Processing Affymetrix Expression Data

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Lab Structure

- Interactive walkthrough of Chapter 3: Processing Affymetrix Expression Data from the book *Bioconductor Case Studies* by Hahne et al.
- Students encouraged to run R during lab and submit commands as we go because there will be exercises along the way.

Outline

1 Background

2 The input data: CEL files

3 Quality Assessment

4 Preprocessing

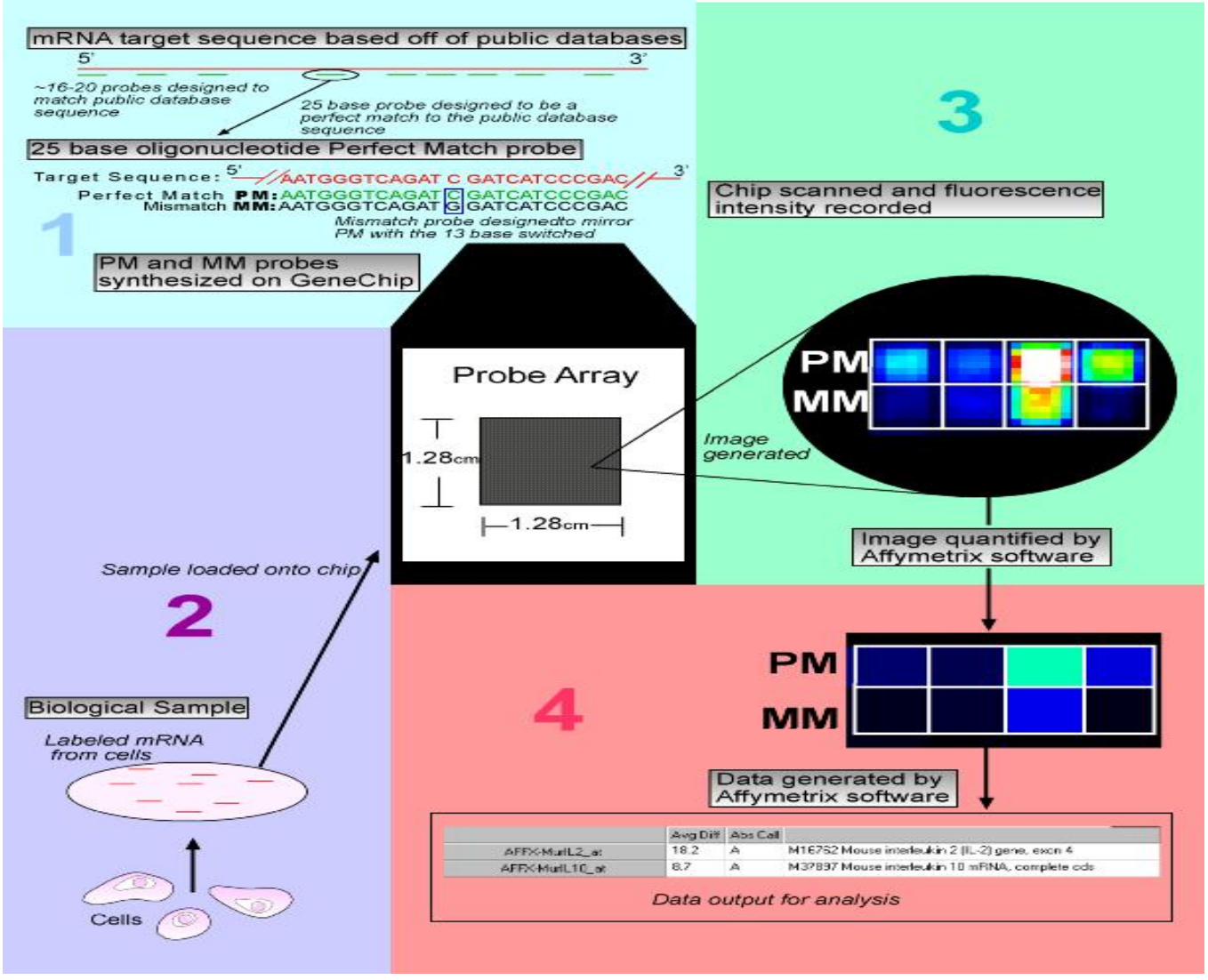
5 Ranking and filtering probe sets

6 Advanced preprocessing

Bioconductor Packages Covered

- **affy** - Methods for Affymetrix oligonucleotide arrays
- **CLL** - Chronic Lymphocytic Leukemia gene expression data
- **simpleaffy** - Simple high-level analysis of Affymetrix data
- **genefilter** - Methods for filtering genes from microarray experiments
- **affyPLM** - Methods for fitting probe-level models
- **limma** - Linear models for microarray data
- **annotate** - Annotation for microarrays
- **annaffy** - Annotation tools for Affymetrix biological metadata
- **hgu95av2.db** - Affymetrix Human Genome U95 Set annotation data (chip hgu95av2)
- **KEGG.db** - A set of annotation maps for KEGG
- **geneplotter** - Graphics related functions for Bioconductor
- **vsn** - Variance stabilization and calibration for microarray data

Affymetrix GeneChip® Microarray Overview



Affymetrix GeneChip®Microarray Terminology

- Each gene or portion of a gene is represented by 1 to 20 oligonucleotides of 25 base-pairs.
- **Probe**: an oligonucleotide of 25 base-pairs, i.e., a 25-mer.
- **Perfect match** (**PM**): A 25-mer complementary to a reference sequence of interest (e.g., part of a gene).
- **Mismatch** (**MM**): same as PM but with a single base change for the middle (13th) base (transversion purine <-> pyrimidine, G <->C, A <->T). Used to measure non-specific binding and background noise.
- **Probe-pair**: a (PM,MM) pair.
