Seminar III: R/Bioconductor

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A Case Study with GOs

Intro

Data and filtering

Complications

Some statistics

GO

More on GO

A Case Study with GOs

Credits

Homework

Packages we'll use today

- You'll probably need to install a few using biocLite.

```
> library("ALL")
> library("Biobase")
> library("annotate")
> library("hgu95av2.db")
> library("genefilter")
> library("annaffy")
> library("GO.db")
> library("GOstats")
> library("biomaRt")
> library("hgu133a.db")
> library("lattice")
```

To start off

- Similar to the 2nd homework, lets create a subset from the ALL dataset.
- Remember that we are working with leukemia samples and the molecular types BCR/ABL and ALL/AF4 are different translocations.

```
> library("ALL")
> data("ALL")
> types <- c("ALL1/AF4", "BCR/ABL")
> bcell <- grep("^B", as.character(ALL$BT))
> ALL_af4bcr <- ALL[, intersect(bcell,
+     which(ALL$mol.biol %in% types))]
> ALL_af4bcr$mol.biol <- factor(ALL_af4bcr$mol.biol)
```

- How many features does our subset have?

To start off

- Samples?

Filtering

- We can make a table to check how many samples we have:

  ```
  > table(ALL_af4bcr$mol.biol)
  ```

  ```
  ALL1/AF4  BCR/ABL
        10       37
  ```
- Our groups are rather different in size, so the outliers of BCR/ABL will dominate the variance.
- There are several options on how to filter the data, but we'll use the 10% and 90% quantiles.
- How do you find that range?

Filtering II

- Lets take advantage of the quantile and diff functions:

```
> qrange <- function(x) diff(quantile(x,
+     c(0.1, 0.9)))
```

- Now we can use the nsFilter function from the genefilter package:

```
> suppressWarnings(library("genefilter"))
> library("hgu95av2.db")
> filt_af4bcr <- nsFilter(ALL_af4bcr,
+     require.entrez = TRUE, require.GOBP = TRUE,
+     var.fun = qrange, var.cutoff = 0.5)
> ALLfilt_af4bcr <- filt_af4bcr$eset
```

- Previously we had used the IQR function instead of our homemade qrange.

Top 100

- Now lets find the top 100 genes by carrying out a two group comparison.
- We'll need to load some packages first:

```
> library("Biobase")
> library("annotate")
```

- Now we can use the rowttests function:

```
> rt <- rowttests(ALLfilt_af4bcr,
+      "mol.biol")
> names(rt)

[1] "statistic" "dm"        "p.value"
```

Quick exercises

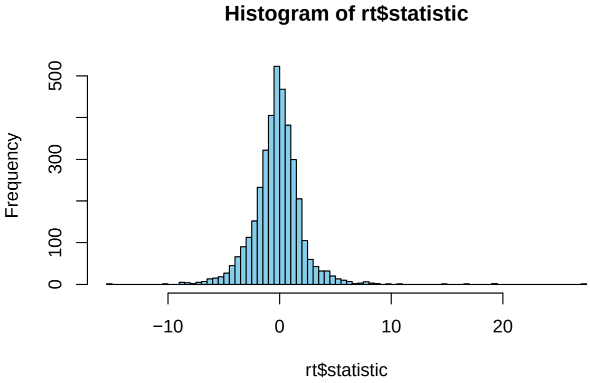
Create a histogram of

- the statistic
- the p values

Solution I

```
> hist(rt$statistic, breaks = 100,
+      col = "skyblue")
```

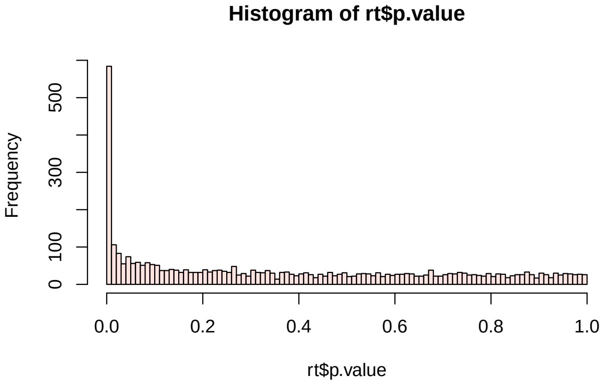
Solution I


Histogram of rt$statistic
Frequency
0
100
300
500
−10
0
10
20
rt$statistic

Solution II

```
> hist(rt$p.value, breaks = 100,
+      col = "mistyrose")
```

Solution II


Histogram of rt$p.value
Frequency
0
100
300
500
0.0
0.2
0.4
0.6
0.8
1.0
rt$p.value

Lowest 400 p values

- Lets create the `ALLsub` *ExpressionSet* with the 400 probe sets with the lowest p values.
- Any ideas?

