Statistical Methods in Genomics - BIOS 7649 Homework 1

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Question 1

Part A

Is it possible to avoid the effects of the experimental factors?

No, it is not possible to avoid the effects of the experimental factors. Different confounding factors including time-of-day and stress from handling can have an effect on the expression of genes. Additionally, even between mice there can be large variation, and because most experiments are based on single samples from each subject (in this case mice) this variable is often impossible to correct for.

Part B

If not, how should we perform the modeling and interpret the reults in light of these effects?

Question 2

Background:

- A two-fold difference between the treated and untreated mice would correspond to a $\delta = 1$, assuming a base 2 logarithm is used.
- Recommended α and σ values from *Design of DNA Microarray Experiment* will be used, which corresponds to $\alpha = 0.001$ and $\sigma = 0.5$ (when using base 2 logarithms).
- Sample size equation is as follows:

$$n = \frac{4(z_{\alpha/2} + z_{\beta})^2}{(\delta/\sigma)^2}$$

Where $\alpha = 0.001$ fixes $z_{\alpha/2} = -3.29$.

These steps are performed in the *pwr.t.test()* from the *pwr* package.

Table 1: Sample Size and Cost of Power Levels

Power	Sample Size	Cost ($)
0.80	24	24000
0.81	24	24000
0.82	24	24000
0.83	24	24000
0.84	24	24000
0.85	26	26000
0.86	26	26000
0.87	26	26000
0.88	26	26000
0.89	28	28000
0.90	28	28000
0.91	28	28000
0.92	28	28000
0.93	30	30000
0.94	30	30000
0.95	30	30000

Note:
delta = 1, *sigma* = 0.5, *alpha* = 0.001
Type = two-sample t-test, Alternative = two-sided

Table 1 illustrates the relationship between increasing power and sample size and cost. Assumptions include that kidney gene expressions between the treated and non-treated mice will be compared by level of expression of each gene. The value $\delta = 1$ represents the difference in means for each gene, where in this study a base 2 logarithm is used meaning that $\delta = 1$ corresponds to a twofold difference in gene expression in the kidneys of treated and non-treated mice. The significance level, α, is set at $\alpha = 0.001$ to limit the number of false discoveries. Power level varies from 0.80 to 0.95. The σ value can be approximated as 0.5 for the Affymetrix GeneChips, and the d parameter is δ/σ.

The null and alternative hypothesis for each gene are as follows:

$$H_0 : \delta = 0$$

$$H_1 : \delta \neq 0$$

The null hypothesis states that there is no meaningful difference between the mean expression of a particular gene, and the alternative is that there is a significant difference between the mean expression of the gene is both groups. A two sample t-test was used as there are two different groups, treated and un-treated, and a two sided test was applied as expression for a particular gene may potentially be higher in either group. 20,000 probe sets corresponds to 20,000 genes, and with a significance level of 0.001 this mean an expected number of false positives is 20. R version 4.3.1 was used in this analysis, and sample size calculations were performed using the *pwr.t.test* function from the *pwr* package in R.

Because sample size cannot be a fraction there are multiple power levels which overlap after rounding up. When considering the relationship between power, cost, and sample size the largest power for each sample size should be considered.

Question 3

Part A

What is the sample size needed based on $\alpha = 0.001$, fold change of 2 ($\delta = 1$ in log_2) and standard deviation of 0.5 to achieve power of at least 0.8 or 0.95? Use *pwr.t.test()* in the *pwr* package. Summarize your findings. Note: The d option in *pwr.t.test()* is δ/sd.

Table 2: Sample size Calculations Using pwr.t.test

Power	Sample Size
0.80	24
0.95	30

Note: $delta = 1$, $sigma = 0.5$, $alpha = 0.001$ Type = two-sample t-test, Alternative = two-sided

For $\delta = 1$, $\sigma = 0.5$, $\alpha = 0.001$, and a two-sample two-sided t-test the sample size needed for a power of 0.80 is 24, and for a power of 0.95 the sample size is 30. This means there are only 6 more subjects required to raise the power from 0.80 to 0.95.

Part B

As in part a), determine the sample size needed, but with a FDR of 0.05 instead. Use *power.t.test.FDR()* in the *ssize* package. Explain π_0 and summarize your findings.