- **Probe-pair set**: a collection of probe-pairs (1 to 20) related to a common gene or fraction of a gene.
- **Affy ID**: an identifier for a probe-pair set.

Affymetrix GeneChip®Files

- **DAT** file: Image file, 10^7 pixels, 50 MB.
- **CEL** file: Cell intensity file, probe level PM and MM values.
- **CDF** file: Chip Description File. Describes which probes go in which probe sets and the location of probe-pair sets (genes, gene fragments, ESTs).

Expression Measures

- 10-20K genes represented by 11-20 pairs of probe intensities (PM & MM).
- Obtain expression measure for each gene on each array by summarizing these pairs.
- Background adjustment and normalization are important issues.
- There are many methods.

Importing Affymetrix GeneChip®Data ...

- Affymetrix GeneChip®CEL files are imported using `ReadAffy` from the `affy` package.

```
> library("affy")
> myAB1 <- ReadAffy()
> myAB2 <- ReadAffy(filenames = c("a1.cel",
+     "a2.cel", "a3.cel"))
```

- By default, all the CEL files from the current working directory (CWD) are imported. CWD can be
  - found using `getwd` and
  - changed using `setwd`.
- Alternatively, the `filenames` argument can be supplied.
- `list.celfiles` can be used to select the list CEL file in the directory.

... Into *AffyBatch* Objects

- Affymetrix GeneChip® probe-level data are stored in *AffyBatch* objects.
- For more information on this class see `help("AffyBatch-class")`.
- We will use pre-imported data from the **CLL** package.

Example Data Set

- Chronic Lymphocytic Leukemia Gene Expression Data
- 24 samples run on HG-U95Av2 Affymetrix GeneChip®arrays
- Large number of clinical measures collected, but we'll use only one.

```
> library("CLL")
> data("CLLbatch")
> CLLbatch
```

```
AffyBatch object
size of arrays=640x640 features (91212 kb)
cdf=HG_U95Av2 (12625 affyids)
number of samples=24
number of genes=12625
annotation=hgu95av2
notes=
```

