Lecture 12

BIOF 339

December 5, 2016

Bioconductor

Bioconductor

Bioconductor provides tools for the analysis and comprehension of high-throughput genomic data, using R.

- 1296 packages
- Covers the bioinformatic pipeline
- Software
- Annotation
- Experiments

Explore Bioconductor website

Installing Bioconductor packages

This is different from the usual `install.packages`

```
source('http://bioconductor.org/biocLite.R')
biocLite('Biobase','limma','hgu95av2.db')
```

Data in Bioconductor

The basic structure in a Bioconductor pipeline is the `ExpressionSet`

```
library(Biobase)
str(sample.ExpressionSet)
```

```
#  Formal class 'ExpressionSet' [package "Biobase"] with 7 slots
#    ..@ experimentData   :Formal class 'MIAME' [package "Biobase"] with 13 s
#    .. .. ..@ name             : chr "Pierre Fermat"
#    .. .. ..@ lab              : chr "Francis Galton Lab"
#    .. .. ..@ contact          : chr "pfermat@lab.not.exist"
#    .. .. ..@ title            : chr "Smoking-Cancer Experiment"
#    .. .. ..@ abstract         : chr "An example object of expression set (E
#    .. .. ..@ url              : chr "www.lab.not.exist"
#    .. .. ..@ pubMedIds        : chr ""
#    .. .. ..@ samples          : list()
#    .. .. ..@ hybridizations   : list()
#    .. .. ..@ normControls     : list()
#    .. .. ..@ preprocessing    : list()
```

Differences with usual R

Instead of storing data in named lists, ExpressionSet objects store data in slots, and we can see what the slots are with `slotNames`:

```
r slotNames(sample.ExpressionSet)

# [1] "experimentData" "assayData" "phenoData" # [4]
"featureData" "annotation" "protocolData" # [7]
".__classVersion__"
```

Differences with usual R

You can access these slots using `@`, instead of the usual `$`:

```
r sample.ExpressionSet@phenoData

# An object of class 'AnnotatedDataFrame' #
sampleNames: A B ... Z (26 total) # varLabels: sex
type score # varMetadata: labelDescription
```

Differences with usual R

However, it's much easier to go with the built-in functions

```
pData(sample.ExpressionSet)
```

```
#        sex    type score
#  A Female Control  0.75
#  B   Male    Case  0.40
#  C   Male Control  0.73
#  D   Male    Case  0.42
#  E Female    Case  0.93
#  F   Male Control  0.22
#  G   Male    Case  0.96
#  H   Male    Case  0.79
#  I Female    Case  0.37
#  J   Male Control  0.63
#  K   Male    Case  0.26
#  L Female Control  0.36
#  M   Male    Case  0.41
#  N   Male    Case  0.80
```

Differences with usual R

```
head(exprs(sample.ExpressionSet))
```

```
#                          A         B        C         D        E       F
#  AFFX-MurIL2_at  192.7420  85.75330 176.7570 135.5750 64.49390 76.3569
#  AFFX-MurIL10_at  97.1370 126.19600  77.9216  93.3713 24.39860 85.5088
#  AFFX-MurIL4_at   45.8192   8.83135  33.0632  28.7072  5.94492 28.2925
#  AFFX-MurFAS_at   22.5445   3.60093  14.6883  12.3397 36.86630 11.2568
#  AFFX-BioB-5_at   96.7875  30.43800  46.1271  70.9319 56.17440 42.6756
#  AFFX-BioB-M_at   89.0730  25.84610  57.2033  69.9766 49.58220 26.1262
#                         G       H       I         J        K       L
#  AFFX-MurIL2_at  160.5050 65.9631 56.9039 135.60800 63.44320 78.2126
#  AFFX-MurIL10_at  98.9086 81.6932 97.8015  90.48380 70.57330 94.5418
#  AFFX-MurIL4_at   30.9694 14.7923 14.2399  34.48740 20.35210 14.1554
#  AFFX-MurFAS_at   23.0034 16.2134 12.0375   4.54978  8.51782 27.2852
#  AFFX-BioB-5_at   86.5156 30.7927 19.7183  46.35200 39.13260 41.7698
#  AFFX-BioB-M_at   75.0083 42.3352 41.1207  91.53070 39.91360 49.8397
#                        M       N       O       P        Q        R
#  AFFX-MurIL2_at  83.0943 89.3372 91.0615 95.9377 179.8450 152.4670
#  AFFX-MurIL10_at 75.3455 68.5827 87.4050 84.4581  87.6806 108.0320
```

