Charlee Cobb - Transcriptomics, Homework 1

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- **PART 1 1.** What is an R object? (1pt) An R object is an instance of either an array, a matrix, a dataframe, a vector, or a list that holds a value of data.
- 2. How many ways can data be assigned to an R object? (1pt) Data can be assigned to an R object by using the "=" symbol, or the "<-" symbol.
- 3. Why do you think it is important to ensure that data objects are of the correct type? (1pt) It's important to ensure that data objects are of the correct type because the type of data limits the number of ways functions can manipulate the object, and limit ways objects interact with each other. For example, if you have a vector of character represented numbers and a vector of numericals, you couldn't run any operations on the two vectors at once because they have different data types. One vector is an object pf characters, and one is an object of numericals.
- 4. What is the relationship between vectors, matrices, and data frames? (1pt) Vectors, matrices, and data frames all hold data points or values. Vectors are one deminsional, and only hold values that are all the same type(ie characters or numerical values). A matrix is made from 2 or vectors that have been coerced together. However, the vectors building the matrix all have to have the same data type. A dataframe is preferred over a matrix when you want to store values that are different data types (ie. numericals and characters together). However for both matrices and data frames, the coerced vectors must always be of the same length to ensure the same number of rows and columns in either the matrix or data frame.
- 5. Why might a data frame be more suitable than a matrix for holding heterogeneous biological data? (1pt) As stated above, a data frame is preferred over a matrix when you want to store values that are different data types. With a data frame, the heterogeneous biological data can be stored because the data frame accepts numerical values, characters, and other data types to remain in the data frame without converting them. So data with gene name, expression value, reference id, and annotations can all be stored in a data frame. A matrix wouldn't allow the expression value to be stored, unless the values were converted to character format.
- PART 2 1. Create a matrix (call it transcriptome) with the values below. The experiments are column names and genes are the row names. (3pts)

```
##creating matrix##

#make vectors of each gene by row:
GeneA <- c(89,78,77,56)
GeneB <- c(90,99,85,97)
GeneC <- c(78,94,99,87)
GeneD <- c(81,83,80,79)
GeneE <- c(62,51,99,88)

#combine above vectors into a matrix named transcriptome. Specify number of rows
#and columns, use 'byrow' condition set to TRUE to ensure data from vectors are entered by row
transcriptome <- matrix(c(GeneA, GeneB, GeneC, GeneD, GeneE), nrow=5, byrow=TRUE, ncol= 4)</pre>
```

```
#add names and columns to the using the transcriptome matrix rownames, colnames function
rownames(transcriptome) <- c("GeneA", "GeneB", "GeneC", "GeneD", "GeneE")
colnames(transcriptome) <- c("Control", "Nitrogen", "Phospate", "Potassium")
print(transcriptome)</pre>
```

```
##
         Control Nitrogen Phospate Potassium
## GeneA
                         78
               89
                                  77
## GeneB
               90
                         99
                                  85
                                             97
               78
                                             87
## GeneC
                         94
                                  99
## GeneD
               81
                         83
                                  80
                                             79
## GeneE
               62
                         51
                                  99
                                             88
```

2. Use R code to calculate the average expression for each gene across all experiments. Call the vector (call it expression_average). (The vector should contain 5 values – one for each gene) (3pts)

```
##make vector of average expression##

#using the apply function, we are going across each row(1) in the data set
#transcriptome, and using the built in mean function to find the average of each
#gene's expression data
expression_average <- apply(transcriptome, 1, mean)
print(expression_average)</pre>
```

```
## GeneA GeneB GeneC GeneD GeneE
## 75.00 92.75 89.50 80.75 75.00
```

3. Sort the matrix so that the gene with the highest average expression value is on top and save it into a new data frame (call it "sorted_genes") (4pts)

```
##sort genes by row and put into a dataframe.##
sorted_genes <-as.data.frame(transcriptome[order(apply(transcriptome, 1, mean), decreasing = T),])
sorted_genes</pre>
```

```
##
         Control Nitrogen Phospate Potassium
## GeneB
               90
                        99
                                  85
                                             97
## GeneC
               78
                         94
                                  99
                                             87
## GeneD
                        83
                                  80
                                             79
               81
## GeneA
               89
                         78
                                  77
                                             56
## GeneE
               62
                        51
                                  99
                                             88
```

#there is an option to make a separate matrix of sorted data and then convert it to #a data frame, but we can do that in one line here.