CLL Sample Information

- The `sampleNames` function extracts the name of the samples.
- The `sampleNames<-` replacement function overwrites the existing names.

```
> head(sampleNames(CLLbatch))

[1] "CLL10.CEL" "CLL11.CEL" "CLL12.CEL"
[4] "CLL13.CEL" "CLL14.CEL" "CLL15.CEL"

> sampleNames(CLLbatch) <- sub("\\.CEL$",
+     "", sampleNames(CLLbatch))
> head(sampleNames(CLLbatch))

[1] "CLL10" "CLL11" "CLL12" "CLL13" "CLL14"
[6] "CLL15"
```

CLL Disease State

```
> data("disease")
> head(disease)

  SampleID  Disease
1    CLL10     <NA>
2    CLL11 progres.
3    CLL12   stable
4    CLL13 progres.
5    CLL14 progres.
6    CLL15 progres.

> table(disease$Disease, useNA = "always")

progres.   stable     <NA>
      14        9        1

> rownames(disease) <- disease$SampleID
```

Managing Phenotypic Data

- Phenotypic data are stored in *AnnotatedDataFrame* objects.
- These are essentially *data.frame* objects with metadata.
- They can be created from other R objects using `new` or imported from a file using `read.AnnotatedDataFrame`.
- The `phenoData` and `phenoData<-` functions get and set the phenotypic data in an *AffyBatch* object.

```
> mt <- match(rownames(disease), sampleNames(CLLbatch))
> vmd <- data.frame(labelDescription = c("Sample ID",
+     "Disease status: progressive or stable disease"))
> phenoData(CLLbatch) <- new("AnnotatedDataFrame",
+     data = disease[mt, ], varMetadata = vmd)
```

Sample Removal

- Phenotypic data can be used to filter *AffyBatch* objects.
- In this case we will remove the sample with an unknown disease state.

```
> CLLbatch <- CLLbatch[, !is.na(CLLbatch$Disease)]
```

QA/QC

- **Quality Assessment**: computation and interpretation of metrics that are intended to measure quality.
- **Quality Control**: possible subsequent actions, such as removing data from bad arrays or re-doing parts of an experiment.

Affymetrix Quality Assessment Metrics

- Average Background: the average of the background values.
- Scale Factor: The constant i which is the ratio of the trimmed mean for array i to the trimmed mean of the reference array.
- Percent Present: the percentage of spots that are present according to Affymetrix detection algorithm.
- $3'/5'$ ratios: for different quality control probe sets, such as Actin and GAPDH, each represented by 3 probesets, one from the $5'$ end, one from the middle and one from the $3'$ end of the targeted transcript. The ratio of the $3'$ expression to the $5'$ expression for these genes serves as a measure of RNA quality.