Making a heatmap

Heatmaps

There are several ways of doing heatmaps in R:

- http://sebastianraschka.com/Articles/heatmaps_in_r.html
- https://plot.ly/r/heatmaps/
- http://moderndata.plot.ly/interactive-heat-maps-for-r/
- http://www.siliconcreek.net/r/simple-heatmap-in-r-with-ggplot2
- https://rud.is/b/2016/02/14/making-faceted-heatmaps-with-ggplot2/

Some example data

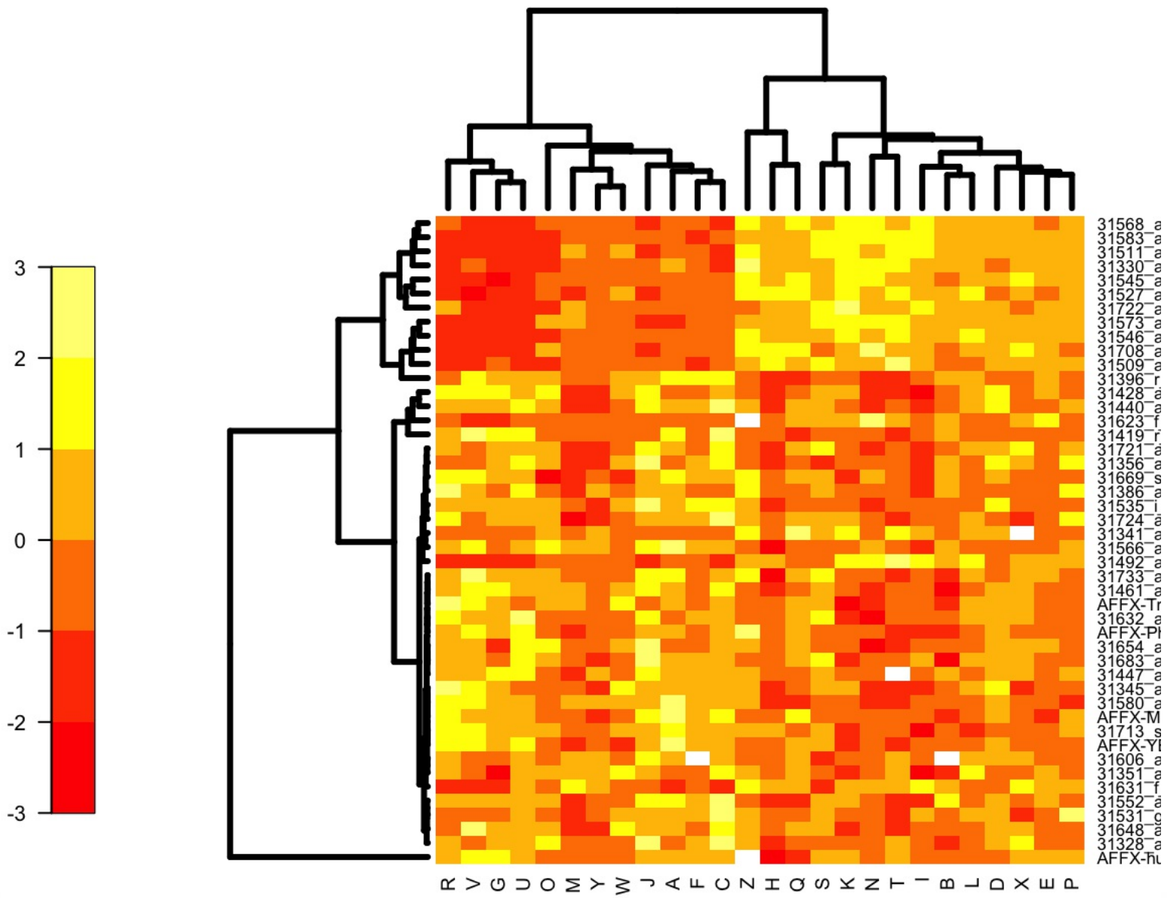
```
library(Biobase)
data(sample.ExpressionSet)
exdat <-  sample.ExpressionSet
library(limma)
design1 <- model.matrix(~type, data=pData(exdat))
lm1 <- lmFit(exprs(exdat), design1)
lm1 <- eBayes(lm1) # compute linear model for each probeset
geneID <- rownames(topTable(lm1, coef=2, num=100, adjust='none',p.value=0.05
exdat2 <- exdat[geneID,] # Keep features with p-values < 0.05
exdat2
```

```
#  ExpressionSet (storageMode: lockedEnvironment)
#  assayData: 46 features, 26 samples
#    element names: exprs, se.exprs
#  protocolData: none
#  phenoData
#    sampleNames: A B ... Z (26 total)
#    varLabels: sex type score
#    varMetadata: labelDescription
```