Table 3: Sample Size Calculation Using power.t.test.FDR

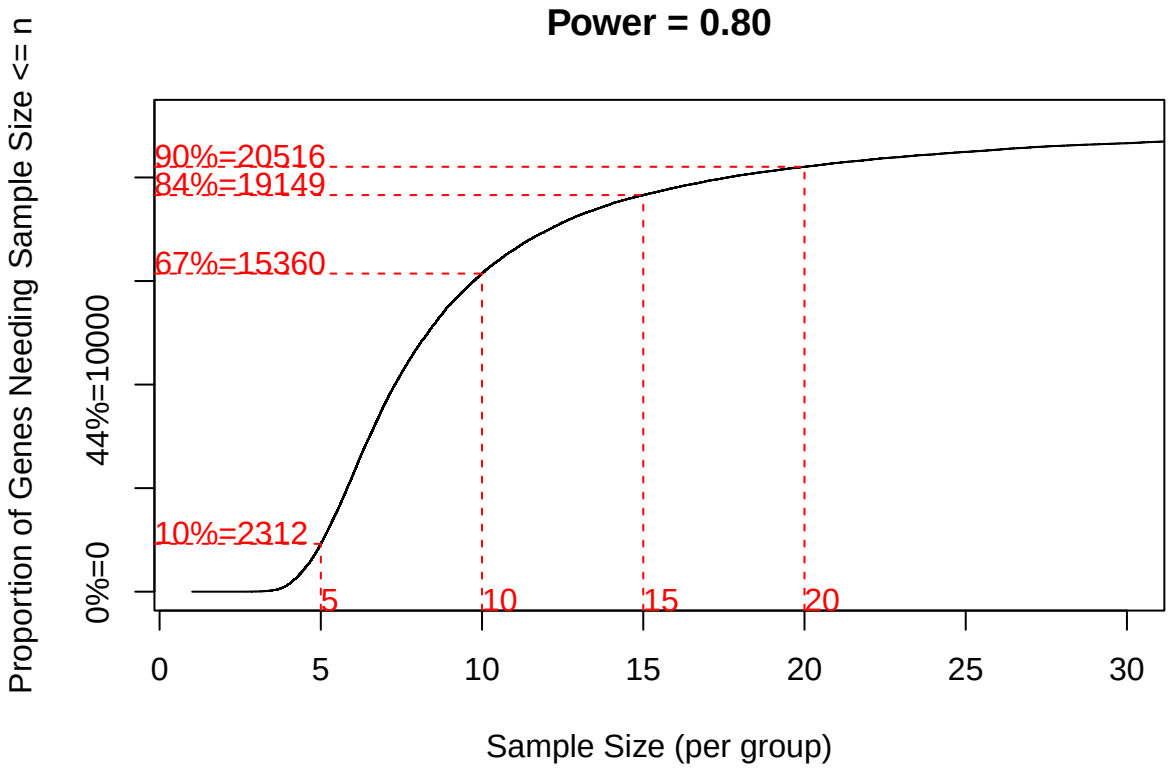
Power	Sample Size
0.80	16
0.95	22

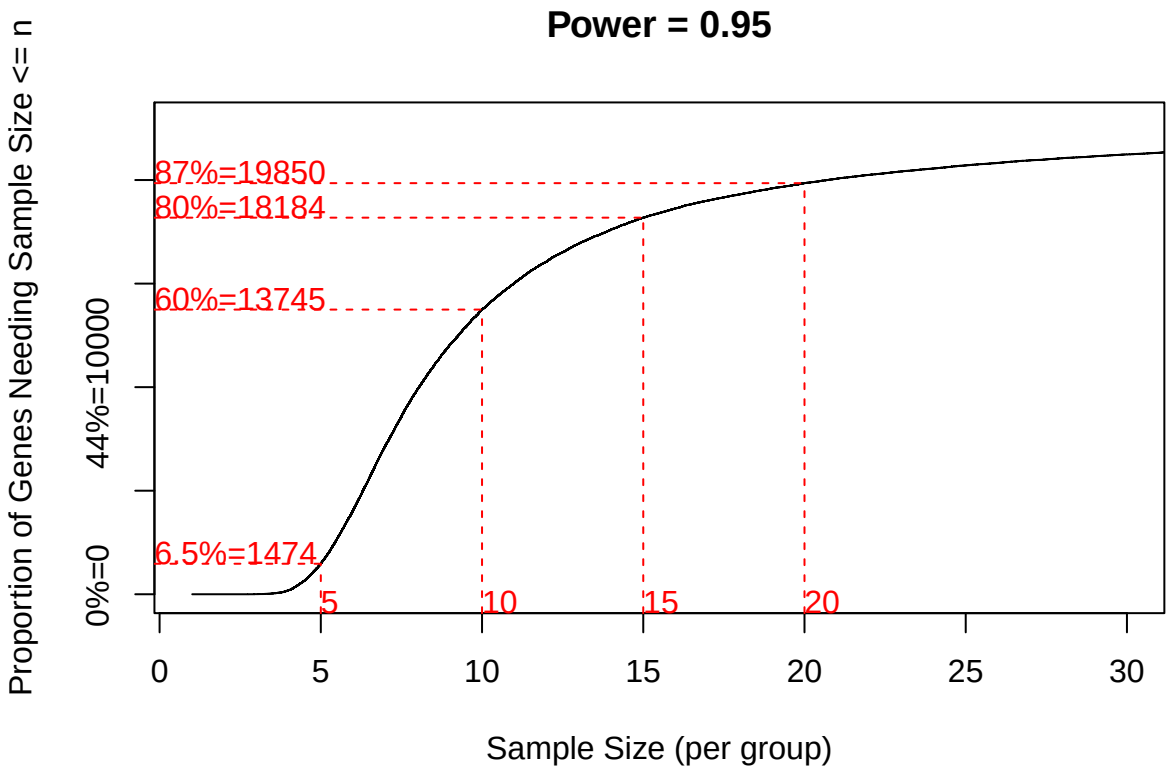
Note: $delta = 1$, $sigma = 0.5$,FDR = 0.05,$pi_0 = 0.80$ Type = two-sample t-test, Alternative = two-sided

