compgen2021: Week 1 exercises

Irem YUCEL

```
library(mosaic)
```

```
## Registered S3 method overwritten by 'mosaic':
##   method                           from
##   fortify.SpatialPolygonsDataFrame ggplot2

##
## The 'mosaic' package masks several functions from core packages in order to add
## additional features.  The original behavior of these functions should not be affected by this.

##
## Attaching package: 'mosaic'

## The following objects are masked from 'package:dplyr':
##
##     count, do, tally

## The following object is masked from 'package:Matrix':
##
##     mean

## The following object is masked from 'package:ggplot2':
##
##     stat

## The following objects are masked from 'package:stats':
##
##     binom.test, cor, cor.test, cov, fivenum, IQR, median, prop.test,
##     quantile, sd, t.test, var

## The following objects are masked from 'package:base':
##
##     max, mean, min, prod, range, sample, sum
```

```
library(matrixStats)
```

```
##
## Attaching package: 'matrixStats'

## The following objects are masked from 'package:mosaic':
##
##     count, iqr
```

```
## The following object is masked from 'package:dplyr':
##
##     count
```

```
library(qvalue)
```

Exercises for Week1

Statistics for genomics

How to summarize collection of data points: The idea behind statistical distributions

1. Calculate the means and variances of the rows of the following simulated data set, and plot the distributions of means and variances using `hist()` and `boxplot()` functions. [Difficulty: **Beginner/Intermediate**]

```
set.seed(100)

#sample data matrix from normal distribution
gset=rnorm(600,mean=200,sd=70)
data=matrix(gset,ncol=6)

row_means=rowMeans(data)
row_vars= apply(data,1,var)

par(mfrow=c(2,2))
hist(row_means)
boxplot(row_means)
hist(row_vars)
boxplot(row_vars)
```

2. Using the data generated above, calculate the standard deviation of the distribution of the means using the `sd()` function. Compare that to the expected standard error obtained from the central limit theorem keeping in mind the population parameters were $\sigma = 70$ and $n = 6$. How does the estimate from the random samples change if we simulate more data with `data=matrix(rnorm(6000,mean=200,sd=70),ncol=6)`? [Difficulty: **Beginner/Intermediate**]

```
gset=rnorm(600,mean=200,sd=70)
data=matrix(gset,ncol=6)
row_means=rowMeans(data)

means_sd = sd(row_means)
ctl_sd=70/sqrt(6)

#The standard deviation of the sample means is similar to the standard deviation from CLT.

data2=matrix(rnorm(6000,mean=200,sd=70),ncol=6)
row_means2=rowMeans(data2)
means_sd2 = sd(row_means2)

#Changing to 6000 did not make a difference, the estimate from the random sampling is still very simila
```

3. Simulate 30 random variables using the `rpois()` function. Do this 1000 times and calculate the mean of each sample. Plot the sampling distributions of the means using a histogram. Get the 2.5th and 97.5th percentiles of the distribution. [Difficulty: **Beginner/Intermediate**]

4. Use the `t.test()` function to calculate confidence intervals of the mean on the first random sample `pois1` simulated from the `rpois()` function below. [Difficulty: **Intermediate**]

```
#HINT
set.seed(100)

#sample 30 values from poisson dist with lambda parameter = 30 (5?)
pois1= do(1000) * mean(rpois(30,lambda=5))
hist(pois1[,1],
     col="cornflowerblue",
     border="white",
     xlab="Sample Means")
q=quantile(pois1[,1],p=c(0.025,0.975))
abline(v=c(q[1], q[2] ),col="red")
text(x=q[1],y=200,round(q[1],2))
text(x=q[2],y=200,round(q[2],2))

t.test(pois1)
```

5. Use the bootstrap confidence interval for the mean on `pois1`. [Difficulty: **Intermediate/Advanced**]

```
#Bootstrapping is when we don't know the population parameter (Lambda in this example).So we take sampl
set.seed(100)
pois1= do(1000) * mean(rpois(30,lambda=5))
boot_samples = do(1000)*sample(pois1,30,replace=T)
quantile(boot_samples[,1],p=c(0.025,0.975))
```

```
##     2.5%    97.5%
## 4.166667 5.866667
```