Solution

- Here is one way:

```
> sel <- order(rt$p.value)[1:400]
> ALLsub <- ALLfilt_af4bcr[sel, ]
```

- Next, lets find how many probe sets in ALL and how many in ALLsub map to the same EntrezGene ID.

A trick

- First lets get the IDs into two separate vectors:

```
> EG <- as.character(hgu95av2ENTREZID[featureNames(ALL)
> EGsub <- as.character(hgu95av2ENTREZID[featureNames(A
```

- Next, lets use a little trick: using two table functions!

```
> head(table(EG))
```

```
EG
   10   100  1000 10000 10001 10002
    1     2     2     1     3     2
```

```
> table(table(EG))
```

A trick

```
   1    2    3    4    5    6    7    8
6891 1495  468   97   25   13    5    5
   9
   1
```

```
> table(table(EGsub))
```

```
  1
400
```

- Why do all the probe sets in ALLsub map to a unique EntrezGene ID?

Looking at a gene

- Now lets look at the expression profile of a given gene, for example, CD44.
- First, lets find out which features belong to our gene:

```
> syms <- as.character(hgu95av2SYMBOL[featureNames(ALLs
> whFeat <- names(which(syms == "CD44"))
```

- Now lets create a subset of ALLsub with the info we want:

```
> ordSamp <- order(ALLsub$mol.biol)
> CD44 <- ALLsub[whFeat, ordSamp]
```

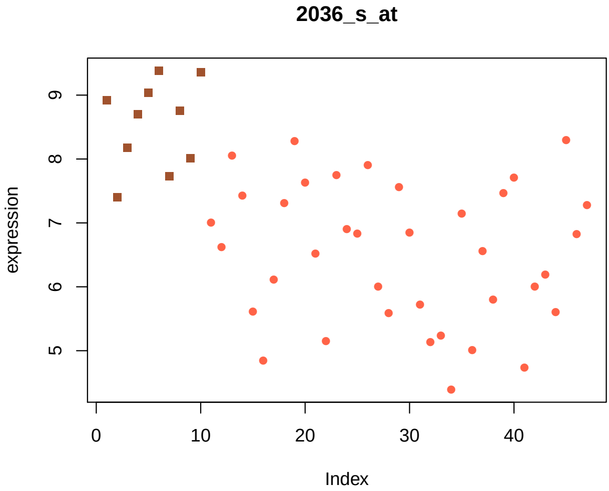
- What kind of plot should we make to visualize the expression profile of CD44?

Simple plot

A simple plot is enough:

```
> plot(as.vector(exprs(CD44)), main = whFeat,
+      col = c("sienna", "tomato")[CD44$mol.biol],
+      pch = c(15, 16)[CD44$mol.biol],
+      ylab = "expression")
```

Simple plot



Now a barplot

We used some mapping tricks to distinguis the two molecular types.
Looks like ALL1/AF4 have higher values than BCR/ABL.
Now lets make a barplot to group the values per chromosome:

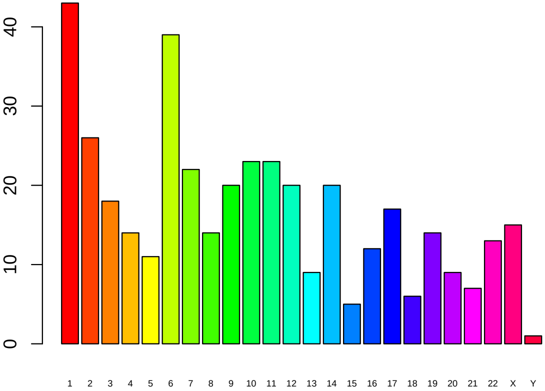
```
> z <- toTable(hgu95av2CHR[featureNames(ALLsub)])
> chrtab <- table(z$chromosome)
> chrtab
```

```
 1 10 11 12 13 14 15 16 17 18 19  2 20
43 23 23 20  9 20  5 12 17  6 14 26  9
21 22  3  4  5  6  7  8  9  X  Y
 7 13 18 14 11 39 22 14 20 15  1
```