For $\delta = 1$, $\sigma = 0.5$, $FDR = 0.05$, $\pi_0 = 0.80$, and a two-sample two-sided t-test the sample size needed for a power of 0.80 is 16, and for a power of 0.95 the sample size required is 22. This means 6 more subjects are required to raise the power from 0.80 to 0.95. π_0 is the proportion of true null hypothesis. In other words, this is the proportion of genes which are not differentially expressed.

Part C

The attached file **sdvalues.txt** contains pooled standard deviations (for the two groups) for the example data set. Read this file and plot the density (or histogram) of the standard deviations. Use *ssize()* and *ssize.plot()* in the *ssize* package to examine the sample size based on these standard deviations. Use *sig.level*, *delta* and *power* as in part a). What do you conclude from the plots?





Part D

The attacked file **arraydata.txt** contains the data for each sample. Use **samr.assess.samplesize()** in the **samr** package, which implements the method from journal club (Tibshirani,2006), to examine the sample size based on these data. What plots are displayed with **samr.assess.samplesize.plot()**? What do you conclude from the plots?

```
library(tidyverse)
library(pwr)
library(kableExtra)
library(ssize)
library(impute)
library(samr)


### START QUESTION 2 CODE ###

# Making a function to calculate sample size and cost for specific power level.
size_cost <- function(power_level){
  # power_level: Refers to the desired power level (will span 0.80 to 0.95).

  power_output <- pwr.t.test(n = NULL,d = 1/0.5,sig.level = 0.001,
                             power = power_level,type = "two.sample",
                             alternative = "two.sided")
```

```r
  # Calculating the total sample size. n from output is the number in each
  # group, meaning we have to multiply by 2 for total sample size.
  sample_size <- ceiling(power_output$n) * 2

  cost <- 1000 * sample_size

  return(c(sample_size,cost))

}


power_vector <- seq(0.80,0.95,by = 0.01) # Vector of different power levels.

# Calculating sample size and cost for various power levels.
cost_size_matrix <- t(sapply(power_vector, function(x) size_cost(x)))

# Combining vector of power levels with cost and sample size results.
cost_size_power <- data.frame(cbind(power_vector,cost_size_matrix))
colnames(cost_size_power) <- c("Power","Sample Size","Cost ($)")

cost_size_power_tbl <- kbl(cost_size_power,
                           caption = "Sample Size and Cost of Power Levels",
                           booktabs = TRUE, align = "lcc") %>%
  kable_styling(latex_options = "HOLD_position") %>%
  footnote(general = c("$\\delta$ = 1, $\\sigma$ = 0.5, $\\alpha$ = 0.001",
           "Type = two-sample t-test, Alternative = two-sided"),escape = FALSE,
           threeparttable = TRUE)

cost_size_power_tbl

### FINISH QUESTION 2 CODE ###


### START QUESTION 3 CODE ###

# Loading in data for question 3.

# Laptop location:"C:/Biostatistics Masters Program/pring 2024/SMG-BIOS7659/Homework 1/arraydata.txt"
# Desktop location:"C:/Users/domin/Documents/Biostatistics Masters Program/Spring 2024/SMG-BIOS7659/Hom
arraydata <- read.table("C:/Biostatistics Masters Program/Spring 2024/SMG-BIOS7659/Homework 1/arraydata


# Laptop location:"C:/Biostatistics Masters Program/Spring 2024/SMG-BIOS7659/Homework 1/sdvalues.txt"
# Desktop location:"C:/Users/domin/Documents/Biostatistics Masters Program/Spring 2024/SMG-BIOS7659/Hom
sdvalues <- read.table("C:/Biostatistics Masters Program/Spring 2024/SMG-BIOS7659/Homework 1/sdvalues.t


