Project Description: Genomic Data Analysis

Jyotishka Datta

November 9, 2019

Final Project : Analyzing Genomic Data

1. This intro will show you how to read the two data-sets and get basic summary. It will also suggest some of the analysis that you can perform.
2. You can use one of the methods taught in class (or try new methods - it's upto you (and your team)).
3. You do not need to present your work. Only submit a well-written report.

Final Project : Analyzing Genomic Data

- Possible Inferential Goals:

1. Compare the baseline expression values between two groups using a nonparametric test, e.g. Wilcoxon/Mann-Whitney test.
2. We have p genes: p simultaneous tests for each of the variables. We need to correct for multiple testing.

Large Scale Testing

- Suppose for the i^{th} variable x_i the two group means are $\theta_{i,1}$ and $\theta_{i,2}$.

$$H_{0i} : \theta_{i,1} = \theta_{i,2} \text{ vs. } H_{1i} : \theta_{i,1} \neq \theta_{i,2}$$

- If H_{0i} is true, the group means $\bar{x}_{i,1}$ and $\bar{x}_{i,1}$ should be close.
- We can do an independent samples test for each of the p variables.
- It is not necessary to compare the means via a t-test. We can compare medians using Mann-Whitney / variations using a Siegel-Tukey or an omnibus test using two-sample Kolmogorov-Smirnon test.

Example from T-cell lymphoma

```
## required for gene expression data classification example
require(ALL)
data(ALL)
dim(ALL)
```

```
## Features  Samples
##    12625      128
```

Simplifying features

We are going to use the first three features.

```r
resp <- gsub("B[0-9]", "B", ALL$BT)
## B-cell tumors of type B, B1,B2, T, T1, T2
resp <- factor(gsub("T[0-9]", "T", resp))
xmat <- t(exprs(ALL))
mydata <- data.frame(y = resp,x1 = xmat[,1],x2=xmat[,2],x3=xmat[,3])
head(mydata,n=3)
```

```
##       y       x1       x2       x3
## 01005 B 7.597323 5.046194 3.900466
## 01010 B 7.479445 4.932537 4.208155
## 03002 B 7.567593 4.799294 3.886169
```

Further Analysis

- Let's look at the results of molecular biology testing for the 128 samples:

```r
table(ALL$mol.biol)
```

```
##
## ALL1/AF4  BCR/ABL E2A/PBX1      NEG   NUP-98  p15/p16
##       10       37        5       74        1        1
```

- Not all levels are frequent !

Filter

Ignoring the samples which came back negative on this test (NEG), most have been classified as (BCR/ABL) or (ALL1/AF4).

For the purposes of this example, we are only interested in these two subgroups, so we will create a filtered version of the dataset using this as a selection criteria:

```r
eset <- ALL[, ALL$mol.biol %in% c("BCR/ABL", "ALL1/AF4")]
dim(eset)
```

```
## Features  Samples
##    12625       47
```

How do we analyze this data?

- We have the expression levels 12,625 genes for 47 samples.
- We also have a factor `ALL$mol.biol` that has two levels: BCR/ABL and ALL1/AF4.
- We can ask for which genes, the gene expression values differ between these two subgroups?
- Two sample tests !

Simple test

- One idea would be use a two sample t-test for equality of group mean for each of the 12,625 genes.
- The `mt.teststat` function from the `multtest` library does this for you.

```r
require(multtest)
```

```
## Loading required package: multtest
```

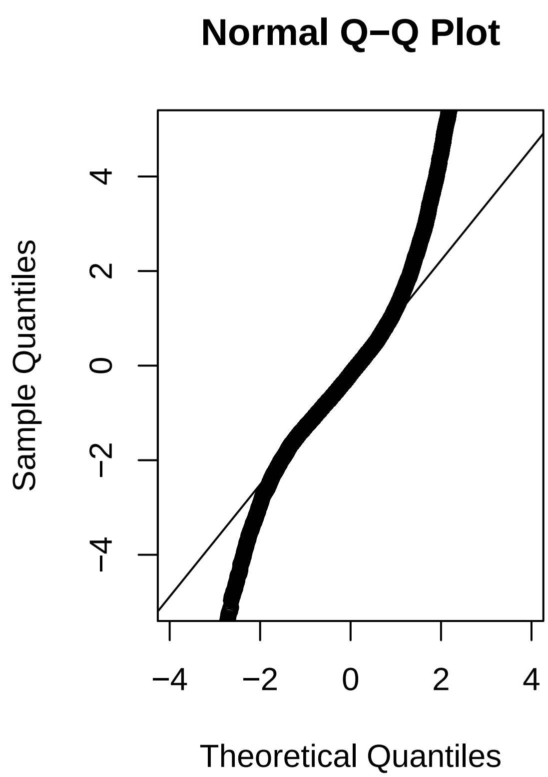
```r
all.mat = exprs(eset)
all.cl <- factor(as.character(eset$mol.biol))
teststat = mt.teststat(all.mat,all.cl,test="t")
```