Heatmaps using `Heatplus`

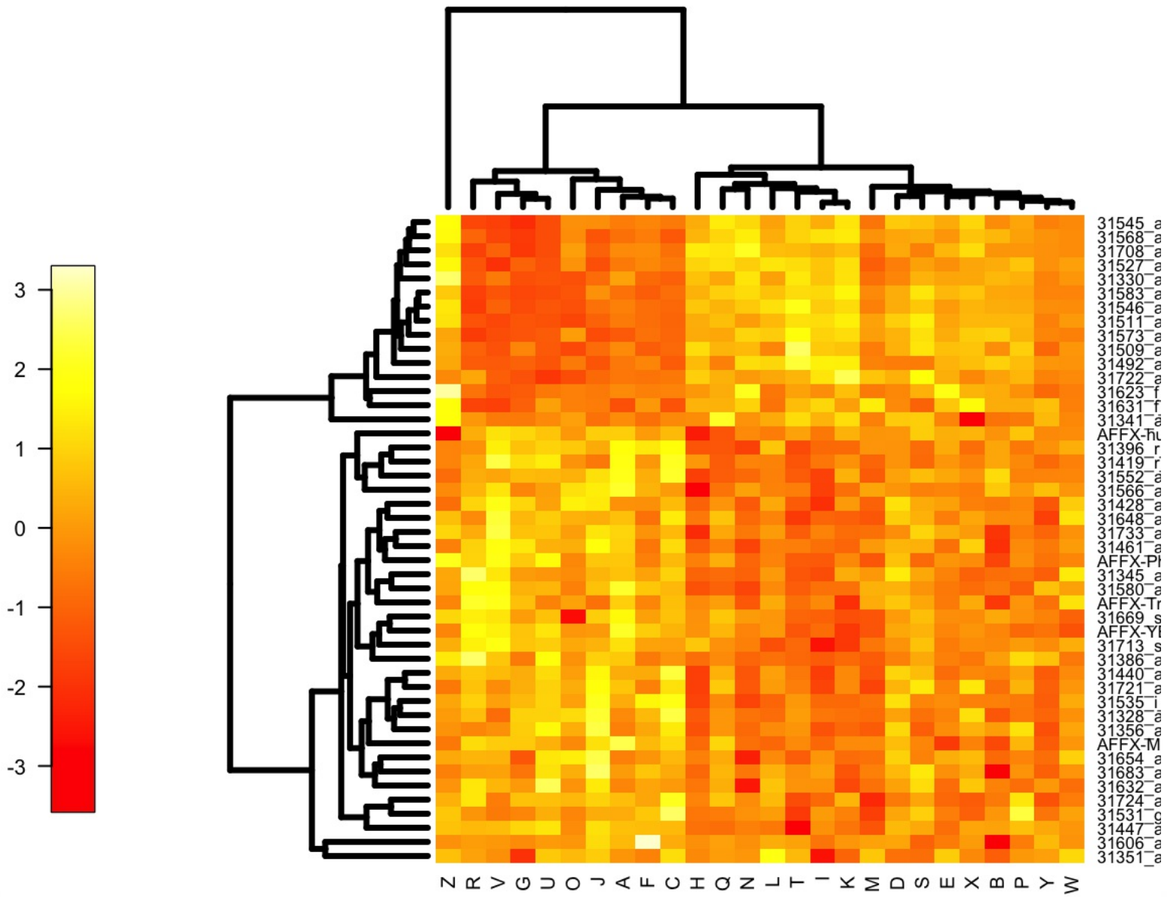
```
source('http://bioconductor.org/biocLite.R')
biocLite('Heatplus')
```

```
library(Heatplus)
reg1 <- regHeatmap(exprs(exdat2), legend=2, col=heat.colors,
                   breaks=-3:3)
plot(reg1)
```