Now a barplot

```
> chridx <- sub("X", "23", names(chrtab))
> chridx <- sub("Y", "24", chridx)
> barplot(chrtab[order(as.integer(chridx))],
+     cex.names = 0.5, col = rainbow(24))
```

Now a barplot



Checking

- Why did I use the `sub` commands?
- Why did I use `order` inside the `barplot` call?

A sweet html table

- Now lets assume that you want to show a table for the 400 genes in ALLsub to someone.
- Lets use the annaffy package to create an html table:

```
> library("annaffy")
> anncols <- aaf.handler(chip = "hgu95av2.db")[c(1:3,
+      8:9, 11:13)]
> anntable <- aafTableAnn(featureNames(ALLsub),
+      "hgu95av2.db", anncols)
> saveHTML(anntable, "ALLsub.html",
+      title = "The Features in ALLsub")
```

- We can open the html file directly from R using:

```
> localURL = file.path(getwd(), "ALLsub.html")
> browseURL(localURL)
```

A sweet html table

- Open the html file :)

Multiple measurements

- A big problem is that multiple probe sets can match to the same gene, which means that for some you have more measurements than for others. Also, alternative splicing can give you headaches.
- These R packages follow the ENCODE Project Consortium.
- Lets look at an example:

```
> probeSetsPerGene <- split(names(EG),
+     EG)
> j <- probeSetsPerGene$"7013"
> j
```

Multiple measurements

```
[1] "1329_s_at"  "1342_g_at"
[3] "1361_at"    "32255_i_at"
[5] "32256_r_at" "32257_f_at"
[7] "32258_r_at"
```

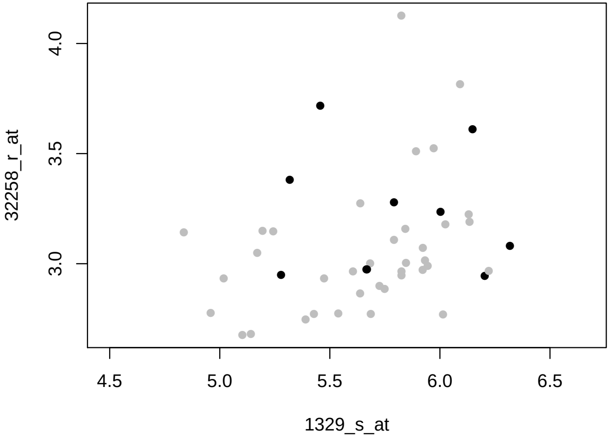
- We found 7 probes matching to the same gene (EntrezGene ID 7013).

Example complication

Lets look at the expression values from 2 of them:

```
> plot(t(exprs(ALL_af4bcr)[j[c(1,
+     7)], ]), asp = 1, pch = 16,
+     col = ifelse(ALL_af4bcr$mol.biol ==
+         "ALL1/AF4", "black", "grey"))
```

