Analysis of NimbleGen Expression Data with the oligo Package

Benilton Carvalho

August 20, 2009

1 Introduction

This document presents a non-trivial use of the oligo Package for the analysis of NimbleGen Expression data. This vignette follows the structure of the chapter **From CEL files to a list of interesting genes** by R. A. Irizarry *in Bioinformatics and Computational Biology Solutions Using R and Bioconductor*, which shows a case study for Affymetrix Expression arrays.

In order to analyze microarray data using oligo, the user is expected to have installed on the system a package with the annotation for the particular array design on which the experiment was performed. For the example in question here, the design is hg18_60mer_expr and the annotation package associated to it is pd.hg18.60mer.expr, which is built by using the pdInfoBuilder package.

2 Initialization of the environment

We start by loading the packages that are going to be used in this session. The maqcExpression4plex package provides a set of six samples on the MAQC Study; the set is comprised of samples on two groups: universal reference and brain. The remaining packages offer additional functionality, like tools for filtering, plotting and visualization.

```
R> library(oligo)
R> library(maqcExpression4plex)
R> library(genefilter)
R> library(limma)
R> library(RColorBrewer)
R> palette(brewer.pal(8, "Dark2"))
```

Once the package is loaded, we can easily get the location of the XYS files that contain the intensities by calling `list.xysfiles`, which takes the same arguments as `list.files`. To minimize the chance of problems, we strongly recommend the use of `full.names=TRUE`.

```
R> extdata <- system.file("extdata",
     package = "maqcExpression4plex")
R> xys.files <- list.xysfiles(extdata,
     full.names = TRUE)
R> basename(xys.files)
```

```
[1] "9868701_532.xys" "9868901_532.xys"
[3] "9869001_532.xys" "9870301_532.xys"
[5] "9870401_532.xys" "9870601_532.xys"
```

To read the XYS files, we provide the `read.xysfiles` function, which also takes `phenoData`, `experimentData` and `featureData` objects and returns an appropriate subclass of *FeatureSet*.

```
R> pd <- dir(extdata, pattern = "phenoData",
     full.names = TRUE)
R> pd <- read.AnnotatedDataFrame(pd)
R> maqc <- read.xysfiles(xys.files, phenoData = pd)
R> class(maqc)
```

```
[1] "ExpressionFeatureSet"
attr(,"package")
[1] "oligoClasses"
```

3 Exploring the feature-level data

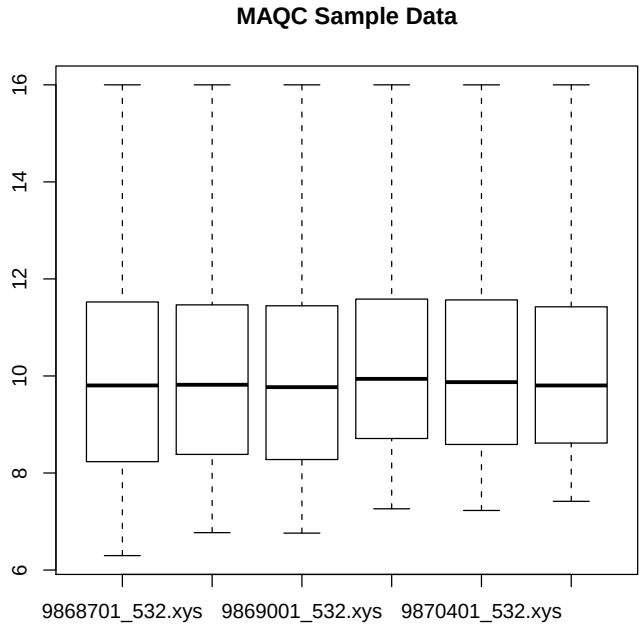
The `read.xysfiles` function returns, in this case, an instance of *ExpressionFeatureSet* and the intensities of these files are stored in its `exprs` slot, which can be accessed with a method with the same name.

```
R> exprs(maqc)[10001:10010, 1:2]
```

```
      9868701_532.xys 9868901_532.xys
10001             735             742
10002            4786            4435
10003           25600           26155
10004            1079            1093
10005            3056            3128
10006             310             385
10007              NA              NA
10008              NA              NA
10009             599             713
10010           28712           29795
```