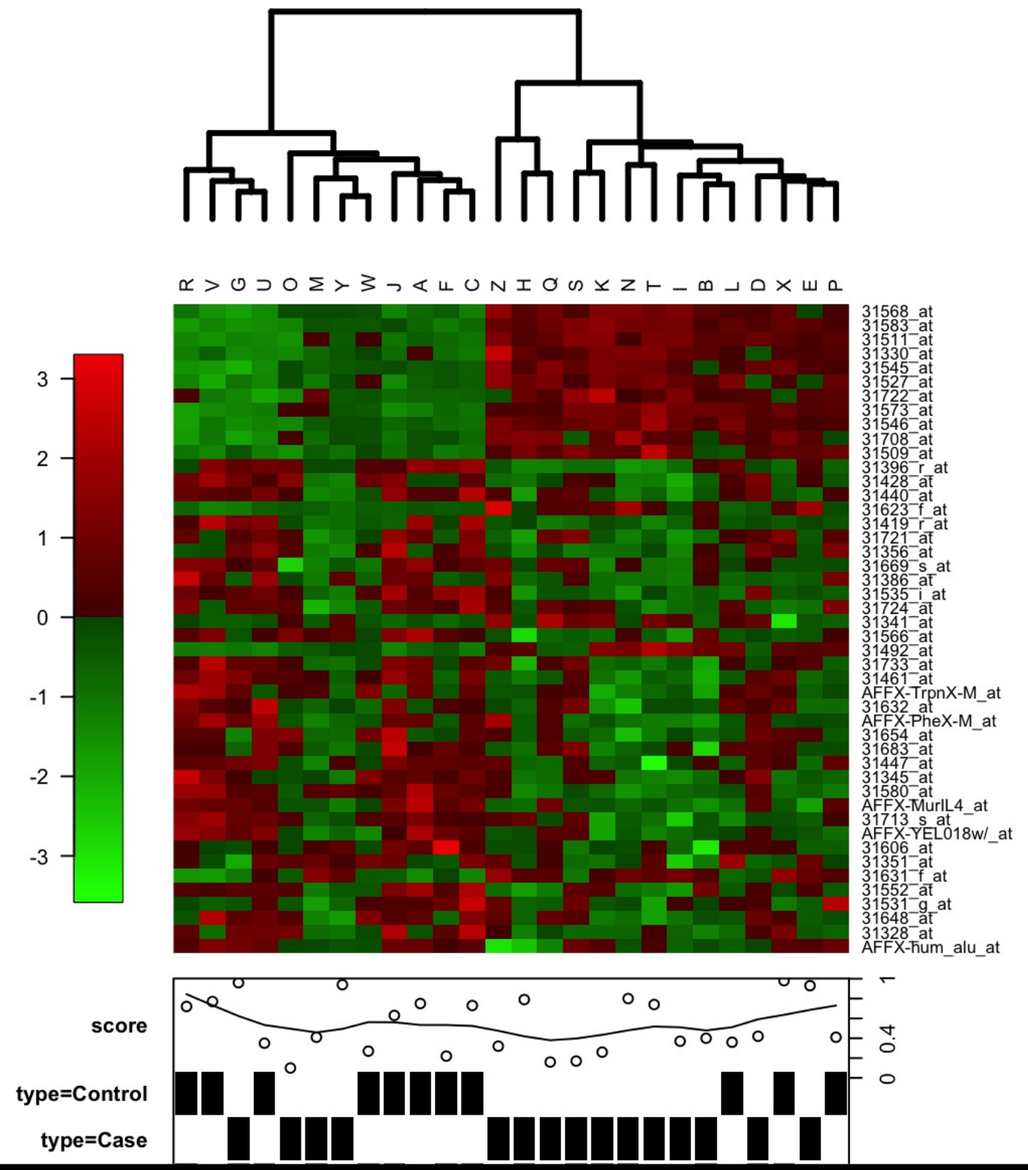

3
2
1
0
-1
-2
-3
R V G U O M Y W J A F C N H Q S K N T I B L D X E P

```
library(Heatplus)
reg1 <- regHeatmap(exprs(exdat2), legend=2, col=heat.colors,
                   breaks=-3:3)
plot(reg1)
```

```
corrdist <- function(x) as.dist(1-cor(x))
hclust.avl <-  function(x) hclust(x, method='average')
reg2 <- regHeatmap(exprs(exdat2), legend=2, col=heat.colors,
                   breaks=-3:3,
                   dendrogram = list(clustfun=hclust.avl, distfun=corrdist)
plot(reg2)
```