How to test for differences in samples

1. Test the difference of means of the following simulated genes using the randomization, `t-test()`, and `wilcox.test()` functions. Plot the distributions using histograms and boxplots. [Difficulty: **Intermediate/Advanced**]

```
set.seed(101)
gene1=rnorm(30,mean=4,sd=3)
gene2=rnorm(30,mean=3,sd=3)

t.test(gene1,gene2)
wilcox.test(gene1,gene2)

#Randomization
#Save the real difference between the gene1 and geen2 groups
#Generate a new data frame using gene1 and gene2 values (in esp column) with 30 test and 30 control lab

org.diff=mean(gene1)-mean(gene2)
```

```
gene.df=data.frame(exp=c(gene1,gene2),
                   group=c(rep("test",30),rep("control",30)))

#Shuffle by the group labels and get the means for control and test, get the difference, repeat 1000 ti
shuffled_means = do(1000) * diff(mosaic::mean(exp ~ shuffle(group), data=gene.df))

hist(shuffled_means[,1],
     xlab="null distribution | no difference in samples",
     main=expression(paste(H[0]," :no difference in means") ),
     xlim=c(-2,2),
     col="cornflowerblue",
     border="white")
abline(v=quantile(exp.null[,1],0.95),col="red" )
abline(v=org.diff,col="blue" )
text(x=quantile(exp.null[,1],0.95),y=200,"0.05",adj=c(1,0),col="red")
text(x=org.diff,y=200,"org. diff.",adj=c(1,0),col="blue")
```

2. Test the difference of the means of the following simulated genes using the randomization, `t-test()` and `wilcox.test()` functions. Plot the distributions using histograms and boxplots. [Difficulty: **Intermediate/Advanced**]

```
set.seed(100)
gene1=rnorm(30,mean=4,sd=2)
gene2=rnorm(30,mean=2,sd=2)

## Same as above

t.test(gene1,gene2)
wilcox.test(gene1,gene2)

#Randomization
#Save the real difference between the gene1 and geen2 groups
#Generate a new data frame using gene1 and gene2 values (in esp column) with 30 test and 30 control lab

org.diff=mean(gene1)-mean(gene2)
gene.df=data.frame(exp=c(gene1,gene2),
                   group=c(rep("test",30),rep("control",30)))

#Shuffle by the group labels and get the means for control and test, get the difference, repeat 1000 ti
shuffled_means = do(1000) * diff(mosaic::mean(exp ~ shuffle(group), data=gene.df))

hist(shuffled_means[,1],
     xlab="null distribution | no difference in samples",
     main=expression(paste(H[0]," :no difference in means") ),
     xlim=c(-2,2),
     col="cornflowerblue",
     border="white")
abline(v=quantile(exp.null[,1],0.95),col="red" )
abline(v=org.diff,col="blue" )
text(x=quantile(exp.null[,1],0.95),y=200,"0.05",adj=c(1,0),col="red")
text(x=org.diff,y=200,"org. diff.",adj=c(1,0),col="blue")
```