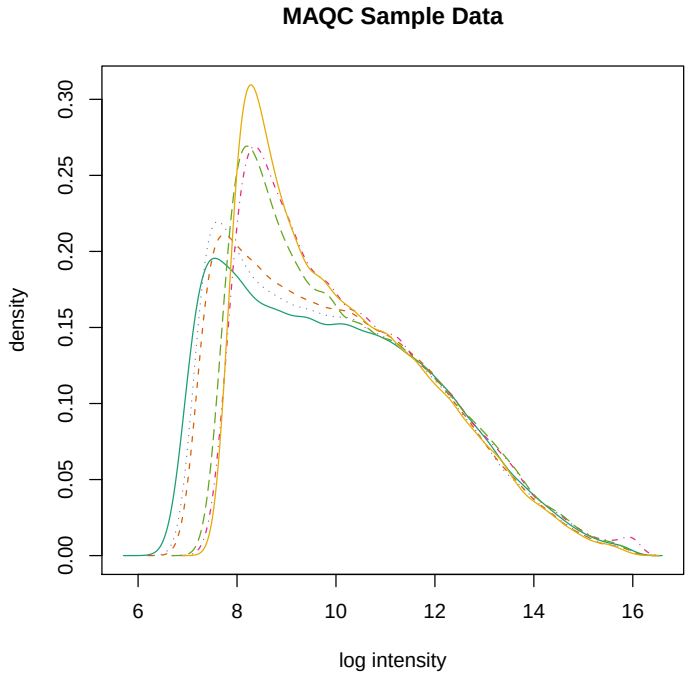
The `boxplot` method can be used to produce boxplots for the feature-level data.

```
R> boxplot(maqc, main = "MAQC Sample Data")
```



Similarly, a smoothed histogram for the feature-level data can be obtained with the `hist` method.

```
R> hist(maqc, main = "MAQC Sample Data")
```



4 RMA algorithm

The RMA algorithm can be applied to the raw data of expression arrays. It is available via the `rma` method. The algorithm will perform background subtraction, quantile normalization and summarization via median polish. The result of `rma` is an instance of *ExpressionSet* class, which also contains an `exprs` slot and method.

```
R> eset <- rma(maqc)

Background correcting
Normalizing
Calculating Expression

R> class(eset)

[1] "ExpressionSet"
attr(,"package")
[1] "Biobase"

R> show(eset)
```

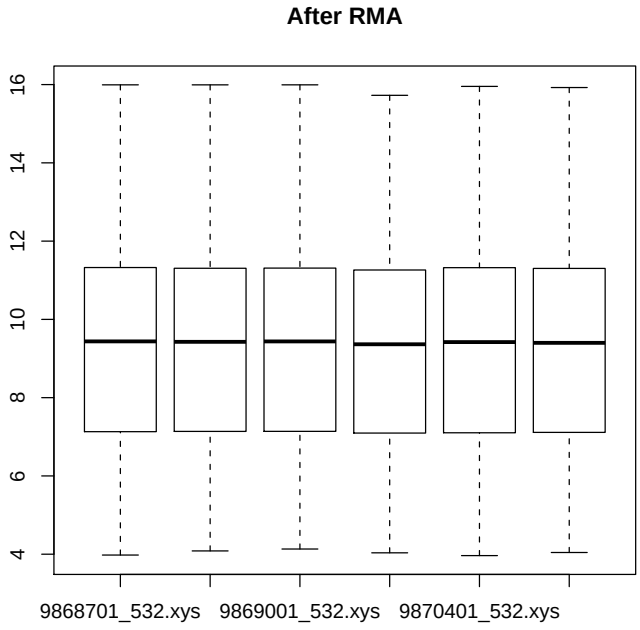
```
ExpressionSet (storageMode: lockedEnvironment)
assayData: 24000 features, 6 samples
  element names: exprs
phenoData
  sampleNames: 9868701_532.xys, 9868901_532.x
  ys, ..., 9870601_532.xys  (6 total)
  varLabels and varMetadata description:
    Key:
  additional varMetadata: channel
featureData
  featureNames: NM_000014, NM_000015, ..., XM
  _928211  (24000 total)
  fvarLabels and fvarMetadata description: none
experimentData: use 'experimentData(object)'
Annotation: pd.hg18.60mer.expr
```

```
R> exprs(eset)[1:10, 1:2]
```