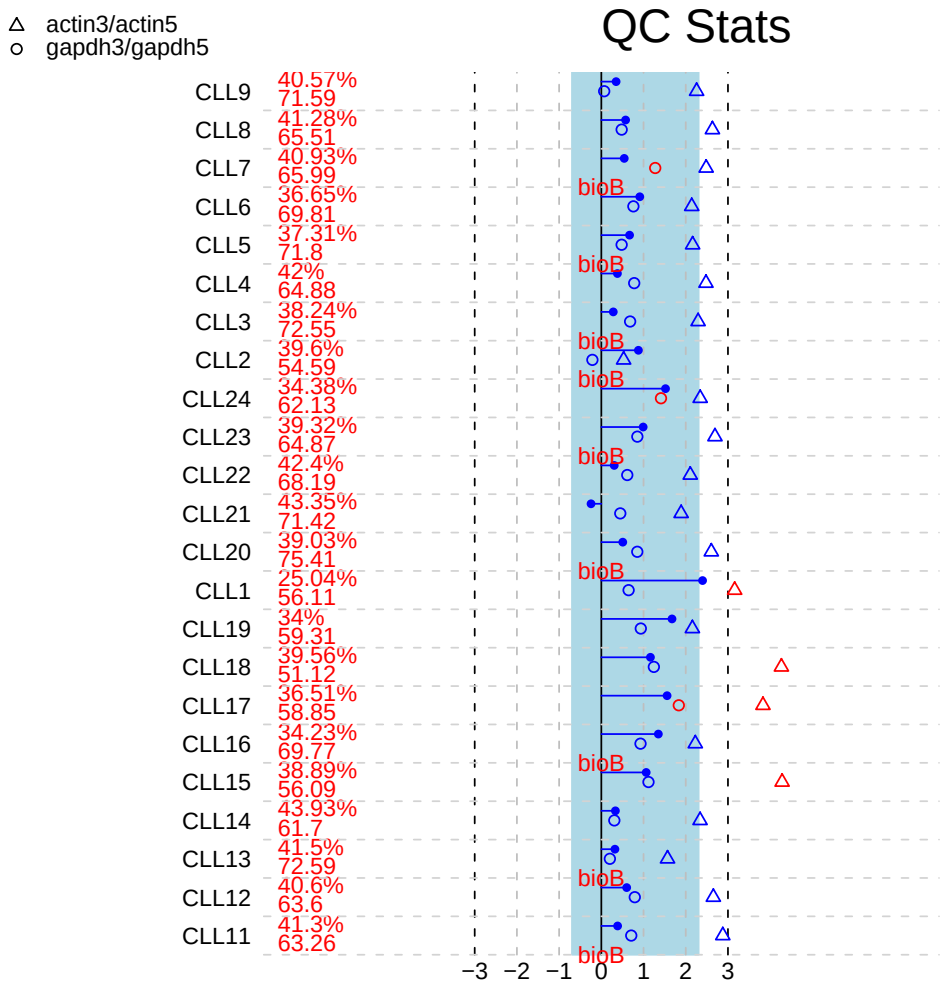
Recommended QA/QC Packages

- The **simpleaffy** package computes a variety of statistics for QA/QC.
- The **affyPLM** package contains methods for fitting probe-level models.
- The **arrayQualityMetrics** and **affyQCReport** packages contain recommended functionality for generating comprehensive QA reports.

```
> library("simpleaffy")
> saqc <- qc(CLLbatch)
```

Quality Control Plot

```
> plot(saqc)
```



Clustering Arrays by Expression Data

- Microarrays can be clustered based on expression profiles to determine outlining samples.
- **genefilter**'s `dist2` calculates pairwise distances that can be fed into cluster analysis software.
  - By default, `dist2` calculates the mean of the absolute differences between pairs of samples.

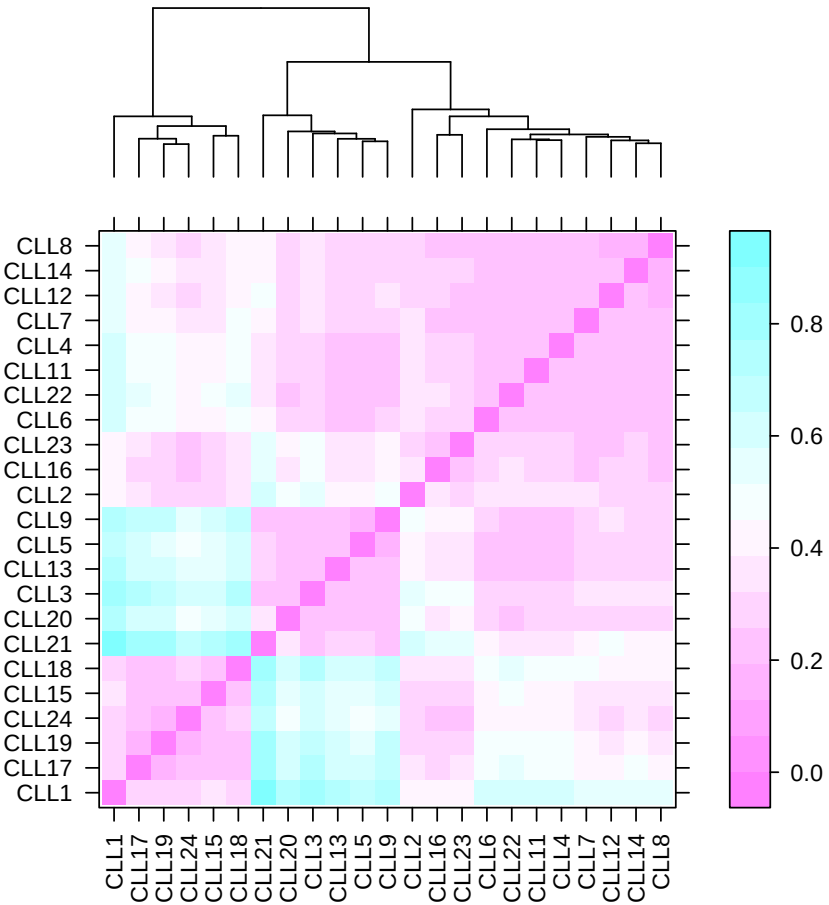
```
> library("genefilter")
> dd <- dist2(log2(exprs(CLLbatch)))
```