#starting with the order() function, we take the apply function we used above to #find a list of means, then use decreasing = T to say we want to order to go from #largest value at the top to smallest value. Putting this inside transcriptome[] #means we are applying this order change to the transcriptome matrix. Finally, #we put this all inside the as.data.frame() function to save the changes in the #data frame sorted genes.

PART 3 a. Load the file expvalues.txt into R. (2pts)

```
#this file is saved in my project directory, so no path is added here
expvalues <- read.table("expvalues.txt")</pre>
head(expvalues)
##
              Control1 Control2 Control3 Treatment1 Treatment2 Treatment3
## 244901_at 229.98565 353.59949 178.49171 112.90800 152.91835 320.83235
## 244902_at 171.14980 84.45094 41.37195 170.17262 134.40814 193.52611
## 244903_at 314.97768 373.31250 52.90873 196.30256 237.51520 253.37774
## 244904_at 24.04366 175.31604 94.68424 82.78488 18.43639 87.00857
## 244905_at 15.86923 40.04125 58.76573 341.35340
                                                      16.80135
                                                                   68.94204
## 244906_at 381.06379 218.90597 307.01488 162.54109 264.47945 263.66098
b. Calculate the mean of the control samples and the mean of the treatment samples for each
gene. (You can use a loop or apply functions) (5pts)
##get control average and treatment average per gene##
#used this line to make sure I was pulling a specific amount of columns:
#print(head(expvalues[,c(1:3)]))
\#(expvalues[,c(1:3)] and (expvalues[,c(4:6)] subset the expvalue data frame to
#control columns only and treatment columns only, respectively.
#using the apply function to find the mean of each gene (per row, 1) in the subset
#data frame of expvalues
control.means <- apply(expvalues[,c(1:3)], 1, mean)</pre>
head(control.means)
## 244901_at 244902_at 244903_at 244904_at 244905_at 244906_at
## 254.02562 98.99090 247.06630 98.01465 38.22541 302.32821
treatment.means <- apply(expvalues[,c(4:6)], 1, mean)</pre>
head(treatment.means)
## 244901_at 244902_at 244903_at 244904_at 244905_at 244906_at
## 195.55290 166.03562 229.06517 62.74328 142.36560 230.22717
#expected means of the 244901_at gene expression to verify results:
#control:
print("control:")
## [1] "control:"
mean(c(229.98565, 353.59949, 178.49171))
## [1] 254.0256
```

```
#treatment:
print("treatment")
## [1] "treatment"
mean(c(112.90800, 152.91835, 320.83235))
## [1] 195.5529
Calculate the fold change for each gene (fold change is the ratio of average treatment to average
control). (2pts)
##calculate fold change##
#fold change == average treatment to average control
#make a data frame of the average treatment values and average control values:
average.expvalues <- matrix(c(treatment.means, control.means), ncol = 2)</pre>
#get row names from expualues and add them to average.expualues
rownames(average.expvalues) <- c(row.names(expvalues))</pre>
#Set column names to treatment and control
colnames(average.expvalues) <- c("treatment", "control")</pre>
head(average.expvalues)
##
             treatment control
## 244901_at 195.55290 254.02562
## 244902_at 166.03562 98.99090
## 244903 at 229.06517 247.06630
## 244904_at 62.74328 98.01465
## 244905_at 142.36560 38.22541
## 244906_at 230.22717 302.32821
#divide column 1(treatment) by column 2(control)
gene.fold.change <- average.expvalues[,1]/average.expvalues[,2]</pre>
head(gene.fold.change)
## 244901_at 244902_at 244903_at 244904_at 244905_at 244906_at
## 0.7698157 1.6772817 0.9271405 0.6401419 3.7243712 0.7615140
#expected fold change of the 244901_at gene expression to verify results:
#control: 254.0256
#treatment: 195.5529
195.5529 / 254.0256
```

