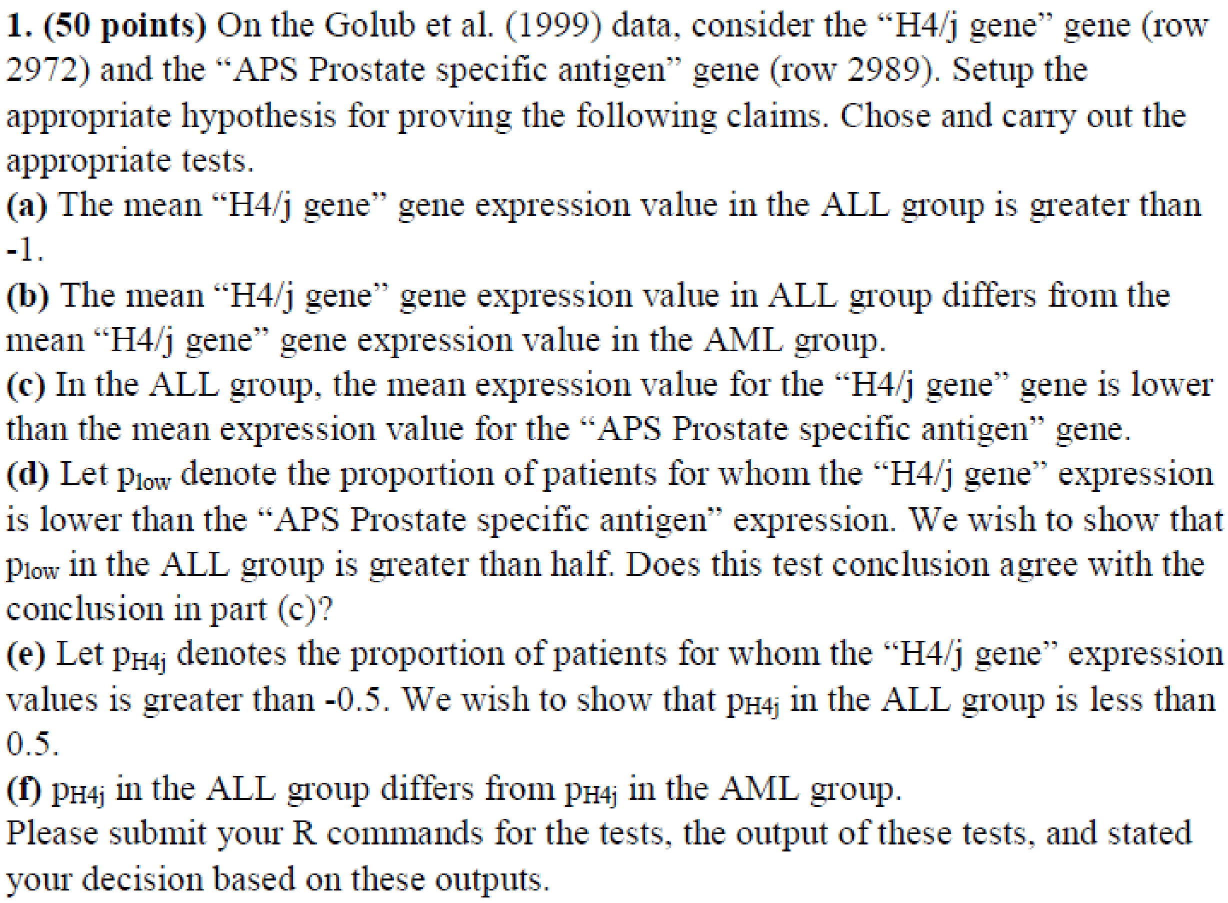
Solutions to Module 6



1. (50 points) On the Golub et al. (1999) data, consider the "H4/j gene" gene (row 2972) and the "APS Prostate specific antigen" gene (row 2989). Setup the appropriate hypothesis for proving the following claims. Chose and carry out the appropriate tests.

(a) The mean "H4/j gene" gene expression value in the ALL group is greater than -1.

(b) The mean "H4/j gene" gene expression value in ALL group differs from the mean "H4/j gene" gene expression value in the AML group.

(c) In the ALL group, the mean expression value for the "H4/j gene" gene is lower than the mean expression value for the "APS Prostate specific antigen" gene.

(d) Let p_{low} denote the proportion of patients for whom the "H4/j gene" expression is lower than the "APS Prostate specific antigen" expression. We wish to show that p_{low} in the ALL group is greater than half. Does this test conclusion agree with the conclusion in part (c)?

(e) Let p_{H4j} denotes the proportion of patients for whom the "H4/j gene" expression values is greater than -0.5. We wish to show that p_{H4j} in the ALL group is less than 0.5.

(f) p_{H4j} in the ALL group differs from p_{H4j} in the AML group.

Please submit your R commands for the tests, the output of these tests, and stated your decision based on these outputs.

Solutions to Module 6

Solutions:

First we setup the golub dataset.

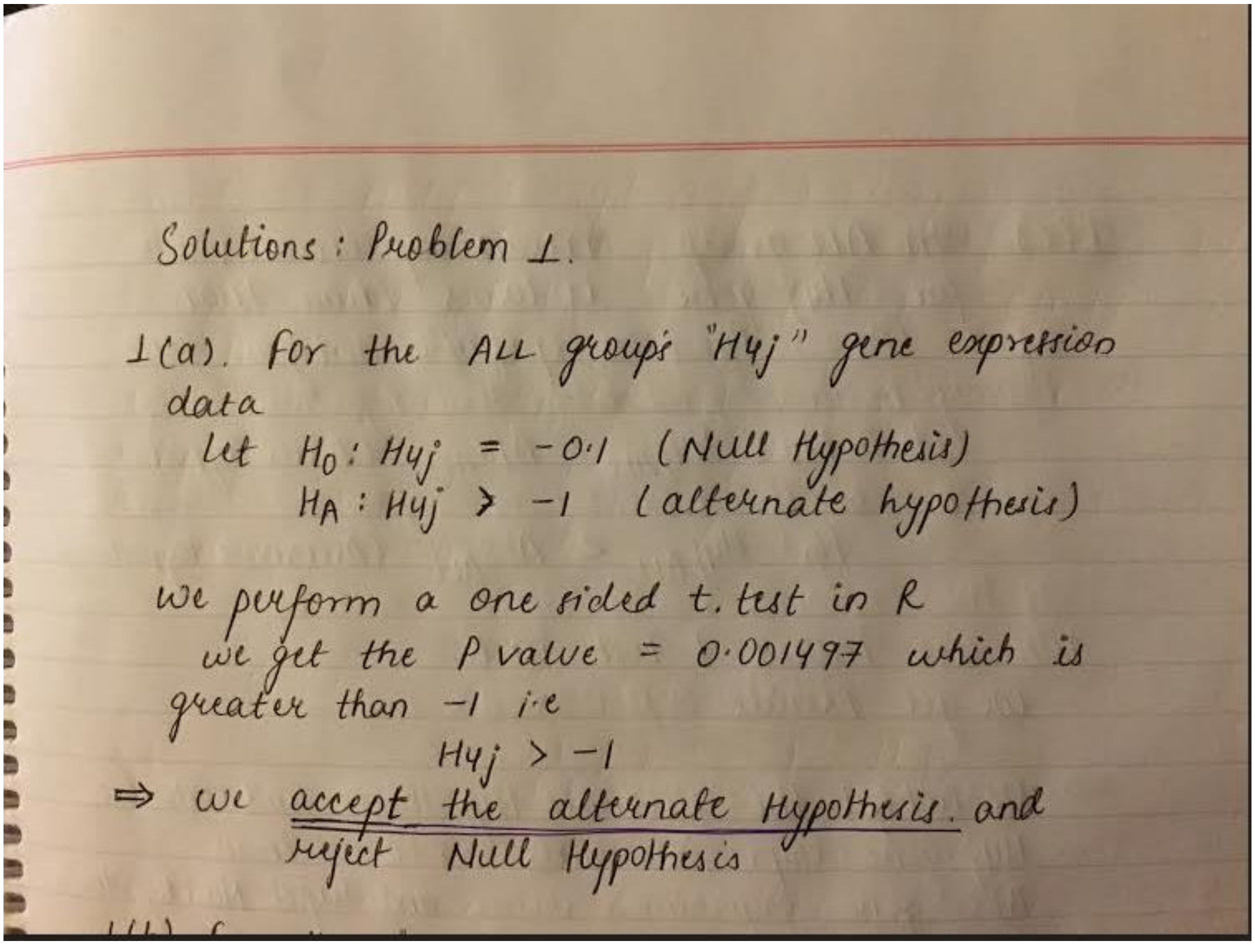
```
> data(golub, package="multtest")
> H4j<-grep("H4/j",golub.gnames[,2])
> APS<-grep("APS Prostate specific antigen", golub.gnames[,2])
> gol.fac<- factor(golub.cl, levels=0:1, labels=c("ALL","AML"))
```

