Gene Expression Data Analysis and Visualization
410.671
HW #3

1.) Load the golub data training set in the multtest library. Also load Biobase and annotate libraries, if they are not loaded with the multtest library. Remember that the golub data training set is in the multtest library, so see the help file for information on this data set (2.5 pts)

```
library(multtest)
library(Biobase)
library(annotate)
data(golub)
```

2.) Cast the matrix to a data frame and label the gene names as numbers (e.g. “g1”,”g2”,etc). (2.5 pts)

```
golub.df <- as.data.frame(golub)
rownames(golub.df) <- paste("g", rownames(golub.df), sep="")
```

3.) Get the sample labels (see lecture notes) and set the sample labels to the data frame. (2.5 pts)

```
colnames(golub.df) <- golub.cl
```

4.) Use the t-test function in the lecture #7 notes and modify it to "wilcox.test" instead of "t.test". Change the "$p.value" argument to "$statistic". Assign the following arguments to the function: (2.5 pts)
exact=F
alternative="two.sided"
correct=T
Run the function on all of the genes in the dataset and save it as "original.wmw.run"

```
wilcox.test.all.genes <- function(x,s1,s2){
  x1 <- x[s1]
  x2 <- x[s2]
  x1 <- as.numeric(x1)
  x2 <- as.numeric(x2)
  wmw.out <- wilcox.test(x1, x2, exact=F, alternative="two.sided", correct=T)
  out <- as.numeric(wmw.out$statistic)
  return(out)
}

original.wmw.run <- apply(golub.df, 1, wilcox.test.all.genes, s1=c(1:27), s2=c(28:38))
```

5.) Now write a for loop to iterate 500 times, where in each iteration, the columns of the data frame are shuffled (class labels mixed up), the WMW test is calculated on all of the genes, and the maximum test statistic (W) is saved in a list. (5 pts)

**Hints:**
Use sample() to sample the number of columns
Get the maximum test statistic across all genes with max()

```
> length(golub.df[colnames(golub.df)==0])
[1] 27
> length(golub.df[colnames(golub.df)==1])
[1] 11

max.test.stats <- c()
for (i in 1:500){
  x1 <- sample(colnames(golub.df),27)
  x2 <- sample(colnames(golub.df), 11)
  wmw.run <- apply(golub.df, 1, wilcox.test.all.genes, s1=x1, s2=x2)
  max.test.stats <- c(max.test.stats, max(wmw.run))
}
```

6.) Once you have the list of maximum test statistics, get the 95% value test statistic. Subset the original.wmw.run list of values with only those that have a higher test statistic than the 95% value that you calculated. Print the gene names and test statistics out. (5 pts)

```
test.stat.95 <- quantile(as.numeric(max.test.stats), 0.95)
original.95 <- original.wmw.run[original.wmw.run > test.stat.95]
> original.95
  g9  g10  g17  g23  g25  g39  g47  g48  g51  g54  g55  g59
 188  191  204  249  210  212  201  193  211  186  251  204
 g63  g66  g74  g77  g78  g79  g81  g83  g84  g89  g94  g96
 198  258  234  220  227  211  244  188  225  195  187  274
 g98 g102 g104 g106 g109 g112 g117 g120 g121 g124 g126 g127
 188  224  236  215  188  209  232  185  225  199  262  256
g128 g135 g138 g142 g148 g149 g151 g153 g154 g156 g158 g159
 216  244  233  216  213  205  208  235  197  196  246  191
g163 g167 g171 g172 g174 g177 g178 g182 g184 g187 g188 g189
 206  215  223  253  262  230  236  261  244  232  232  216
g192 g193 g194 g195 g201 g202 g204 g205 g206 g207 g210 g214
 241  220  215  228  188  255  263  228  221  246  202  211
g218 g220 g225 g226 g227 g228 g230 g232 g233 g235 g237 g239
 200  240  202  244  205  233  230  250  188  220  237  247
g240 g241 g242 g244 g246 g248 g250 g253 g254 g258 g259 g265
 206  202  186  236  254  244  189  266  213  233  266  188
g270 g271 g277 g282 g283 g285 g286 g288 g289 g290 g291 g295
 232  227  185  242  271  226  260  238  213  225  221  205
```