## START QUESTION 3 PART A CODE ##

# Calculating sample size for power = 0.80.
lower_power_a <- pwr.t.test(n = NULL,d = 1/0.5,sig.level = 0.001,
                            power = 0.8,type = "two.sample",
                            alternative = "two.sided")
```

```r
# Calculating sample size for power = 0.95.
upper_power_a <- pwr.t.test(n = NULL,d = 1/0.5,sig.level = 0.001,
                            power = 0.95,type = "two.sample",
                            alternative = "two.sided")

# Extracting sample size for each group, rounding up, and multiplying by 2
# in order to get the full sample size.
lower_size_a <- ceiling(lower_power_a$n) * 2
upper_size_a <- ceiling(upper_power_a$n) * 2

pa_size_df <- data.frame(power = c(0.80,0.95),
                         samplesize = c(lower_size_a,upper_size_a))

pa_size_tbl <- kbl(pa_size_df,
                   caption = "Sample size Calculations Using pwr.t.test",
                   col.names = c("Power","Sample Size"),
                   booktabs = TRUE, align = "cc") %>%
  kable_styling(latex_options = "HOLD_position") %>%
  footnote(general = c("$\\delta$ = 1, $\\sigma$ = 0.5, $\\alpha$ = 0.001",
           "Type = two-sample t-test, Alternative = two-sided"),escape = F,
           threeparttable = TRUE)

pa_size_tbl

## FINISH QUESTION 3 PART A CODE ##


## START QUESTION 3 PART B CODE ##

# Calculating sample size using specified FDR level, power = 0.80.
lower_power_b <- power.t.test.FDR(sd = 0.5,n = NULL,delta = 1,
                                  FDR.level = 0.05,pi0 = 0.80,power = 0.80,
                                  type = "two.sample",alternative = "two.sided")

# Calculating sample size using specified FDR level, power = 0.95.
upper_power_b <- power.t.test.FDR(sd = 0.5,n = NULL,delta = 1,
                                  FDR.level = 0.05,pi0 = 0.80,power = 0.95,
                                  type = "two.sample",alternative = "two.sided")

# Extracting sample size for each group, rounding up, and multiplying by 2
# in order to get the full sample size.
lower_size_b <- ceiling(lower_power_b$n) * 2
upper_size_b <- ceiling(upper_power_b$n) * 2

pb_size_df <- data.frame(power = c(0.80,0.95),
                         samplesize = c(lower_size_b,upper_size_b))

pb_size_tbl <- kbl(pb_size_df,
                   caption = "Sample Size Calculation Using power.t.test.FDR",
                   col.names = c("Power","Sample Size"),
                   booktabs = TRUE, align = "cc") %>%
  kable_styling(latex_options = "HOLD_position") %>%
  footnote(general = c("$\\delta$ = 1, $\\sigma$ = 0.5,FDR = 0.05,$\\pi_0$ = 0.80",
```

```
           "Type = two-sample t-test, Alternative = two-sided"),escape = FALSE,
           threeparttable = TRUE)

pb_size_tbl

## FINISH QUESTION 3 PART B CODE ##


## START QUESTION 3 PART C CODE ##

# Using ssize function to make a vector of powers. From the prompt n = 4 per
# group, alpha = 0.001, delta = 1, and sd comes from the provided file.
ssize_vec_lower <- ssize(sdvalues$V2,delta = 1,sig.level = 0.001,
                         power = 0.80,alpha.correct = "Bonferonni")

ssize_vec_upper <- ssize(sdvalues$V2,delta = 1,sig.level = 0.001,
                         power = 0.95,alpha.correct = "Bonferonni")


ssize.plot(ssize_vec_lower,xlim = c(1,30),marks = c(5,10,15,20),
           main = "Power = 0.80")
ssize.plot(ssize_vec_upper,xlim = c(1,30),marks = c(5,10,15,20),
           main = "Power = 0.95")

## FINISH QUESTION 3 PART C CODE ##
```