R script for a)

```
> print(t.test(golub[H4j, gol.fac=="ALL"], mu= -1, alternative="greater"))

        One Sample t-test

data:  golub[H4j, gol.fac == "ALL"]
t = 3.2743, df = 26, p-value = 0.001497
alternative hypothesis: true mean is greater than -1
95 percent confidence interval:
 -0.844439       Inf
sample estimates:
 mean of x
-0.6753033
```



Solutions : Problem 1.

1(a). For the ALL groups "H4j" gene expression data

let $H_0 : H4j = -0.1$ (Null Hypothesis)

$H_A : H4j > -1$ (alternate hypothesis)

we perform a one sided t. test in R

we get the P value = 0.001497 which is greater than -1 i.e

$H4j > -1$

⇒ we accept the alternate Hypothesis. and reject Null Hypothesis

Solutions to Module 6

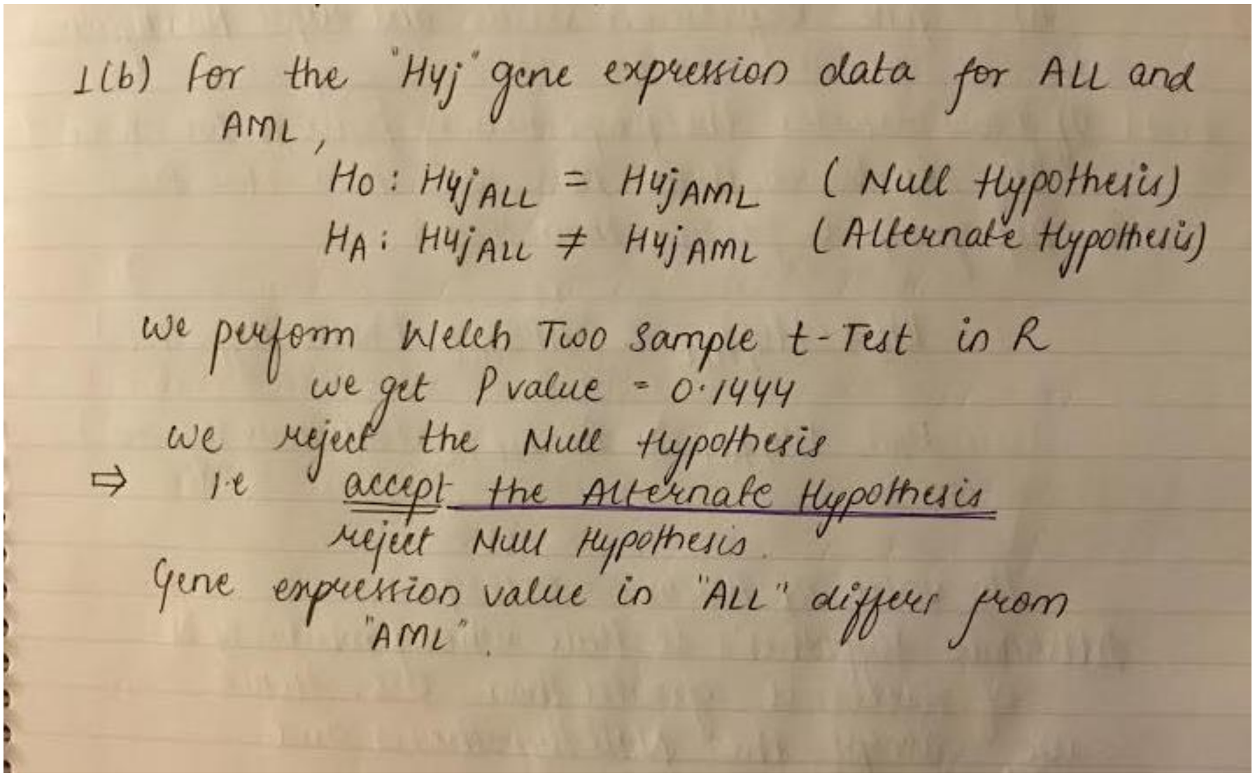
1(b)

R script for b)

```
> print(t.test(golub[H4j, gol.fac=="ALL"], golub[H4j, gol.fac=="AML"]))

        Welch Two Sample t-test

data:  golub[H4j, gol.fac == "ALL"] and golub[H4j, gol.fac == "AML"]
t = -1.4988, df = 29.978, p-value = 0.1444
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.48627436  0.07463315
sample estimates:
 mean of x  mean of y 
-0.6753033 -0.4694827 
```



1(b) for the "H4j" gene expression data for ALL and AML,

$H_0 : H4j_{ALL} = H4j_{AML}$ (Null Hypothesis)

$H_A : H4j_{ALL} \neq H4j_{AML}$ (Alternate Hypothesis)

we perform Welch Two Sample t-Test in R
we get P value = 0·1444
we reject the Null Hypothesis
⇒ i·e <u>accept the Alternate Hypothesis</u>
reject Null Hypothesis.
Gene expression value in "ALL" differs from "AML".