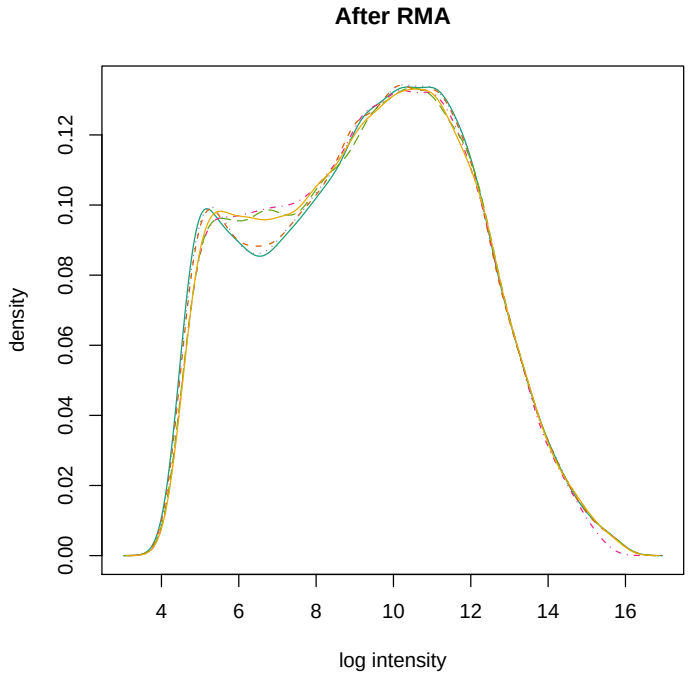
```
          9868701_532.xys 9868901_532.xys
NM_000014            12.3            12.3
NM_000015             4.5             4.6
NM_000016            12.4            12.2
NM_000017             8.5             8.5
NM_000018            12.6            12.4
NM_000019            11.7            11.6
NM_000020             8.9             9.2
NM_000021            11.8            11.8
NM_000022             8.9             8.4
NM_000023             8.9             9.1
```

The `boxplot` and `hist` methods are also implemented for *ExpressionSet* objects. Note that `rma`'s output is in the $\log_2$ scale, so we call such methods using the argument `transfo=identity`, so the data are not transformed in any way.

```
R> boxplot(eset, transfo = identity,
     main = "After RMA")
```




```
R> hist(eset, transfo = identity, main = "After RMA")
```



5 Assessing differential expression

One simple approach to assess differential expression is to flag units with log-ratios greater (in absolute value) than 1, i.e. a change greater than 2-fold when comparing brain vs. universal reference.

```
R> e <- exprs(eset)
R> index <- which(eset[["Key"]] == "brain")
R> d <- rowMeans(e[, index]) - rowMeans(e[,
     -index])
R> a <- rowMeans(e)
R> sum(abs(d) > 1)
```