Example complication

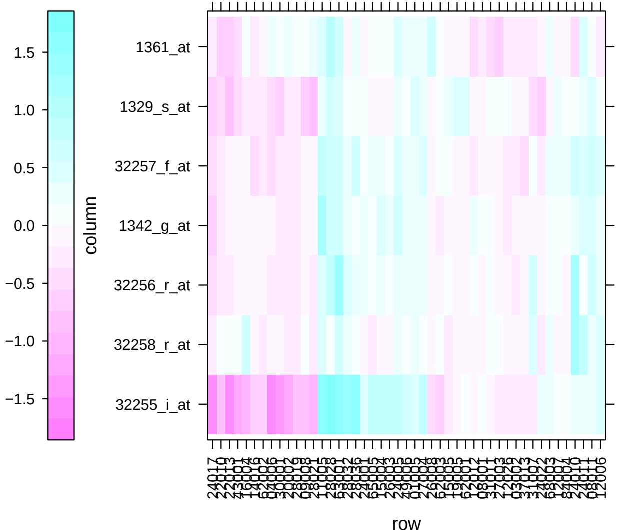


A complicated plot

We now used a different trick to map the colors: the ifelse function. A better plot in this case is the heatmap using the `lattice` function levelplot. Lets make one for the our gene 7013.

```
> library("lattice")
> mat <- exprs(ALL_af4bcr)[j, ]
> mat <- mat - rowMedians(mat)
> ro <- order.dendrogram(as.dendrogram(hclust(dist(mat))))
> co <- order.dendrogram(as.dendrogram(hclust(dist(t(mat)))
> at <- seq(-1, 1, length = 21) *
+     max(abs(mat))
> lp <- levelplot(t(mat[ro, co]),
+     aspect = "fill", at = at, scales = list(x = list(rot
+     colorkey = list(space = "left"))
> print(lp)
```

A complicated plot



chr

- One of the tests we can make now is to check for every chromosome, the low and high p values.
- To do so we can use `chisq.test` and `fisher.test`.
- First we need to create a data frame to map for every EntrezGene ID to which chromosome it belongs:

```
> ps_chr <- toTable(hgu95av2CHR)
> ps_eg <- toTable(hgu95av2ENTREZID)
> chr <- merge(ps_chr, ps_eg)
> dim(chr)
```

```
[1] 11972     3
```

- We don't need the first column, so lets take it out:

chr

```
> chr <- unique(chr[, colnames(chr) !=
+     "probe_id"])
> dim(chr)

[1] 9009    2

> head(chr)

  chromosome gene_id
1         14    5875
2         16    5595
3          1    7075
4         10    1557
5         11     643
7          5    1843
```

chr

- What problem do you notice? You might need to explore `chr` in full.

Duplications

- Look at this table:

```
> table(table(chr$gene_id))

   1     2
8985    12
```

- Lets take out those complicated genes that have duplicated entries.

```
> chr <- chr[!duplicated(chr$gene_id),
+      ]
```

Checking for association

- Now we can do the a contigency table for the association between the EntrezGene ID with their chromosome mapping and with being differently expressed.
- Lets re-use our EGsub object which had those differently expressed.

```
> isdiff <- chr$gene_id %in% EGsub
> tab <- table(isdiff, chr$chromosome)
> tab
```

Checking for association

```
isdiff    1  10  11  12  13  14  15  16
  FALSE 898 304 498 474 150 271 256 366
  TRUE   43  23  23  20   9  20   5  12

isdiff   17  18  19   2  20  21  22   3
  FALSE 512 122 543 547 221  93 249 461
  TRUE   17   6  14  26   9   7  13  18

isdiff    4   5   6   7   8   9  Un   X
  FALSE 326 390 490 406 297 311   4 384
  TRUE   14  11  39  22  14  20   0  15

isdiff    Y
```

Checking for association

```
  FALSE  24
  TRUE    0
```

- Once we have this table, we can do a Fisher's exact test:

  ```
  > fisher.test(tab, simulate.p.value = TRUE)
  ```

  ```
  Fisher's Exact Test for Count Data
  with simulated p-value (based on
  2000 replicates)

  data:  tab
  p-value = 0.01499
  alternative hypothesis: two.sided
  ```

- And a Chi squared test:

  ```
  > chisq.test(tab)
  ```

Checking for association

```
Pearson's Chi-squared test

data:  tab
X-squared = 42.2405, df = 24,
p-value = 0.01213
```

- What can we conclude?