Solutions to Module 6

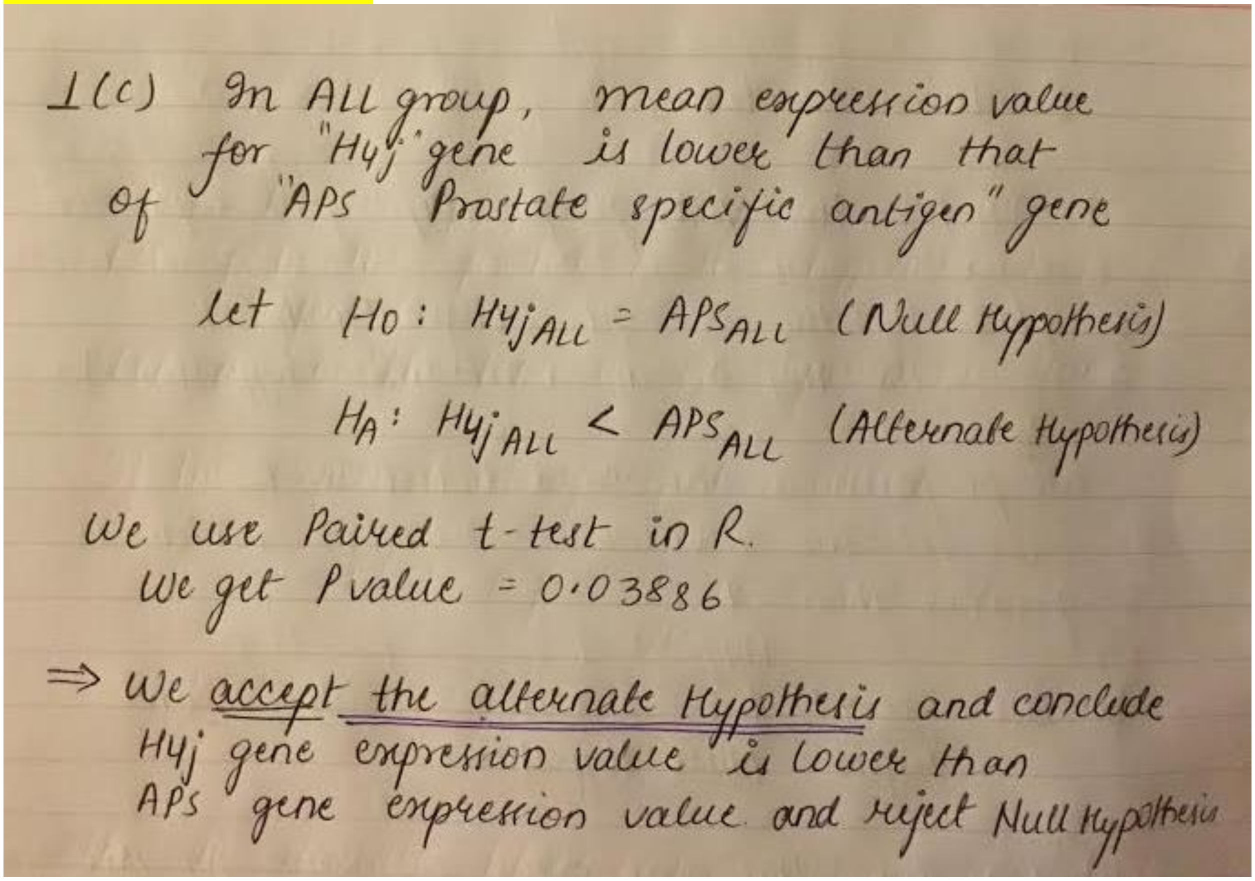
1c)

R script for 1c)

```
> print(t.test(golub[H4j, gol.fac=="ALL"], golub[APS, gol.fac=="ALL"], altern
ative="less", paired=T))

        Paired t-test

data:  golub[H4j, gol.fac == "ALL"] and golub[APS, gol.fac == "ALL"]
t = -1.8366, df = 26, p-value = 0.03886
alternative hypothesis: true difference in means is less than 0
95 percent confidence interval:
        -Inf -0.02175309
sample estimates:
mean of the differences
             -0.3050307
```



1(c) In ALL group, mean expression value for "H4j" gene is lower than that of "APS Prostate specific antigen" gene

let $H_0: H4j_{ALL} = APS_{ALL}$ (Null Hypothesis)

$H_A: H4j_{ALL} < APS_{ALL}$ (Alternate Hypothesis)

We use Paired t-test in R.
We get Pvalue = 0·03886

⇒ We accept the alternate Hypothesis and conclude H4j gene expression value is lower than APS gene expression value and reject Null Hypothesis

Solutions to Module 6

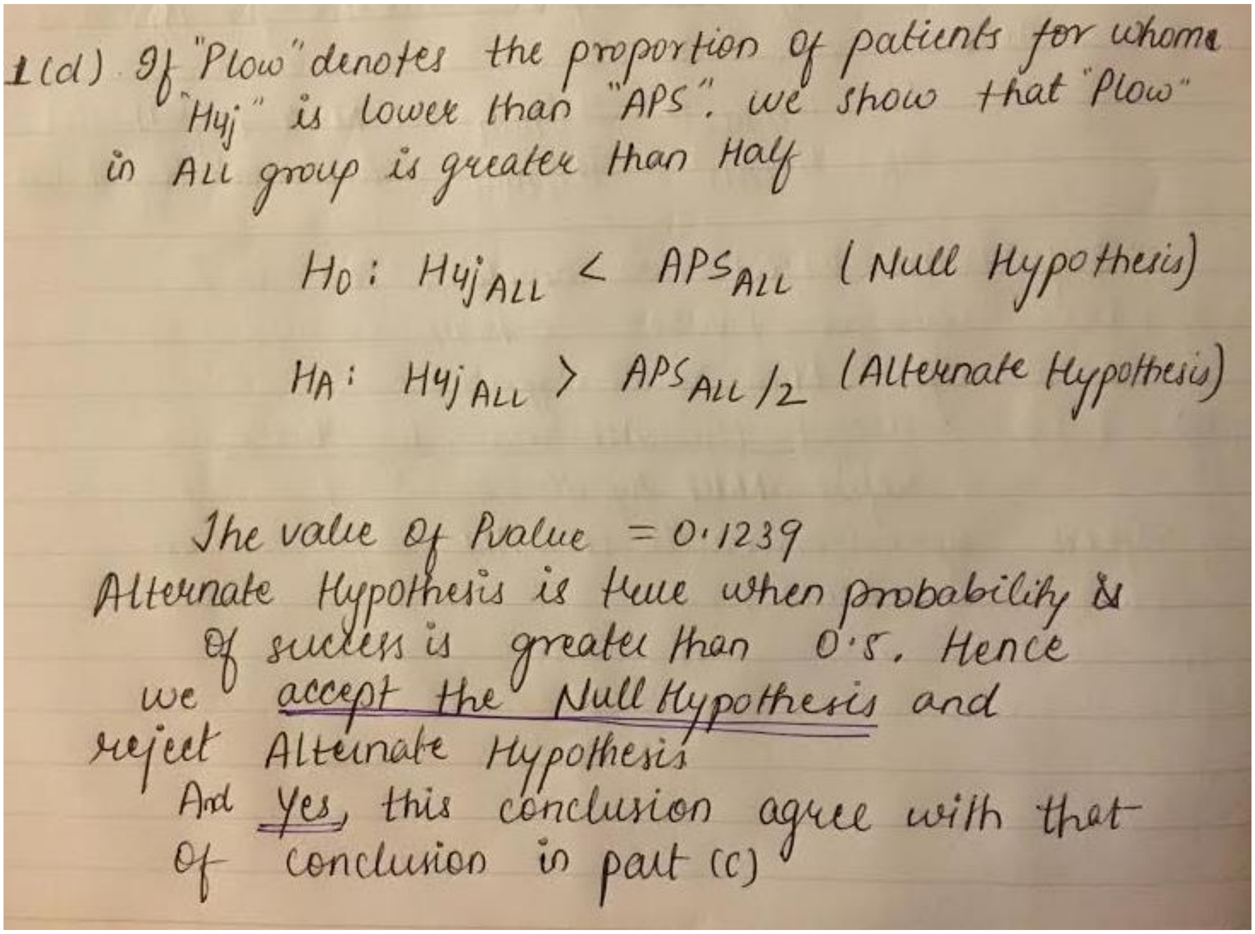
1d)