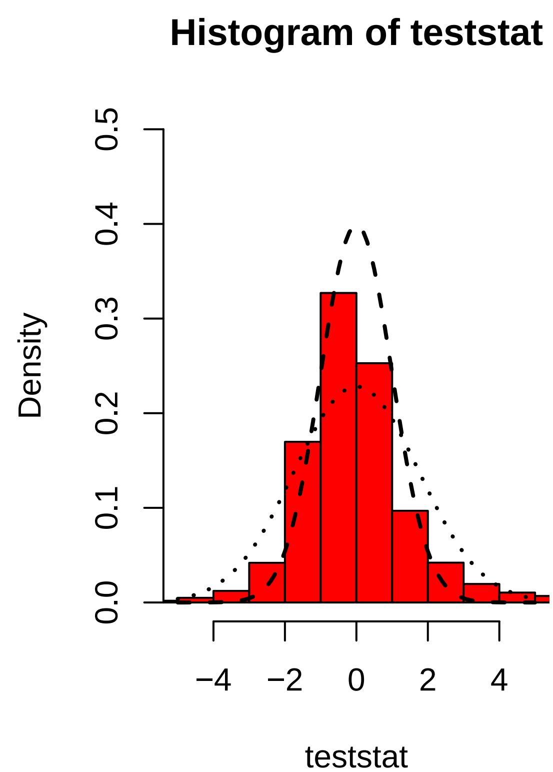
- The two datasets `all.mat` and `all.cl` are all you need.
- `all.mat` has the gene expression values and `all.cl` gives the class labbels.
- mt.teststat {multtest}: **Package for computing test statistics for each row of a data frame**.

Why t-test?

- These functions provide a convenient way to compute test statistics, e.g., two-sample Welch t-statistics, **Wilcoxon statistics**, F-statistics, paired t-statistics, block F-statistics, for each row of a data frame.
- Should we use a t-test or a different test?
- How do you know?

Visualize





- Histogram and N(0,1) density different on the tails - a few interesting genes?

Multiple Testing Issues

- We have a large number of tests: 12,625. If we use standard hypothesis testing at a 5% significance level, 5% of all tests will be falsely rejected (type 1 error) just by pure chance.
- We need some kind of multiplicity control.
- The most stringent is Bonferroni: Divide each α by the total number of tests $p = 12,625$.

Bonferroni

- Bonferroni's correction controls for the familywise error rate (FWER) instead of each α.

$$FWER = P(\text{at least one false rejection}) \leq \alpha$$

- Bonferroni leads to a stringent test, since α/M could be very small if we are carrying out a large number of M tests simultaneously.
- In R, we can apply the `p.adjust` function for this task.
- `p.adjust` also has other useful methods such as "Benjamini-Hochberg False Discovery Rate control procedure".

```r
rawp = 2 * (1 - pnorm(abs(teststat)))
selected  <- p.adjust(rawp, method = "bonferroni") <0.05
esetSel <- eset [selected, ]
sum(selected)
```

```
## [1] 343
```

- Bonferroni's correction leads to rejection of 343 tests - these genes significantly differ between two groups

Bonferroni Procedure

- M hypothesis tests: H_{0m} vs. H_{1m} for $m = 1, \ldots, M$.
- Let $p_1, \ldots, p_M$ be the p-values for these M tests.
- In our case $M = p$ (no. of genes)
- Bonferroni method:

$$\text{Reject null hypothesis } H_{0m} \text{ if } p_m \leq \frac{\alpha}{M}$$

- Outcome: The probability of falsely rejecting any null hypothesis is less than or equal to α.