3. We need an extra data set for this exercise. Read the gene expression data set as fol-

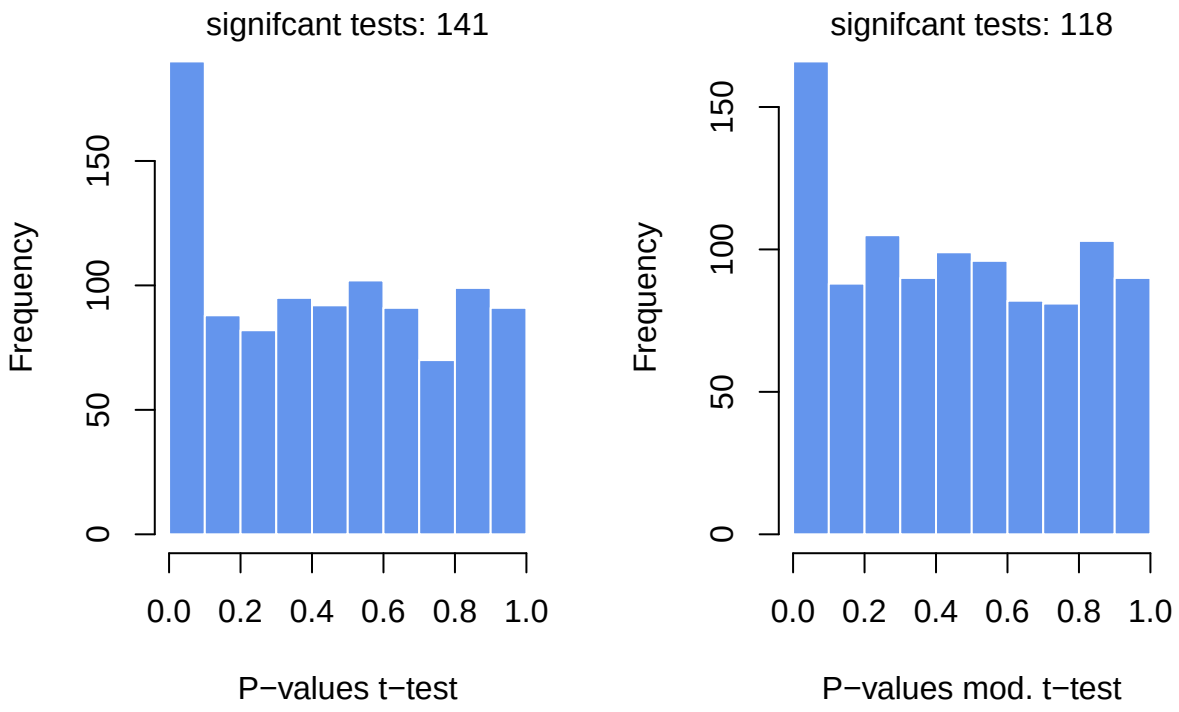
lows: `gexpFile=system.file("extdata","geneExpMat.rds",package="compGenomRData")` `data=readRDS(gexpFile)`. The data has 100 differentially expressed genes. The first 3 columns are the test samples, and the last 3 are the control samples. Do a t-test for each gene (each row is a gene), and record the p-values. Then, do a moderated t-test, as shown in section "Moderated t-tests" in this chapter, and record the p-values. Make a p-value histogram and compare two approaches in terms of the number of significant tests with the 0.05 threshold. On the p-values use FDR (BH), Bonferroni and q-value adjustment methods. Calculate how many adjusted p-values are below 0.05 for each approach. [Difficulty: **Intermediate/Advanced**]

```
#Read the data
gexpFile=system.file("extdata","geneExpMat.rds",package="compGenomRData")
data=readRDS(gexpFile)

#For the whole data
#Check if the variances are similar for the t.test:
col_vars= apply(data,2,var)

#Use Welch Two Sample t-test since variances are different:
test=data[,1:3]
control=data[,4:6]
n1=3
n2=3
stats::t.test(test,control)
```

```
##
##  Welch Two Sample t-test
##
## data:  test and control
## t = -14.74, df = 4115, p-value < 2.2e-16
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.2222141 -0.1700416
## sample estimates:
##     mean of x     mean of y
## -0.0004465826  0.1956812552
```

```
#Testing each gene:
#The formula for the Welch's t-test is: Differences of means/ variance
#Calculate the differences of the means of 2 groups:
diff_means=rowMeans(test)-rowMeans(control)

#Get the estimate of pooled variance
pooled_var = sqrt(
  (rowVars(test)*(n1-1) + rowVars(control)*(n2-1)) / (n1+n2-2) * ( 1/n1 + 1/n2 )
  )

#Estimate t statistic without moderated variance and calculate P-value of rejecting null
t = diff_means / pooled_var
p = 2*pt( -abs(t), n1+n2-2 )

#Same with the moderated variance
mod.pooled_var = (pooled_var + median(pooled_var)) / 2 # moderation in variation
t.mod <- diff_means / mod.pooled_var
```

```r
p.mod = 2*pt( -abs(t.mod), n1+n2-2 )

par(mfrow=c(1,2))
hist(p,col="cornflowerblue",border="white",main="",xlab="P-values t-test")
mtext(paste("signifcant tests:",sum(p<0.05))  )
hist(p.mod,col="cornflowerblue",border="white",main="",
     xlab="P-values mod. t-test")
mtext(paste("signifcant tests:",sum(p.mod<0.05))  )
```




```r
#Moderated t-test reduced the number of significant tests to 118 from 141


#Pvalue correction for the t-test and the moderated t-test:
qvalues <- qvalue(p)
qvaluesmod <- qvalue(p.mod)
bonf.pval=p.adjust(p,method ="bonferroni")
bonf.pvalmod=p.adjust(p.mod,method ="bonferroni")
fdr.adj.pval=p.adjust(p,method ="fdr")
fdr.adj.pvalmod=p.adjust(p.mod,method ="fdr")

#For t-test

num_sig_q<- sum(qvalues$qvalues<0.05)
num_sig_bonf<-sum(bonf.pval<0.05)
num_sig_fdr<- sum(fdr.adj.pval<0.05)

paste("Qvals:", num_sig_q)
```

```
## [1] "Qvals: 100"
```

```r
paste("Bonferroni:", num_sig_bonf)
```

```
## [1] "Bonferroni: 25"
```

```r
paste("FDR:", num_sig_fdr)
```

```
## [1] "FDR: 99"
```

```r
#For the moderated t-test

num_sig_q<- sum(qvaluesmod$qvalues<0.05)
num_sig_bonf<-sum(bonf.pvalmod<0.05)
num_sig_fdr<- sum(fdr.adj.pvalmod<0.05)

paste("Qvals:", num_sig_q)
```

```
## [1] "Qvals: 100"
```

```r
paste("Bonferroni:", num_sig_bonf)
```

```
## [1] "Bonferroni: 2"
```

```r
paste("FDR:", num_sig_fdr)
```

```
## [1] "FDR: 100"
```