R script for 1 d)

```
> Plow<-sum(golub[H4j, gol.fac=="ALL"] < golub[APS, gol.fac=="ALL"])
> H4jlength<-length(golub[H4j, gol.fac=="ALL"])
> print(binom.test(x=Plow, n=H4jlength, p=0.5, alternative="greater"))

        Exact binomial test

data:  Plow and H4jlength
number of successes = 17, number of trials = 27,
p-value = 0.1239
alternative hypothesis: true probability of success is greater than 0.5
95 percent confidence interval:
 0.4533598 1.0000000
sample estimates:
probability of success
             0.6296296
```



1(d) If "Plow" denotes the proportion of patients for whom "H4j" is lower than "APS", we show that "Plow" in ALL group is greater than Half

$H_0 : H4j_{ALL} < APS_{ALL}$ (Null Hypothesis)

$H_A : H4j_{ALL} > APS_{ALL}/2$ (Alternate Hypothesis)

The value of Pvalue = 0·1239
Alternate Hypothesis is true when probability & of success is greater than 0·5. Hence we accept the Null Hypothesis and reject Alternate Hypothesis
And Yes, this conclusion agree with that of conclusion in part (c)

Solutions to Module 6

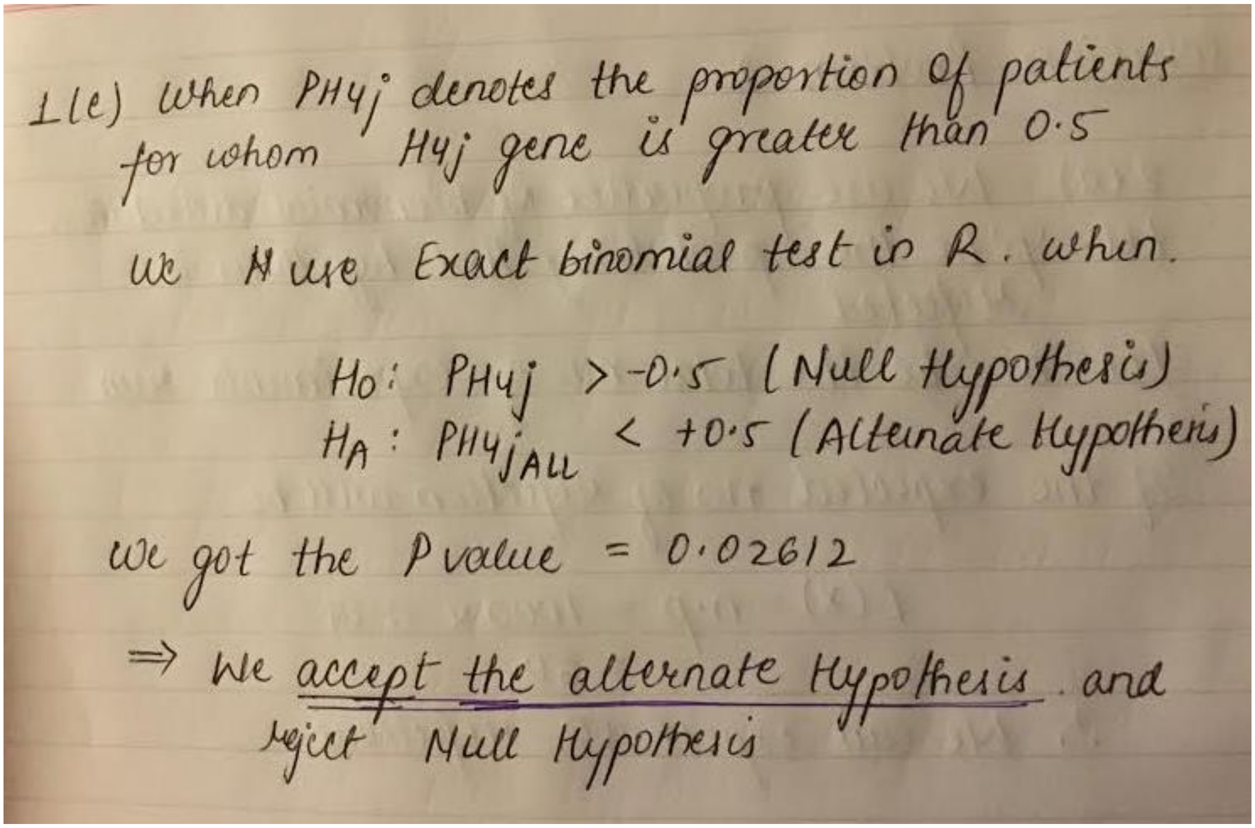
1e)

R script for 1e

```
> PH4j<-sum(golub[H4j, gol.fac=="ALL"] > -0.5)
> PH4jlen<-length(golub[H4j, gol.fac=="ALL"])
> print(binom.test(x=PH4j, n=PH4jlen, p=0.5, alternative="less"))

        Exact binomial test

data:  PH4j and PH4jlen
number of successes = 8, number of trials = 27,
p-value = 0.02612
alternative hypothesis: true probability of success is less than 0.5
95 percent confidence interval:
 0.0000000 0.4713915
sample estimates:
probability of success
             0.2962963
```



1(e) When PH4j denotes the proportion of patients for whom H4j gene is greater than 0.5

We ~~H~~ use Exact binomial test in R. when.

H_0: $PH4j > -0.5$ (Null Hypothesis)

H_A: $PH4j_{ALL} < +0.5$ (Alternate Hypothesis)

We got the Pvalue = 0.02612

⇒ We accept the alternate Hypothesis and reject Null Hypothesis

Solutions to Module 6

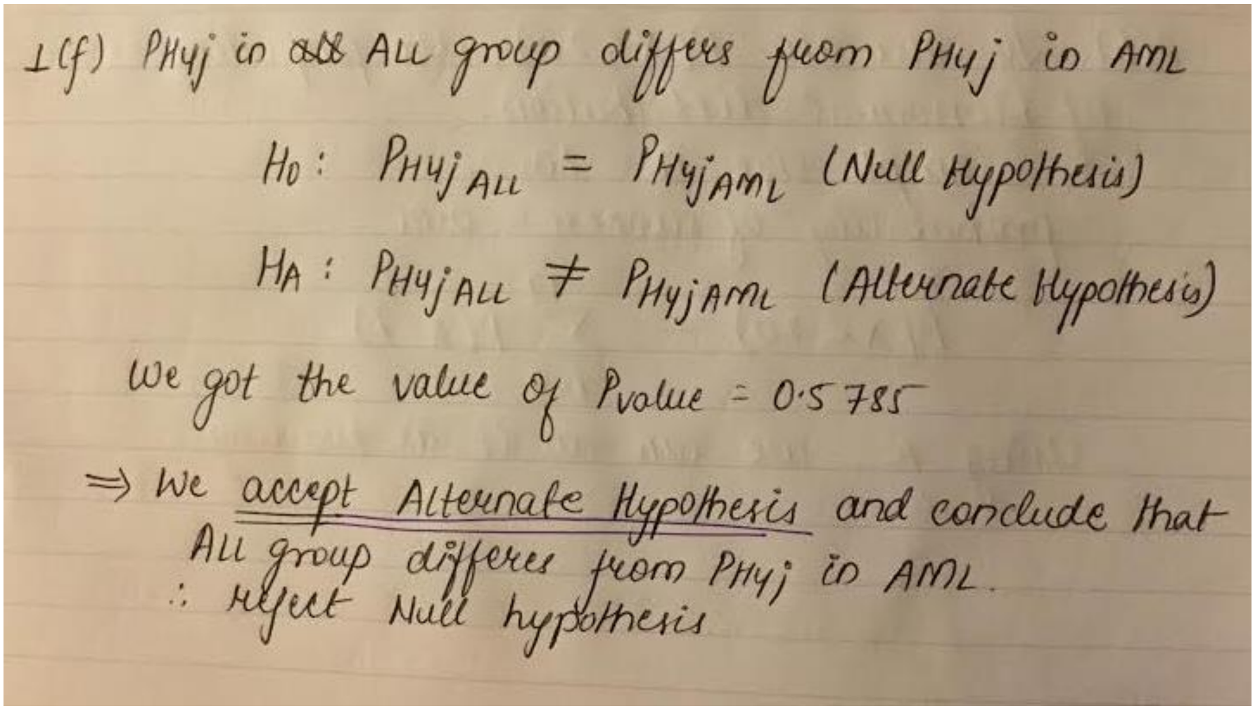
1f)