Benjamini-Hochberg

The Benjamini and Hochberg procedure for multiple testing offers an alternative to the Bonferroni's FWER control procedure, that offers a less stringent testing mechanism for high-dimensional data. The main idea is that instead of controlling the familywise error rate, i.e. the probability of at least one false rejection, we control the average proportion of false discoveries, also called false discovery rate or FDR. It turns out that the Benjamini-Hochberg method is less stringent and has many attractive properties for high-dimensional data and often recognized as a state-of-the-art procedure for multiple testing. We describe the testing procedure below.

- Let M_0 be the number of null hypotheses that are true, $M_0 = M - M_1$.

	H_0 acc	H_0 rej	Total
H_0 true	U	V	M_0
H_0 false	T	S	M_1
Total	M-R	R	M

Define the false discovery proportion (FDP):

$$FDP = \begin{cases} V/R \text{ if } R > 0 \\ 0 \text{ otherwise} \end{cases}$$

Benjamini-Hochberg procedure

- M hypothesis tests We order the p-values in increasing order. $p_{(1)} \leq \dots \leq p_{(M)}$.
- *Benjamini-Hochberg Method*
  1. For a given α find the largest k such that

$$p_{(k)} \leq k\frac{\alpha}{M}$$

  2. Then reject all H_{0m} for $m = 1, \dots, k$.
- *Theorem* :

$$FDR = E(FDP) \leq \frac{M_0}{M}\alpha \leq \alpha$$

- *Outcome*: For a given significance level α, the Benjamini Hochberg method bounds the false discovery rate.

Benjamini-Hochberg in R

```
rawp = 2 * (1 - pnorm(abs(teststat)))
selected  <- p.adjust(rawp, method = "BH") <0.05
esetSel <- eset [selected, ]
sum(selected)
```

```
## [1] 947
```

- Benjamini-Hochberg method leads to rejection of 947 tests - less stringent than Bonferroni.

Literature for Multiple Testing

- The original paper by Benjamini & Hochberg is here: Benjamini & Hochberg (1995)..
- Manual for the R package `multtest`: multtest manual.
- For more details about large scale inference including multiple testing, an excellent reference is Prof. Bradley Efron's monograph on this subject. You can download the pdf copy of the book from Prof. Efron's webpage here: Large Scale Inference - Efron (2010). The first 4 chapters are pertinent for this project and the whole book is a very nice read if you want to learn about large scale inferences.

Nonparametric test

- We can use the `mt.teststat` function from the `multtest` package as shown above but choose a different test other than the Student's `t` test.
- Since we know how to do any nonparametric test covered in class for two independent samples, we can use them on each gene and calculate P-values for each gene, using a for loop.

```
dim(all.mat)
```

```
## [1] 12625    47
```

```
dim(all.cl)
```

```
## NULL
```

```
str(all.cl)
```

```
##  Factor w/ 2 levels "ALL1/AF4","BCR/ABL": 2 2 1 2 2 2 2 2 2 2 ...
```

A simple two-sample nonparametric test for the first row, i.e. one single gene is shown below:

```
all.mat.af4 = all.mat[,all.cl=='ALL1/AF4']
all.mat.abl = all.mat[,all.cl=='BCR/ABL']

wilcox.test(all.mat.af4[1,],all.mat.abl[1,])
```

```
##
##  Wilcoxon rank sum test
##
## data:  all.mat.af4[1, ] and all.mat.abl[1, ]
## W = 110, p-value = 0.05179
## alternative hypothesis: true location shift is not equal to 0
```

Goal 1: Multiple Testing

- You need to perform this test for all the 12,625 rows, get P-values for each of them and then apply multiple testing correction as shown above.
- Perform at least two different tests and two different multiplicity corrections, e.g. Bonferroni and Benjamini-Hochberg corrections.

Goal 2: Dependence?

- The Benjamini–Hochberg procedure assumes that the P-values (and the underlying test statistics) are independent of each other. This is not true in real life, and as a result the outcomes of Benjamini-Hochberg often tend to be anti-conservative. Benjamini & Yekutieli (2001) proposed a modification for the BH procedure that takes dependence into account. You can apply their approach by simply changing `method = BH` to `method = BY` in your `p.adjust` function.

- Your goal would be investigate whether there is a strong dependence in your data (i.e. dependence between genes), and whether applying BY instead of BH changes your conclusion.

- Write your conclusions clearly. Submit your R codes along with your report.

Help

- If you get stuck with any of the steps, please let me know at jd033@uark.edu.