Between-Array Distance Plot (1/2)

```
> diag(dd) <- 0
> dd.row <- as.dendrogram(hclust(as.dist(dd)))
> row.ord <- order.dendrogram(dd.row)
> library("latticeExtra")
> legend <- list(top = list(fun = dendrogramGrob,
+     args = list(x = dd.row, side = "top")))
> lp <- levelplot(dd[row.ord, row.ord],
+     scales = list(x = list(rot = 90)),
+     xlab = "", ylab = "", legend = legend)
```

Between-Array Distance Plot (2/2)

```
> plot(lp)
```



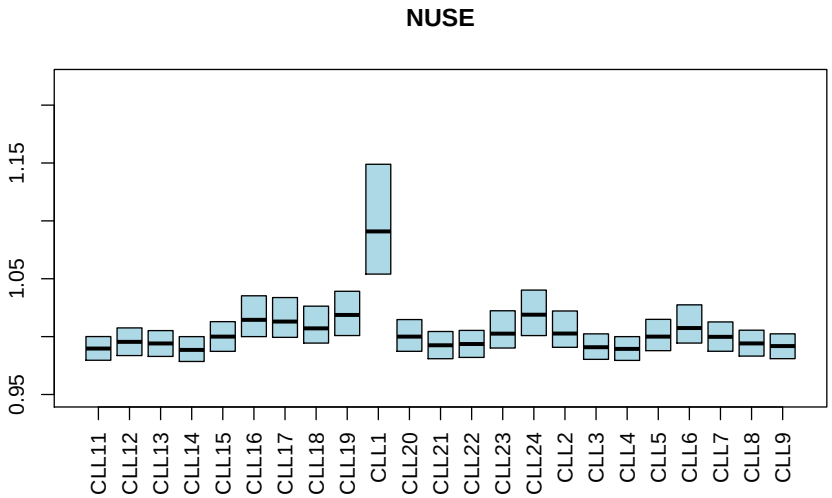
QA/QC Through Probe-Level Modeling

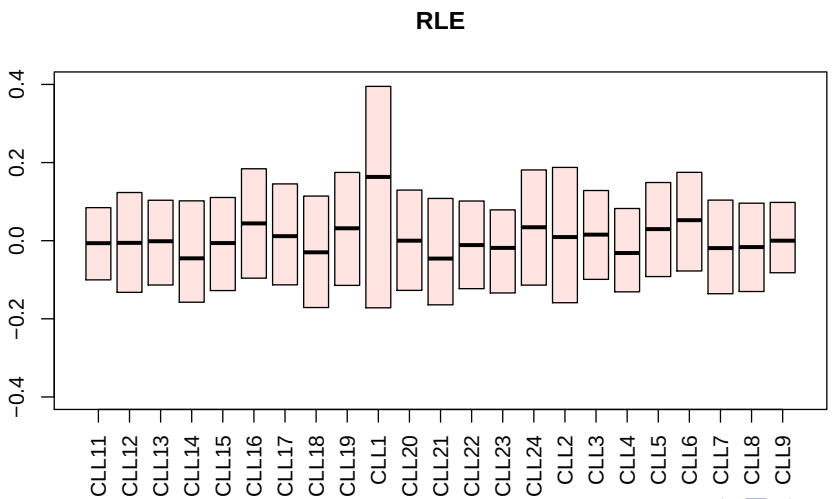
- The **affyPLM** package provides another set of QA/QC diagnostic measures:
  - Relative Log Expression (RLE) - For each gene, the across array median log expression is subtracted from each individual measurement.
  - Normalize Unscaled Standard Error (NUSE) - see documentation for mathematical definition.
- For both these measures, boxplots are used to highlight aberrant samples.