R script for 1f

```
> PH4j.ALL<-sum(golub[H4j, gol.fac=="ALL"] > -0.5)
> PH4j.AML<-sum(golub[H4j, gol.fac=="AML"] > -0.5)
> ALL.length<- length(golub[H4j, gol.fac=="ALL"])
> AML.length<- length(golub[H4j, gol.fac=="AML"])
> print(prop.test(x= c(PH4j.ALL,PH4j.AML), n=c(ALL.length, AML.length), alter
native="two.sided"))

        2-sample test for equality of proportions with
        continuity correction

data:  c(PH4j.ALL, PH4j.AML) out of c(ALL.length, AML.length)
X-squared = 0.3086, df = 1, p-value = 0.5785
alternative hypothesis: two.sided
95 percent confidence interval:
 -0.5631762  0.2466779
sample estimates:
   prop 1    prop 2 
0.2962963 0.4545455 
Warning message:
In prop.test(x = c(PH4j.ALL, PH4j.AML), n = c(ALL.length, AML.length),  :
  Chi-squared approximation may be incorrect
```



1(f) PH4j in ~~all~~ ALL group differs from PH4j in AML

H_0 : $PH4j_{ALL} = PH4j_{AML}$ (Null Hypothesis)

H_A : $PH4j_{ALL} \neq PH4j_{AML}$ (Alternate Hypothesis)

We got the value of Pvalue = 0.5785

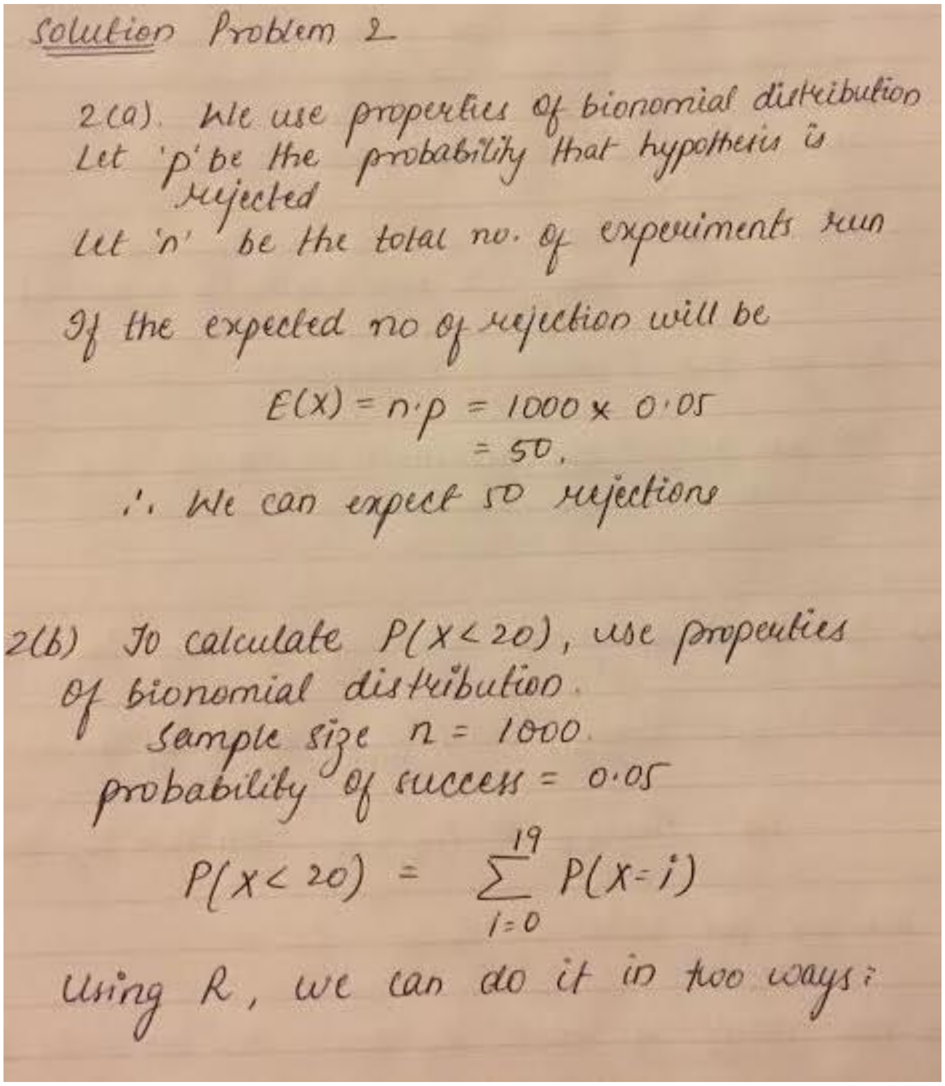
⇒ We accept Alternate Hypothesis and conclude that ALL group differes from PH4j in AML.
∴ reject Null hypothesis

Solutions to Module 6

2. (10 points) Suppose that the probability to reject a biological hypothesis by the results of a certain experiment is 0.05. Suppose that this experiment is repeated 1000 times.

(a) How many rejections do you expect?

(b) What is the probability of less than 20 rejections?



Solution Problem 2

2(a) We use properties of bionomial distribution
Let 'p' be the probability that hypothesis is rejected
Let 'n' be the total no. of experiments run