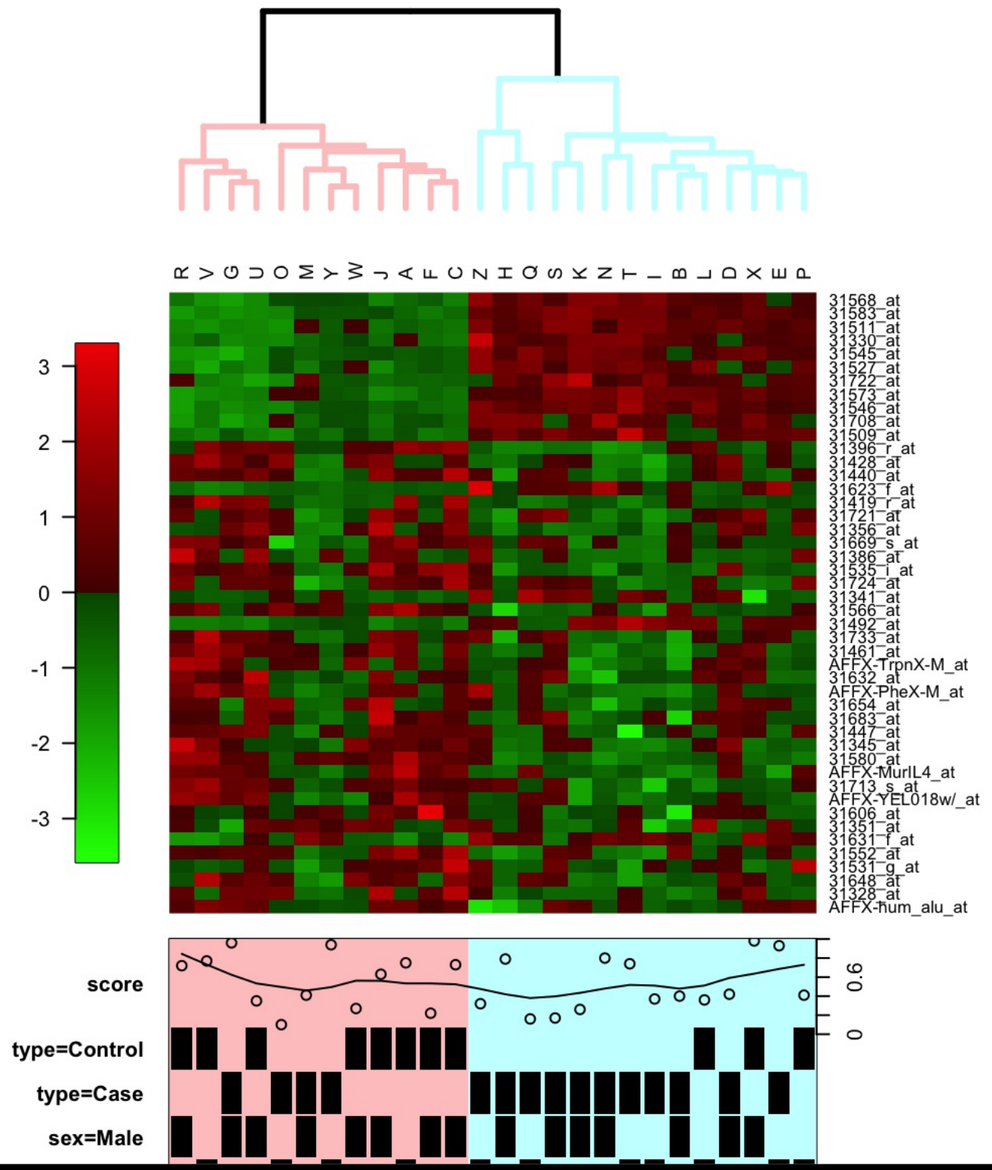

3
2
1
0
-1
-2
-3
Z R V G U O J A F C H Q N L T I K M D S E X B P Y W

```
ann1 <- annHeatmap(exprs(exdat2), ann=pData(exdat2))
plot(ann1)
```


R V G U O M Y W J A F C N H Q S K N T L B L D X E P
3
2
1
0
-1
-2
-3
31568_at
31583_at
31511_at
31330_at
31545_at
31527_at
31722_at
31573_at
31546_at
31708_at
31509_at
31396_r_at
31428_at
31440_at
31623_f_at
31419_r_at
31721_at
31356_at
31669_s_at
31386_at
31535_i_at
31724_at
31341_at
31566_at
31492_at
31733_at
31461_at
AFFX-TrpnX-M_at
31632_at
AFFX-PheX-M_at
31654_at
31683_at
31447_at
31345_at
31580_at
AFFX-MurIL4_at
31713_s_at
AFFX-YEL018w/_at
31606_at
31351_at
31631_f_at
31552_at
31531_g_at
31648_at
31328_at
AFFX-hum_alu_at
score
type=Control
type=Case
0
0.4
1

```
ann1 <- annHeatmap(exprs(exdat2), ann=pData(exdat2))
plot(ann1)
```

```
ann2 <- annHeatmap(exprs(exdat2), ann=pData(exdat2),
                   cluster = list(cuth=7500,
                                  label=c('Control-like','Case-like')))
plot(ann3)
```


R V G U O M Y W J A F C Z H Q S K N T I B L D X E P
3
2
1
0
-1
-2
-3
31568_at
31583_at
31511_at
31330_at
31545_at
31527_at
31722_at
31573_at
31546_at
31708_at
31509_at
31396_r_at
31428_at
31440_at
31623_f_at
31419_r_at
31721_at
31356_at
31669_s_at
31386_at
31535_i_at
31724_at
31341_at
31566_at
31492_at
31733_at
31461_at
AFFX-TrpnX-M_at
31632_at
AFFX-PheX-M_at
31654_at
31683_at
31447_at
31345_at
31580_at
AFFX-MurIL4_at
31713_s_at
AFFX-YEL018w/_at
31606_at
31351_at
31631_f_at
31552_at
31531_g_at
31648_at
31328_at
AFFX-hum_alu_at
score
type=Control
type=Case
sex=Male
0.6
0