g296 g297 g298 g304 g307 g309 g312 g313 g314 g316 g318 g320
185 257 234 243 252 245 189 248 246 234 210 241
g323 g325 g327 g328 g329 g330 g331 g332 g333 g334 g335 g336
262 228 195 202 275 245 212 186 188 221 254 215
g337 g338 g342 g344 g345 g347 g357 g359 g360 g362 g363 g364
232 188 201 259 274 187 247 235 202 200 230 215
g365 g366 g368 g369 g370 g373 g376 g377 g385 g389 g391 g394
212 190 215 209 205 189 244 229 228 227 245 290
g395 g398 g399 g402 g407 g421 g422 g423 g426 g435 g441 g445
250 235 247 209 240 211 270 207 237 210 197 225
g446 g447 g449 g453 g454 g455 g460 g462 g464 g472 g478 g479
198 202 185 242 213 226 195 259 191 185 237 248
g486 g489 g490 g493 g494 g500 g504 g514 g515 g516 g520 g522
187 250 248 191 251 207 212 194 264 210 208 257
g523 g527 g529 g538 g542 g543 g544 g546 g551 g555 g557 g559
283 194 229 211 238 201 224 274 234 193 221 222
g560 g561 g563 g564 g567 g571 g573 g579 g590 g591 g603 g605
262 281 255 196 219 237 209 195 245 185 195 213
g610 g611 g615 g620 g621 g627 g634 g640 g645 g648 g651 g652
218 227 207 239 269 236 243 192 229 271 189 193
g655 g661 g663 g671 g675 g678 g679 g685 g686 g688 g689 g692
192 187 192 199 230 190 201 232 234 258 217 185
g693 g695 g696 g698 g701 g702 g703 g704 g710 g711 g713 g715
199 251 221 241 240 186 285 273 199 226 266 240
g717 g718 g720 g725 g726 g730 g733 g738 g742 g746 g753 g758
283 210 233 252 238 214 220 271 218 277 223 206
g763 g764 g770 g772 g776 g777 g785 g793 g797 g800 g801 g804
248 241 186 204 199 201 256 224 217 220 263 193
g807 g811 g828 g835 g837 g838 g839 g842 g846 g849 g852 g856
248 249 186 272 210 283 272 229 242 272 216 191
g858 g862 g864 g866 g867 g871 g877 g882 g885 g886 g889 g890
235 259 225 269 190 195 236 187 233 220 201 224
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229 257 197 254 220 232 260 187 265 239 185 194 200 241

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g2373
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g2422
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g2459
 216   225   259   214   215   262   190   216   227   233   227   191   199   244
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g2506
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g2576
 213   187   205   219   217   240   207   209   232   227   195   195   233   242
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g2638
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g2716
 240   272   214   186   194   230   250   209   253   244   279   185   193   187
g2720 g2727 g2729 g2736 g2747 g2751 g2753 g2755 g2770 g2776 g2783 g2784 g2786
g2787
 189   211   215   261   194   187   260   203   238   213   217   197   246   222
g2790 g2794 g2798 g2801 g2802 g2803 g2804 g2815 g2817 g2818 g2828 g2829 g2833
g2841
 223   228   229   274   224   247   228   223   220   199   195   273   193   193
g2842 g2844 g2845 g2851 g2860 g2861 g2863 g2867 g2871 g2876 g2879 g2888 g2889
g2890
 231   236   189   281   272   192   202   201   203   211   272   229   232   190
g2894 g2902 g2903 g2907 g2925 g2939 g2947 g2950 g2955 g2959 g2961 g2973 g2982
g2983
 216   236   242   232   212   291   219   258   267   211   203   203   194   222
g2984 g2985 g2988 g2997 g3000 g3001 g3006 g3024 g3033 g3037 g3046
 204   252   230   190   190   195   211   189   207   198   276
```

7.) Now we want to compare these results to those using the empirical Bayes method in the limma package. Load this library and calculate p-values for the same dataset using the eBayes() function. (5 pts)

```
library(limma)
design <- cbind(Grp1=1, Grp2v1=c(rep(1,27),
            rep(0,11)))
fit <- lmFit(golub.df,design)
fit <- eBayes(fit)
```