Probe-Level Diagnostics (1/3)

```
> library("affyPLM")
> dataPLM <- fitPLM(CLLbatch)

> boxplot(dataPLM, main = "NUSE", ylim = c(0.95,
+     1.22), outline = FALSE, col = "lightblue",
+     las = 3, whisklty = 0, staplelty = 0)

> Mbox(dataPLM, main = "RLE", ylim = c(-0.4,
+     0.4), outline = FALSE, col = "mistyrose",
+     las = 3, whisklty = 0, staplelty = 0)
```

Probe-Level Diagnostics (2/3)





Probe-Level Diagnostics (3/3)

- Both diagnostic boxplots indicate that array CLL1 is problematic.
- We drop it from our further analysis.

```
> badArray <- match("CLL1", sampleNames(CLLbatch))
> CLLB <- CLLbatch[, -badArray]
```

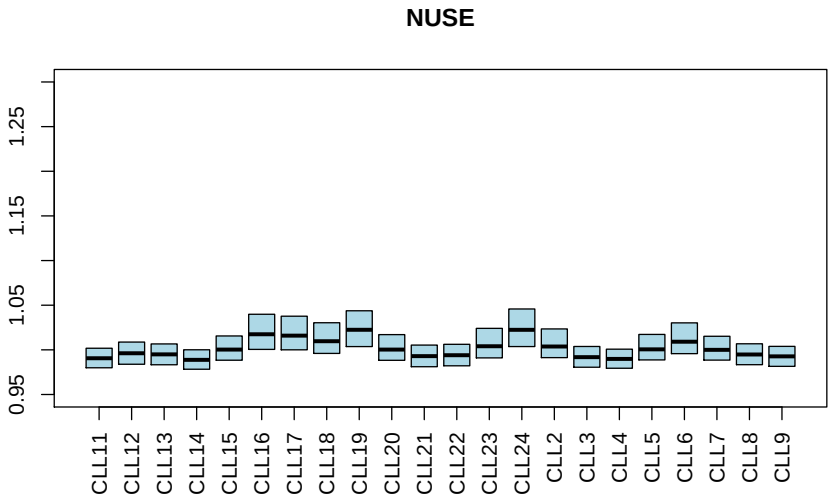
Exercise 3.1

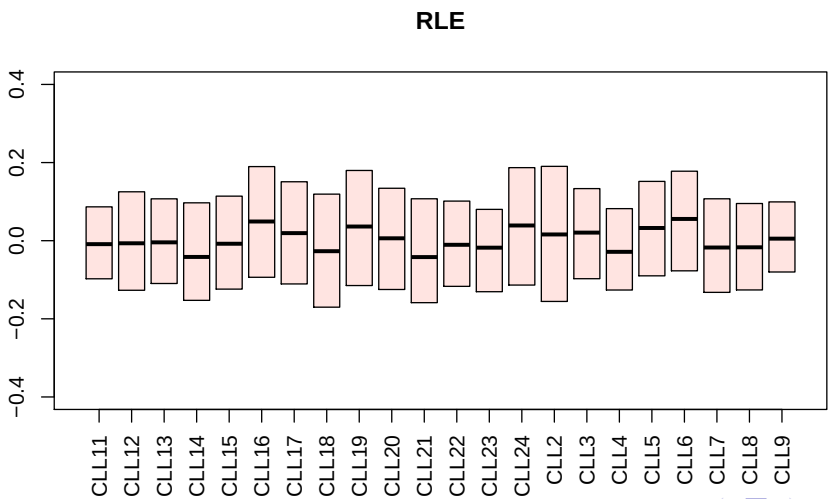
- *Repeat the calculation of the NUSE and RLE plots for the data with the array CLL1 removed.*