Relationship between variables: Linear models and correlation

Below we are going to simulate X and Y values that are needed for the rest of the exercise.

```r
# set random number seed, so that the random numbers from the text
# is the same when you run the code.
set.seed(32)

# get 50 X values between 1 and 100
x = runif(50,1,100)

# set b0,b1 and variance (sigma)
b0 = 10
b1 = 2
sigma = 20
# simulate error terms from normal distribution
eps = rnorm(50,0,sigma)
# get y values from the linear equation and addition of error terms
y = b0 + b1*x+ eps



# 1. Run the code then fit a line to predict Y based on X. [Difficulty:**Intermediate**]
```

```r
model = lm(y ~ x)
summary(model)



# 2. Plot the scatter plot and the fitted line. [Difficulty:**Intermediate**]

plot(x,y)
abline(model,col="red")


# 3. Calculate correlation and R^2. [Difficulty:**Intermediate**]

corr = cor(x,y)
r_sqr=corr^2


# 4. Run the `summary()` function and
#try to extract P-values for the model from the object
#returned by `summary`. See `?summary.lm`. [Difficulty:**Intermediate/Advanced**]

model_summary = summary(model)
model_summary


# 5. Plot the residuals vs. the fitted values plot, by calling the `plot()`
#function with `which=1` as the second argument. First argument
#is the model returned by `lm()`. [Difficulty:**Advanced**]

plot(lm(y~x),which = 1)


# 6. For the next exercises, read the data set histone modification data set. Use the following to get

hmodFile=system.file("extdata",
                     "HistoneModeVSgeneExp.rds",
                      package="compGenomRData")

# There are 3 columns in the dataset. These are measured levels of H3K4me3,
# H3K27me3 and gene expression per gene. Once you read in the data, plot the scatter plot for H3K4me3 v

histone_data=readRDS(hmodFile)
plot(histone_data$H3k4me3, histone_data$measured_log2, xlab = "Measured levels of H3K4me3", ylab= " Gene



# 7. Plot the scatter plot for H3K27me3 vs. expression. [Difficulty:**Beginner**]

plot(histone_data$H3k27me3, histone_data$measured_log2, xlab = "Measured levels of H3k27me3", ylab= " G



# 8. Fit the model for prediction of expression data using: 1) Only H3K4me3 as explanatory variable, 2)
```

```r
fit1 = lm(histone_data$measured_log2 ~ histone_data$H3k4me3)
fit2 = lm(histone_data$measured_log2 ~ histone_data$H3k27me3)
fit3 = lm(histone_data$measured_log2 ~ histone_data$H3k4me3 + histone_data$H3k27me3)

summary(fit1)
summary(fit2)
summary(fit3)

#All terms are significant



# 10. Is using H3K4me3 and H3K27me3 better than the model with only H3K4me3? [Difficulty:**Intermediate

#Comparing R-squared values, which is: R-squared = Explained variation / Total variation. So higher R-s
```