If the expected no of rejection will be

$$E(X) = n \cdot p = 1000 \times 0{\cdot}05 = 50.$$

∴ We can expect 50 rejections

2(b) To calculate $P(X<20)$, use properties of bionomial distribution.
Sample size $n = 1000$.
probability of success = 0·05

$$P(X<20) = \sum_{i=0}^{19} P(X=i)$$

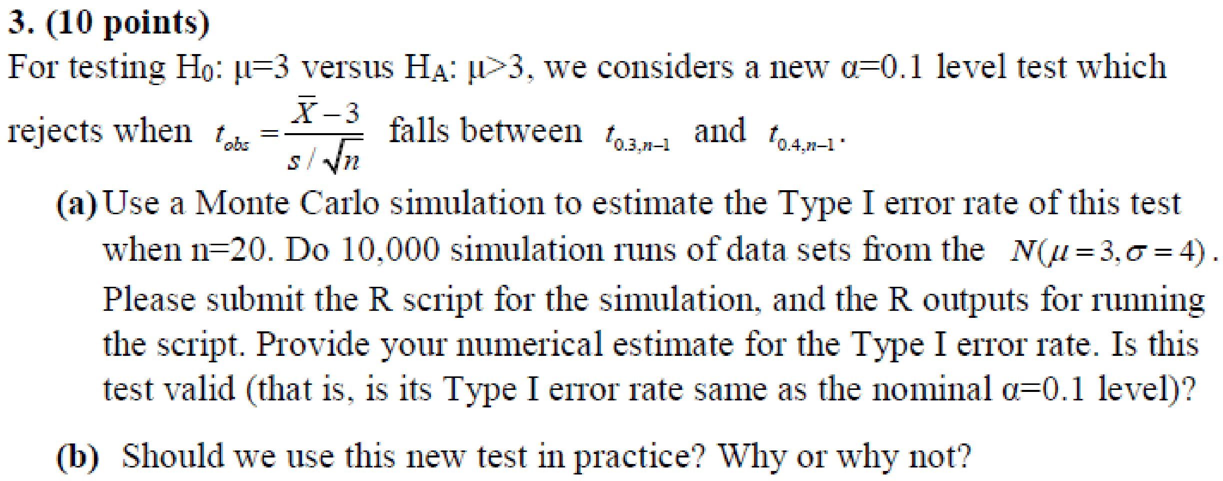
Using R, we can do it in two ways:

```
> # using dbinom to find the probability of fewer than 20 rejects.
> sum(dbinom(x=0:19, size= 1000, prob =0.05))
[1] 2.879692e-07

> # using pbinom to find the probability of fewer than 20 rejects.
> pbinom(q=19, size=1000, prob=0.05)
[1] 2.879692e-07
```

The probability of less than 20 rejection is 2.879692e-07 i.e approximately = 0

Solutions to Module 6



3. (10 points)

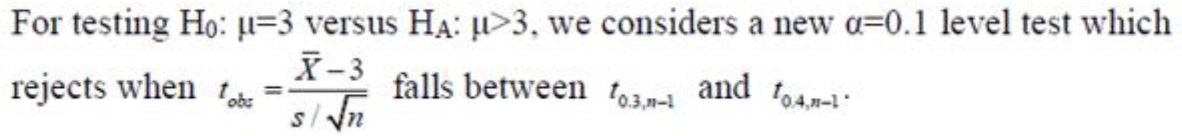
For testing H$_0$: μ=3 versus H$_A$: μ>3, we considers a new α=0.1 level test which rejects when $t_{obs} = \frac{\bar{X}-3}{s/\sqrt{n}}$ falls between $t_{0.3,n-1}$ and $t_{0.4,n-1}$.

(a) Use a Monte Carlo simulation to estimate the Type I error rate of this test when n=20. Do 10,000 simulation runs of data sets from the $N(\mu = 3, \sigma = 4)$. Please submit the R script for the simulation, and the R outputs for running the script. Provide your numerical estimate for the Type I error rate. Is this test valid (that is, is its Type I error rate same as the nominal α=0.1 level)?

(b) Should we use this new test in practice? Why or why not?

Solution:

3a)



For testing H$_0$: μ=3 versus H$_A$: μ>3, we considers a new α=0.1 level test which rejects when $t_{obs} = \frac{\bar{X}-3}{s/\sqrt{n}}$ falls between $t_{0.3,n-1}$ and $t_{0.4,n-1}$.

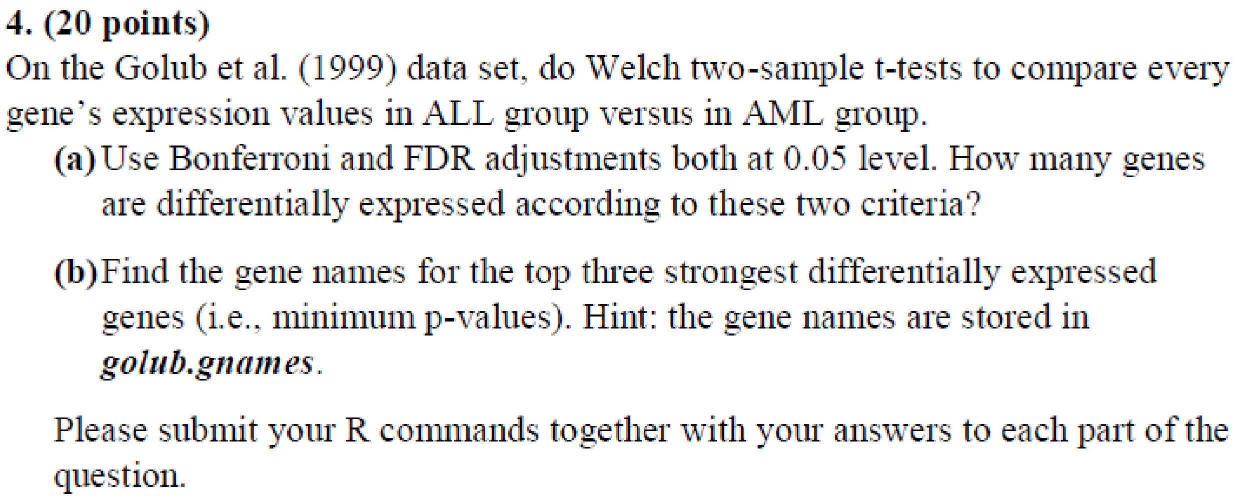
```
> #creating the data set
> x.simul<- matrix(rnorm(10000*20, mean=3, sd=4), ncol=20)
> # t-test
> tstat<- function(x) (mean(x)-3)/(sd(x)/sqrt(length(x)))
> tstat.simul<-apply(x.simul,1,tstat)
> #calculating the rejection rate
> power.simul<- mean(tstat.simul > qt(0.3, df=19) & tstat.simul < qt(0.4, df=19) )
> # type I error rate with its 95% CI
> print(power.simul+ c(-1,0,1)* qnorm(0.975)*sqrt(power.simul*(1-power.simul)/10000))
[1] 0.09100468 0.09680000 0.10259532
```

The rejection rate is 0.09680 with 95% CI of (0.0910, 0.1025)

3b)

We should not use this as , for type I error, we reject the null hypothesis ≈ 10% . And also because to prove the significance of alternate hypothesis H$_A$ the α = 0.10 is not sufficient enough.

Solutions to Module 6



4. (20 points)

On the Golub et al. (1999) data set, do Welch two-sample t-tests to compare every gene's expression values in ALL group versus in AML group.

(a) Use Bonferroni and FDR adjustments both at 0.05 level. How many genes are differentially expressed according to these two criteria?

(b) Find the gene names for the top three strongest differentially expressed genes (i.e., minimum p-values). Hint: the gene names are stored in ***golub.gnames***.

Please submit your R commands together with your answers to each part of the question.