Solution Exercise 3.1 (1/2)

```
> dataPLMx <- fitPLM(CLLB)

> boxplot(dataPLMx, main = "NUSE", ylim = c(0.95,
+     1.3), outline = FALSE, col = "lightblue",
+     las = 3, whisklty = 0, staplelty = 0)

> Mbox(dataPLMx, main = "RLE", ylim = c(-0.4,
+     0.4), outline = FALSE, col = "mistyrose",
+     las = 3, whisklty = 0, staplelty = 0)
```

Solution Exercise 3.1 (2/2)





Expression Microarray Preprocessing Tasks

- Background correction; increases sensitivity by removing non-specific signal
- Between-array normalization; adjusts for technical variability within an experiment. **Between experiment variability should be modeled, not normalized away.**
- Reporter summarization; summarize gene expression value for each gene from all array features that target its transcripts.

RMA Preprocessing

- Robust multi-array analysis (RMA) techniques provides all three of the preprocessing tasks.
- Works with $n = 2$ or more chips.
- Method provided and documented in **affy** package as the `rma` function.
- The `threestep` function in the `affyPLM` package allows for more flexibility in performing these three tasks.

```
> CLLrma <- rma(CLLB)
```

RMA Results

- The `rma` function returns an *ExpressionSet* object.
- These expression values are on the $\log_2$ scale.
- The sample information is transferred to the output.

```
> exprs(CLLrma)[1:3, 1:3]
```

```
            CLL11    CLL12    CLL13
100_g_at 7.997251 7.939522 8.068330
1000_at  8.351710 8.560025 8.206671
1001_at  4.565553 4.466520 4.645425
```

```
> pData(CLLrma)[1:3, ]
```

```
      SampleID  Disease
CLL11    CLL11 progres.
CLL12    CLL12   stable
CLL13    CLL13 progres.
```

Exercise 3.2

- *How many probe sets are there in this dataset?*

Solution Exercise 3.2

- There are lots of solutions. Here are some:

```
> e <- exprs(CLLrma)
> dim(e)[1]
[1] 12625
> nrow(e)
[1] 12625
> dim(exprs(CLLrma))[1]
[1] 12625
> nrow(CLLrma)
Features
   12625
> length(featureNames(CLLrma))
[1] 12625
```

Non-Specific Filtering After RMA

- Genome-wide microarrays are sensitive up to 50% of the genes being differentially expressed.
- Non-informative genes add noise and filtering them out benefits downstream analyses.
- The `nsFilter` function from the **genefilter** package filters probe sets on various criteria.
- Let's filter out genes with small variance across samples, no Entrez Gene identifiers, and Affymetrix control probes.

```
> CLLf <- nsFilter(CLLrma, remove.dupEntrez = FALSE,
+     var.cutof = 0.5)$eset
```

Individual *t*-tests

- Average log-fold change within phenotypic groups are a good naive measure to compare.
- The `rowMeans` function can calculate these statistics from an expression matrix.
- The `rowttests` function from the **genefilter** performs Student's *t*-test on each row of a matrix using the average log-fold changes from two groups.
- Similarly, the `rowFtests` function performs *F*-tests for comparing multiple groups.

```
> a <- rowMeans(exprs(CLLf))
```

```
> CLLtt <- rowttests(CLLf, "Disease")
> names(CLLtt)
```