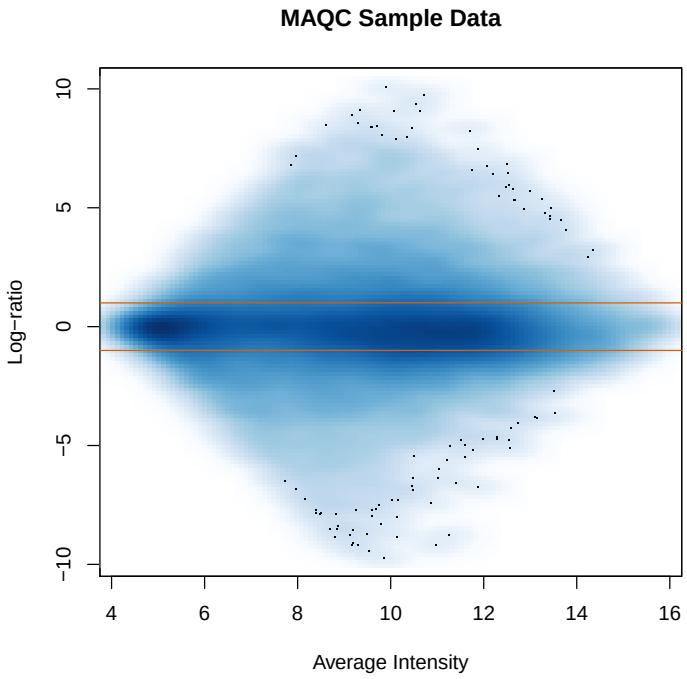
```
[1] 10043
```

Another approach is to use t-tests to infer whether or not there is differential expression.

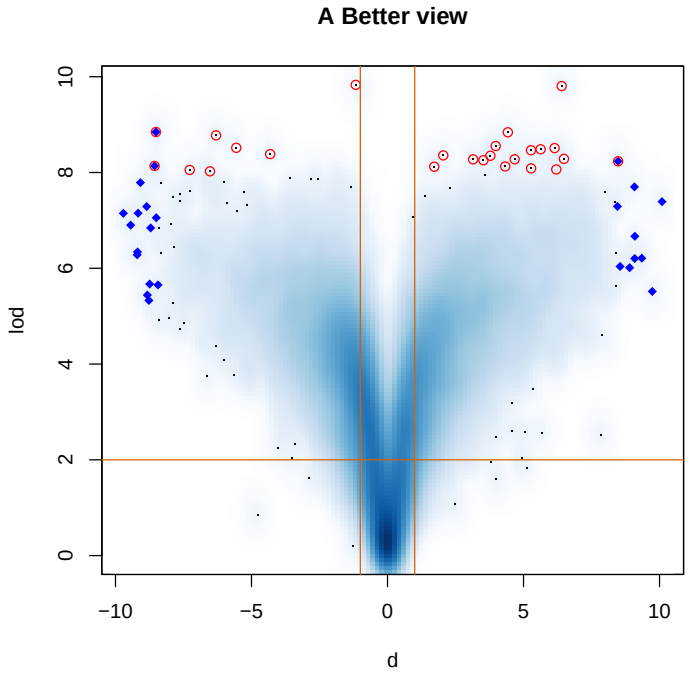
```
R> tt <- rowttests(e, factor(eset[["Key"]]))
R> lod <- -log10(tt[["p.value"]])
```

The MA plot can be used to visualize the behavior of the log-ratio as a function of average log-intensity. Features with log-ratios greater (in absolute value) than 1 are candidates for being classified as differentially expressed.

```
R> smoothScatter(a, d, xlab = "Average Intensity",
     ylab = "Log-ratio", main = "MAQC Sample Data")
R> abline(h = c(-1, 1), col = 2)
```



The use of t-tests allows us to use the volcano plot to visualize candidates for differential expression. Below, we highlight, in blue, the top 25 in log-ratio and,



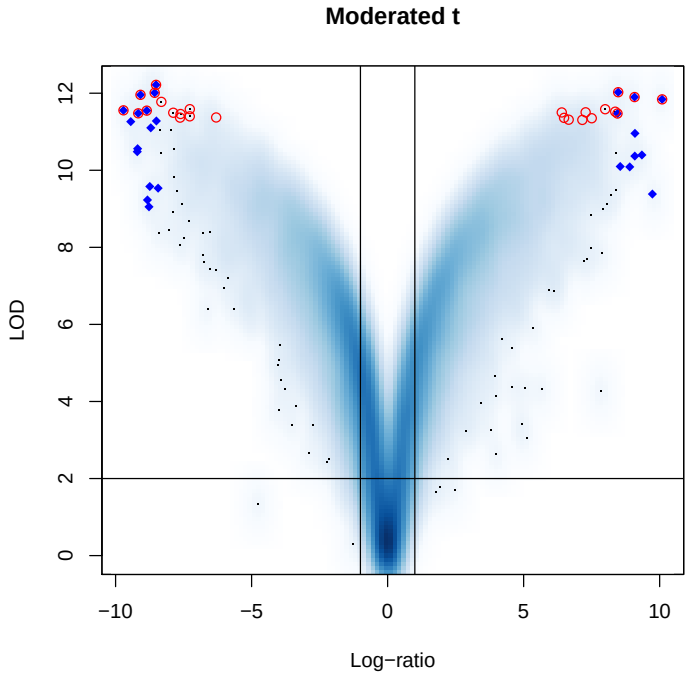
in red, the top 25 in effect size.