Strand bias

- We can also check for where the genes are located, what other genes are nearby, grouping genes by location before another test, . . .
- Lets check if our differentially expressed genes are on the same strand:

```
> chrloc <- toTable(hgu95av2CHRLOC[featureNames(ALLsub)
> head(chrloc)
```

Strand bias

```
  probe_id start_location Chromosome
1  1635_at      132579088          9
2  1635_at      132700651          9
3 39329_at      -68410592         14
4 40797_at      -56675801         15
5 33800_at       -3952652         16
6 34777_at       10283217         11
```

- Alternative splicing will give us some problems:

```
> table(table(chrloc$probe_id))
```

```
  1   2   3   4   5   6   9
285  66  33   9   3   3   1
```

Strand bias

- Lets collapse the information so that we only record the strand, which should be the same even if there is alternative splicing:

```
> strds <- with(chrloc, unique(cbind(probe_id,
+     sign(start_location))))
> table(strds[, 2])
```

```
 -1   1
194 206
```

- What do we conclude?

Quick review

- GO, short for Gene Ontology, classifies genes products according to
  1. Molecular function
  2. Biological process
  3. Cellular component
- GO terms are represented in a graph where there are two types of relationships:
  1. is as
  2. part of
- To facilitate the mapping, GO terms are identified in 7 numbers.
- All the descendants of a given GO term are called *offspring*. The immediate ones are called *children*.
- All the parental GO terms are called *ancestor*.

GO.db

- In R, the package `GO.db` enables us to browse the GO tree:

```
> library("GO.db")
> as.list(GOMFCHILDREN["GO:0008094"])

$`GO:0008094`
        isa          isa          isa
"GO:0004003" "GO:0015616" "GO:0033170"
        isa          isa          isa
"GO:0033676" "GO:0033680" "GO:0043142"

> as.list(GOMFOFFSPRING["GO:0008094"])
```

GO.db

```
$`GO:0008094`
 [1] "GO:0003689" "GO:0004003"
 [3] "GO:0015616" "GO:0017116"
 [5] "GO:0033170" "GO:0033676"
 [7] "GO:0033680" "GO:0033681"
 [9] "GO:0033682" "GO:0043140"
[11] "GO:0043141" "GO:0043142"
```

Hyper Geometric GO test

- The packages annotate and GOstats are the basic ones to carry out GO analysis.
- Other related packages are topGO and goTools.
- Lets make the basic GO test. We want to compare the frequency of a GO term on a subset versus the frequency of the same GO term on the overall universe.
- Things get complicated because some GO terms have more offspring than others. . .
- Lets do the test (actually, lots of tests) for our data:

Hyper Geometric GO test

```
> library("GOstats")
> affyUniverse <- featureNames(ALLfilt_af4bcr)
> uniId <- hgu95av2ENTREZID[affyUniverse]
> entrezUniverse <- unique(as.character(uniId))
> params <- new("GOHyperGParams",
+     geneIds = EGsub, universeGeneIds = entrezUniverse
+     annotation = "hgu95av2", ontology = "BP",
+     pvalueCutoff = 0.001, conditional = FALSE,
+     testDirection = "over")
```

- After building up all the parameters we can now make the actual test:

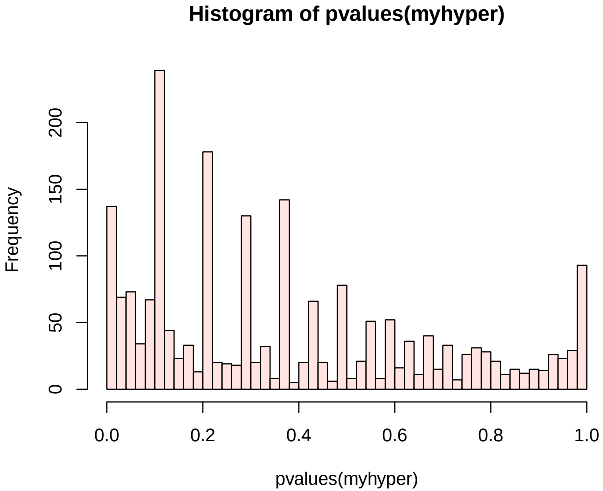
```
> myhyper <- hyperGTest(params)
```

P values

We didn't adjust our p values as it can complicated. Instead, lets visualize the histogram:

```
> hist(pvalues(myhyper), breaks = 50,
+      col = "mistyrose")
```

P values


Histogram of pvalues(myhyper)
Frequency
0
50
100
150
200
0.0
0.2
0.4
0.6
0.8
1.0
pvalues(myhyper)