Solution:

4a) The R code for problem 4a) is :

```
> # problem 4
>
> rm(list=ls())
>
> # 4(a) Use Bonferroni and FDR adjustments both at 0.05 level. How many gene
s are differentially expressed according to these two criteria?
>
> # load the golub data set and create factor
> data(golub, package="multtest")
> gol.fac<- factor(golub.cl, levels=0:1, labels=c("ALL","AML"))
>
> # to get the no. of genes and apply welch two-sample test
>
> length<- length(golub.gnames[,2])
> pvalues <- NULL
> for (i in 1:length){
+   pvalue <- t.test(golub[i,gol.fac=="ALL"], golub[i,gol.fac=="AML"])$p.valu
e
+   pvalues <- c(pvalues, pvalue)
+ }
>
> # performing Bonferroni and FDR adjustment
> p.bon<-p.adjust(p=pvalues, method="bonferroni")
> p.fdr<-p.adjust(p=pvalues, method="fdr")
>
```

Solutions to Module 6

The output showing the number of genes expressed is

```
> print("total number of genes differentially expressed at 0.05 level  no adj
ustment")
[1] "total number of genes differentially expressed at 0.05 level  no adjustm
ent"
> sum(pvalues < 0.05 )
[1] 1078
> print("total number of genes differentially expressed at 0.05 level  with b
onferroni ")
[1] "total number of genes differentially expressed at 0.05 level  with bonfe
rroni "
> sum(p.bon<0.05)
[1] 103
> print("total number of genes differentially expressed at 0.05 level  with F
DR")
[1] "total number of genes differentially expressed at 0.05 level  with FDR"
> sum(p.fdr<0.05)
[1] 695
```

When there were no adjustments the number of genes differentially expressed = 1078

With bonferroni , no of genes differentially expressed = 103

With FDR , no of genes differentially expressed = 695

4b) the R code for 4b) is :

```
> # 4(b) Find the gene names for the top three strongest differentially expre
ssed genes (i.e., minimum p-values). Hint: the gene names are stored in golub
.gnames
>
> # load the golub data set and create factor
> data(golub, package="multtest")
> gol.fac<- factor(golub.cl, levels=0:1, labels=c("ALL","AML"))
>
> # to get the no. of genes and apply welch two-sample test
>
> length<- length(golub.gnames[,2])
> pvalues <- NULL
> for (i in 1:length){
+   pvalue <- t.test(golub[i,gol.fac=="ALL"], golub[i,gol.fac=="AML"])$p.valu
e
+   pvalues <- c(pvalues, pvalue)
+ }
>
> # performing Bonferroni and FDR adjustment
> p.bon<-p.adjust(p=pvalues, method="bonferroni")
> p.fdr<-p.adjust(p=pvalues, method="fdr")
>
```

Solutions to Module 6

```
> #printing results
>
> print("top three strongest differentially espressed genes for
FDR")
[1] "top three strongest differentially espressed genes for FDR
"
> p.fdr<-p.adjust(p=pvalues,method="fdr")
> orderAML<-order(p.fdr, decreasing=FALSE)
> golub.gnames[orderAML[1:3],2]
[1] "Zyxin"
[2] "FAH Fumarylacetoacetate"
[3] "APLP2 Amyloid beta (A4) precursor-like protein 2"
>
> print("top three strongest differentially espressed genes for
Bonferroni")
[1] "top three strongest differentially espressed genes for Bon
ferroni"
> p.bon<-p.adjust(p=pvalues,method="bon")
> orderAL<-order(p.bon, decreasing=FALSE)
> golub.gnames[orderAML[1:3],2]
[1] "Zyxin"
[2] "FAH Fumarylacetoacetate"
[3] "APLP2 Amyloid beta (A4) precursor-like protein 2"
```

The output showing the top three strongest differentially expressed gene are:

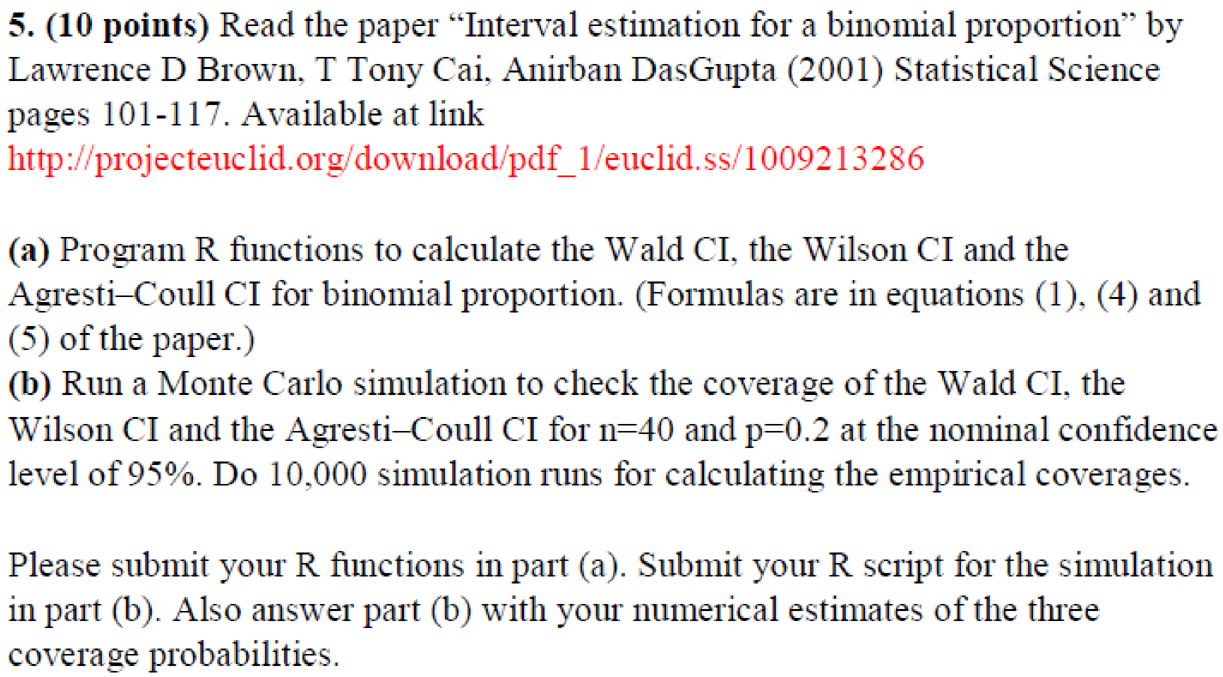
The three strongest differentially expressed genes after FDR and Bonferroni adjustment are

"Zyxin"

"FAH Fumarylacetoacetate"

"APLP2 Amyloid beta (A4) precursor-like protein 2"