The limma Package can also be used to assess difference in expression between the two groups.

```
R> design <- model.matrix(~factor(eset[["Key"]]))
R> fit <- lmFit(eset, design)
R> ebayes <- eBayes(fit)
R> lod <- -log10(ebayes[["p.value"]][,
     2])
R> mtstat <- ebayes[["t"]][, 2]
```

The Empirical Bayes approach implemented in limma provides moderated t-statistic, shown to have a better performance when compared to the standard t-statistic. Below, we reconstruct the volcano plot, but using the moderated t-statisic.

```
R> o1 <- order(abs(d), decreasing = TRUE)[1:25]
R> o2 <- order(abs(mtstat), decreasing = TRUE)[1:25]
R> o <- union(o1, o2)
R> smoothScatter(d, lod, main = "Moderated t",
     xlab = "Log-ratio", ylab = "LOD")
R> points(d[o1], lod[o1], pch = 18, col = "blue")
R> points(d[o2], lod[o2], pch = 1, col = "red")
R> abline(h = 2, v = c(-1, 1))
```



The `topTable` command provides us a way of ranking genes for further evaluation. In the case below, we adjust for multiple testing by FDR and look at the Top-10 genes.

```
R> tab <- topTable(ebayes, coef = 2,
     adjust = "fdr", n = 10)
R> tab
```

```
             ID logFC AveExpr    t P.Value
13761 NM_021871   8.5     8.7  118 6.1e-13
746   NM_000806  -8.5     8.6 -111 9.4e-13
169   NM_000184   8.6     9.2  111 9.8e-13
13760 NM_021870   9.1     9.2  109 1.1e-12
10465 NM_014841  -9.1    10.1 -107 1.3e-12
7467  NM_005277 -10.1     9.9 -105 1.4e-12
3286  NM_001034   8.3     8.9  103 1.7e-12
4919  NM_002421   7.3     8.4   96 2.6e-12
9238  NM_007325  -8.0     9.1  -96 2.6e-12
4201  NM_001622   9.7     9.9   96 2.8e-12
      adj.P.Val  B
13761   3.8e-09 19
746     3.8e-09 19
169     3.8e-09 19
```

```
13760   3.8e-09 19
10465   3.8e-09 19
7467    3.8e-09 19
3286    3.8e-09 18
4919    3.8e-09 18
9238    3.8e-09 18
4201    3.8e-09 18
```

6 Session Info

This document was created using the following:

```
R> sessionInfo()

R version 2.9.1 (2009-06-26)
i386-pc-mingw32

locale:
LC_COLLATE=English_United States.1252;LC_CTYPE=English_United States.1252;LC_MONETARY=Englis

attached base packages:
[1] tools     stats     graphics  grDevices
[5] utils     datasets  methods   base

other attached packages:
 [1] pd.hg18.60mer.expr_2.4.1
 [2] RColorBrewer_1.0-2
 [3] limma_2.18.2
 [4] genefilter_1.24.2
 [5] maqcExpression4plex_1.2
 [6] pd.mapping50k.xba240_0.4.1
 [7] RSQLite_0.7-2
 [8] DBI_0.2-4
 [9] hapmap100kxba_1.3.2
[10] oligo_1.8.3
[11] preprocessCore_1.6.0
[12] oligoClasses_1.6.0
[13] Biobase_2.4.1

loaded via a namespace (and not attached):
 [1] affxparser_1.16.0   affyio_1.12.0
 [3] annotate_1.22.0     AnnotationDbi_1.6.1
 [5] Biostrings_2.12.8   IRanges_1.2.3
 [7] KernSmooth_2.23-2   splines_2.9.1
 [9] survival_2.35-4     xtable_1.5-5
```