```
#p-values
p.values <- fit$p.value[,2]
```

8.) Sort the empirical Bayes p-values and acquire the lowest *n* p-values, where *n* is defined as the number of significant test statistics that you found in problem 6. Intersect the gene names for your two methods and report how many are in common between the two differential expression methods, when choosing the top *n* genes from each set. (2.5 pts)

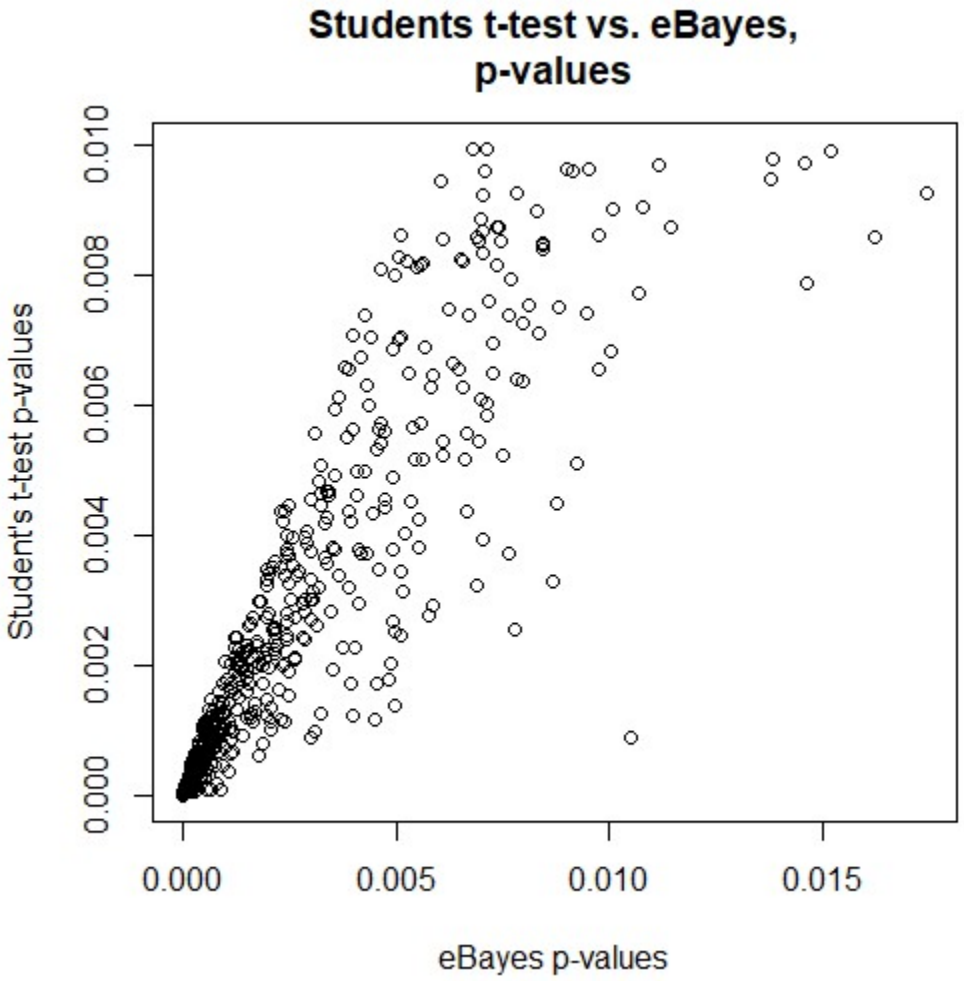
```
p.values.sorted <- sort(p.values)
n.lowest.p <- p.values.sorted[1:length(original.95)]
interesct.genes <- intersect(names(original.95),names(n.lowest.p))
> length(interesct.genes)
[1] 492
```

9.) Finally, compare the results from a Student's t-test with the empirical Bayes method. To do this, first calculate a two sample (two-tailed) Student's t-test on all genes. Make sure that you are running a Student's t-test and not a Welch's t-test. Then extract only those genes with a p-value less than 0.01 from this test. Plot the gene p-values<0.01 for the Student's t-test vs. the same genes in the empirical Bayes method. Make sure to label the axes and title appropriately. (7.5 pts)

```
t.test.all.genes <- function(x,s1,s2){
  x1 <- x[s1]
  x2 <- x[s2]
  x1 <- as.numeric(x1)
  x2 <- as.numeric(x2)
  t.out <- t.test(x1, x2, alternative="two.sided", var.equal=T )
  out <- as.numeric(t.out$p.value)
  return(out)
}

pv <- apply(golub.df, 1, t.test.all.genes, s1=c(1:27),s2=c(28:38))
pv.01 <- pv[pv < 0.01]

plot(p.values[names(pv.01)],pv.01, main="Students t-test vs. eBayes,\np-values",
    xlab="eBayes p-values", ylab="Student's t-test p-values")
```


Students t-test vs. eBayes,
p-values
Student's t-test p-values
0.000
0.002
0.004
0.006
0.008
0.010
0.000
0.005
0.010
0.015
eBayes p-values