Summary for myhyper

- As you can notice, we have a peak on the left side. Meaning that we have several low p values.
- Lets look deeper into the results from our test:

```
> sum <- summary(myhyper, p = 0.001)
> head(sum)
```

```
      GOBPID       Pvalue OddsRatio
1 GO:0007154 3.683084e-09  1.903807
2 GO:0007165 9.991034e-09  1.883483
3 GO:0006955 3.396946e-07  2.416384
4 GO:0019882 8.991223e-07  6.479221
5 GO:0002376 5.214422e-06  1.998862
6 GO:0006687 9.127024e-06 50.939086
    ExpCount Count Size
1 116.390817   168 1090
2 109.663641   159 1027
3  27.442605    54  257
4   3.737320    15   35
```

Summary for myhyper

```
5  39.829151    67  373
6   0.747464     6    7
                                 Term
1                  cell communication
2                 signal transduction
3                     immune response
4 antigen processing and presentation
5               immune system process
6 glycosphingolipid metabolic process
```

- What do you notice? What can you conclude?

Longer definitions

- Even though the GO term definition is better than the GO ID, it is not sufficient.
- So lets take a look at the actual definitions using the GO.db package:

```
> GOTERM[["GO:0032945"]]
```

```
GOID: GO:0032945
Term: negative regulation of
    mononuclear cell proliferation
Ontology: BP
Definition: Any process that stops,
    prevents or reduces the
    frequency, rate or extent of
    mononuclear cell proliferation.
```

Longer definitions

```
Synonym: negative regulation of
    PBMC proliferation
Synonym: negative regulation of
    peripheral blood mononuclear
    cell proliferation
```

biomaRt

- Remember that you can use biomaRt to get GO IDs or to use them as a query and get more information on your genes / proteins.
- For instance, take a look at the getGo function.
- You can find GO IDs from `biomaRt` in PFAM, Prosite, and InterPro besides the usual, ENSEMBL.

SQL based packages

- Several packages, for example hgu133a and hgu95av2 were changed from being *environment* based to SQL based packages.
- They did this change to facilitate mapping between different identifiers.
- This was specially useful in cases where you have incomplete data.
- Plus it made everything faster :)

An example:

- Old way:

```
> goCats <- unlist(eapply(GOTERM,
+     Ontology))
> old <- table(goCats)[c("BP", "CC",
+     "MF")]
```

- New way WAY faster:

```
> query <- "select ontology from go_term"
> goCats <- dbGetQuery(GO_dbconn(),
+     query)
> new <- table(goCats)[c("BP", "CC",
+     "MF")]
```

- Comparing:

An example:

```
> identical(old, new)
[1] TRUE
```

Credits

- Bioconductor Case Studies by Florian Hahne, Wolfgang Huber, Robert Gentleman and Seth Falcon.
- Specially chapter 8.

Homework

- Choose a different EntrezGene ID (not 7013) that has different probes.
- Make a scatterplot compairing the expression values from two probe sets.
- Make the heatmap showing all the probe sets.
- Add your conclusions.

SessionInfo

```
> sessionInfo()

R version 2.9.0 (2009-04-17)
i386-pc-mingw32

locale:
LC_COLLATE=English_United States.1252;LC_CTYPE=English_United States.1252;LC_MO

attached base packages:
[1] stats     graphics  grDevices
[4] utils     datasets  methods
[7] base

other attached packages:
 [1] GOstats_2.10.0
 [2] graph_1.22.2
 [3] Category_2.10.1
 [4] lattice_0.17-22
 [5] annaffy_1.16.0
 [6] KEGG.db_2.2.11
```

SessionInfo

```
 [7] GO.db_2.2.11
 [8] annotate_1.22.0
 [9] hgu95av2.db_2.2.12
[10] RSQLite_0.7-1
[11] DBI_0.2-4
[12] AnnotationDbi_1.6.0
[13] genefilter_1.24.3
[14] ALL_1.4.5
[15] Biobase_2.4.1

loaded via a namespace (and not attached):
[1] grid_2.9.0      GSEABase_1.6.1
[3] RBGL_1.20.0     splines_2.9.0
[5] survival_2.35-4 tools_2.9.0
[7] XML_2.5-1       xtable_1.5-5
```