Solutions to Module 6



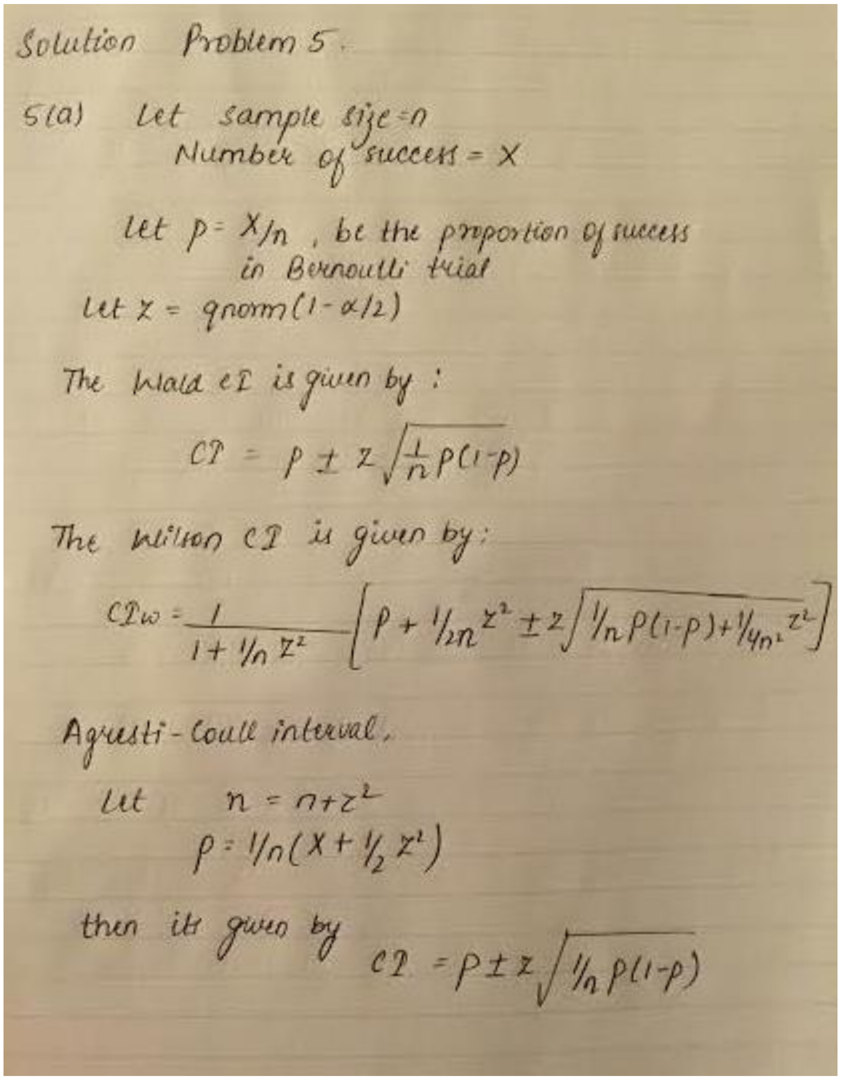
5. (10 points) Read the paper "Interval estimation for a binomial proportion" by Lawrence D Brown, T Tony Cai, Anirban DasGupta (2001) Statistical Science pages 101-117. Available at link
http://projecteuclid.org/download/pdf_1/euclid.ss/1009213286

(a) Program R functions to calculate the Wald CI, the Wilson CI and the Agresti–Coull CI for binomial proportion. (Formulas are in equations (1), (4) and (5) of the paper.)
(b) Run a Monte Carlo simulation to check the coverage of the Wald CI, the Wilson CI and the Agresti–Coull CI for n=40 and p=0.2 at the nominal confidence level of 95%. Do 10,000 simulation runs for calculating the empirical coverages.

Please submit your R functions in part (a). Submit your R script for the simulation in part (b). Also answer part (b) with your numerical estimates of the three coverage probabilities.

Solution:

Solutions to Module 6



Solution Problem 5.

5(a) Let sample size = n

Number of success = X

Let $p = X/n$, be the proportion of success in Bernoulli trial

Let $z = qnorm(1-\alpha/2)$

The Wald CI is given by:

$$CI = p \pm z\sqrt{\frac{1}{n}p(1-p)}$$

The Wilson CI is given by:

$$CI_w = \frac{1}{1+1/n\, z^2}\left[p + 1/2n\, z^2 \pm z\sqrt{1/n\, p(1-p) + 1/4n^2\, z^2}\right]$$

Agresti-Coull interval.

Let $n = n+z^2$

$p = 1/n(X + 1/2\, z^2)$

then its given by $CI = p \pm z\sqrt{1/n\, p(1-p)}$

The code for problem 5a) is :

```
> # 5 (a) Program R functions to calculate the Wald CI, the Wilson CI and the Agresti-Cou
on.
>
> wald.CI<- function(X,n,alpha=0.05){
+    p<- X/n
+    z<- qnorm(1-alpha/2)
+    c(c(p,(p + c(-1,1) * z *sqrt((p*(1-p))/n))))
+ }
>
>
> wilson.CI<- function(X,n,alpha=0.05){
+    p<- X/n
+     z<- qnorm(1-alpha/2)
+    c(p,((1/(1+z^2/n))* (p+(z^2/(2*n))+ c(-1,1)*z*sqrt((p*(1-p))/n+z^2/(4*n^2)))))
+ }
>
>
```

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```
> AgC.CI <- function(X,n,alpha=0.05){
+   z<- qnorm(1-alpha/2)
+   N<- n + z^2
+   p<- (X + z^2/2)/N
+    c(p,(p + c(-1,1)*z*sqrt((p*(1-p)))))
+ }
```

Solution for problem 5 b) : the code for the program is

```
> n.40<- rbinom(n=1, size=40, p=0.2)
> n.40.wald<-wald.CI(n.40,40)
> n.40.wilson<-wilson.CI(n.40,40)
> n.40.AgC<-AgC.CI(n.40,40)
> # run 10000 simulations to calculate the emprical changes.
>
> simul<- rbinom(n=10000, size=40, p=0.2)
> simul.wald<- NULL
> simul.wilson<- NULL
> simul.AgC<- NULL
> for(i in simul){
+   simul.wald<- rbind(simul.wald, wald.CI(i,40))
+   simul.wilson<- rbind(simul.wilson, wilson.CI(i,40))
+   simul.AgC<- rbind(simul.AgC, AgC.CI(i,40))
+ }
>
```

The point estimates and CI's for single run when n= 40 p=0.2

```
> print(" the proportion of success for n=40 & p=0.2 ")
[1] " the proportion of success for n=40 & p=0.2 "
> print(n.40)
[1] 8
> print("The 95% CI for n=40 & p=0.2 ")
[1] "The 95% CI for n=40 & p=0.2 "
> print(" for Wald CI")
[1] " for Wald CI"
> print(rbind(n.40.wald))
          [,1]       [,2]     [,3]
n.40.wald  0.2 0.07604099 0.323959
> print(" for wilson CI")
[1] " for wilson CI"
> print(rbind(n.40.wilson))
            [,1]      [,2]      [,3]
n.40.wilson  0.2 0.1049999 0.3475731
> print(" for AgC CI")
[1] " for AgC CI"
> print(rbind(n.40.AgC))
              [,1]       [,2]     [,3]
n.40.AgC 0.2262865 -0.5938148 1.046388
```

Solutions to Module 6

the proportion of success =8
for Wald cI p=0.0764 and 95% cI is (0.2,0.32)
for Wilson CI p=0.104 and 95% CI is (0.2,0.34)
for AgC CI p= -0.59 and 95% CI is (0.226,1.046)

The coverage of CI intervals after 10000 simulations

```
> # calculating the coverage of CI intervals and printing the results
>
> print("The estimated coverage after 10000 simulations of n=40, p=0.2")
[1] "The estimated coverage after 10000 simulations of n=40, p=0.2"
> print("for wald CI coverage")
[1] "for wald CI coverage"
> wald.coverage<- mean(0.2 > simul.wald[,2] & 0.2 < simul.wald[,3])
> print(wald.coverage)
[1] 0.9037
> print("for wilson CI coverage")
[1] "for wilson CI coverage"
> wilson.coverage<- mean(0.2 > simul.wilson[,2] & 0.2 < simul.wilson[,3])
> print(wilson.coverage)
[1] 0.9266
> print("for AgC CI coverage")
[1] "for AgC CI coverage"
> AgC.coverage<- mean(0.2 > simul.AgC[,2] & 0.2 < simul.AgC[,3])
> print(AgC.coverage)
[1] 1
```

The estimated coverage after 10000 simulations of n=40 , p=0.2
For wald coverage: 90.35%
For Wilson CI coverage: 92.66%
For AgC CI coverage: 100%