[1] 0.7698157

d. Take the log2 of the fold change. You have just calculated log fold change(LFC).(2pts)

```
##calculate log fold change##
#use the log2() function to apply log2 to each value in the gene.fold.change object
gene.log.fold.change <- log2(gene.fold.change)</pre>
head(gene.log.fold.change)
## 244901_at 244902_at 244903_at 244904_at 244905_at 244906_at
## -0.3774151 0.7461250 -0.1091402 -0.6435364 1.8969969 -0.3930575
e. How many genes have a LFC > 1 OR < -1? (2pts) There are 1850 genes with a LFC greater
than(>) 1, and 2394 with an LFC less than (<) -1
##find genes with values >1 and values < -1
#> 1
LFC.greater <- gene.log.fold.change[gene.log.fold.change > 1]
head(LFC.greater)
                 244914_at
##
     244905 at
                             244916_at
                                         244917_at
                                                     244945_at 244956_s_at
##
      1.896997
                  1.208409
                              1.069662
                                          1.002224
                                                      1.007064
                                                                  1.814538
length(LFC.greater) #length shows number of values (genes) in the LFC.greater object
## [1] 1850
#< -1
LFC.less <- gene.log.fold.change[gene.log.fold.change < -1]
length(LFC.less)
## [1] 2394
f. Save the names of the genes that have a LFC > 1 into a file called "Induced_genes.txt"
(2pts)
##save the names of the genes in LFC.greater
#the LFC greater object has the gene names as an attribute under $names, so when
#the object is printed, we see the gene names listed
#extract the gene names from the attribute using the attribute function
#?attribute()
LFC.greater.attr <- attributes(LFC.greater)</pre>
head(LFC.greater.attr$names)
## [1] "244905 at"
                     "244914 at" "244916 at" "244917 at" "244945 at"
## [6] "244956 s at"
#write the gene names in the file called "Induced_genes.txt" with the write()
#function
write((LFC.greater.attr$names), file = "Induced_genes.txt")
```

Using the same set of induced genes in the previous question, create a boxplot to show the distribution of values for each induced gene in each experiment. The x-axis should have all six experiments, and the y-axis is the expression level. Save the boxplot as a pdf file called "boxplot.pdf" (5pts)

```
##make a box plot of induced genes##
#subset the data from expvalues to induced.gene.expvalues so only the induced
#qenes from the above chunk are present. Using the %in% pipe to subset data
induced.gene.expvalues <- expvalues[rownames(expvalues) %in% LFC.greater.attr$names, ]</pre>
colnames(induced.gene.expvalues) <- c("Control1", "Control2", "Control3", "Treatment1", "Treatment2", "</pre>
head(induced.gene.expvalues)
                Control1 Control2 Control3 Treatment1 Treatment2 Treatment3
##
## 244905 at
               15.869235 40.04125 58.76573 341.35340 16.801354
                                                                    68.94204
## 244914_at
              9.206320 23.66153 37.63685 25.38165 7.404548 130.13789
## 244916 at
                3.371939 38.96267 16.94395 68.91271
                                                         2.800184
                                                                   52.70934
## 244917_at
               12.290308 16.83069 19.14878 48.15070 10.206957 38.33083
## 244945 at 157.299361 204.15104 24.23849 552.28230 93.524026 129.35776
## 244956 s at 95.024495 40.62732 28.03325 459.47534 92.258511 24.02345
#make a pdf
pdf("boxplot.pdf")
par(mar=c(14, 4.1, 4.1, 2.1))
#make a boxplot with the subseted data above, save it the pdf open
boxplot(induced.gene.expvalues$Control1,
       induced.gene.expvalues$Control2,
       induced.gene.expvalues$Control3,
       induced.gene.expvalues$Treatment1,
       induced.gene.expvalues$Treatment2,
       induced.gene.expvalues$Treatment3,
       main = "Induced Gene Expression Distribution",
       xlab = "experiments", ylab = "expression value",
       show.names = TRUE) #show the distribution of all the 6 measurements
#save plots
dev.off()
## pdf
##
#check <- induced.gene.expvalues[induced.gene.expvalues > 15000]
#length(check)
```