not optimized for multithreading
- Good for proofing code, but not a scalable solution for HPC
- Not currently available as an O2, but under consideration as a standalone service accessing O2 filesystems

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