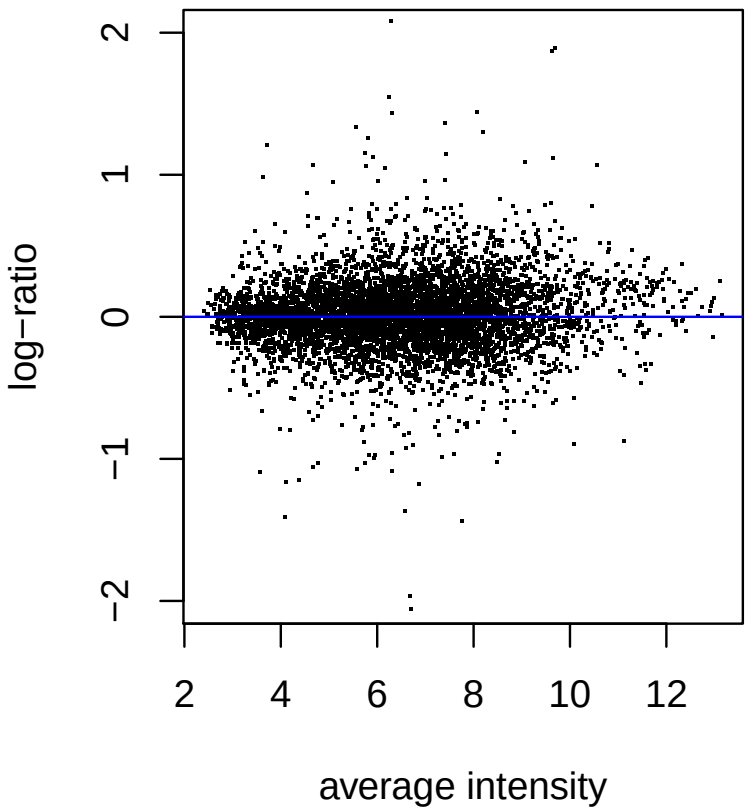
```
[1] "statistic" "dm"                "p.value"
```

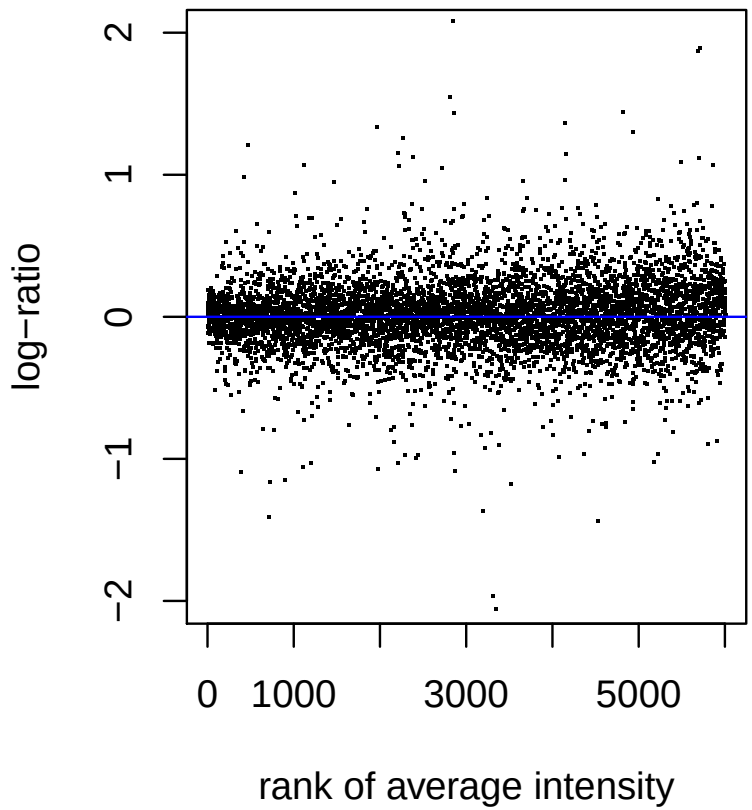
Exercise 3.3

- Does the variability of the log-ratio values depend on the average intensity?
- Plot the log-ratio against the average intensity
- Plot log-ratio versus the rank of the average intensity

```
> par(mfrow = c(1, 2))
> myPlot <- function(...) {
+     plot(y = CLLtt$dm, pch = ".", ylim = c(-2,
+         2), ylab = "log-ratio", ...)
+     abline(h = 0, col = "blue")
+ }
> myPlot(x = a, xlab = "average intensity")
> myPlot(x = rank(a), xlab = "rank of average intensity")
```

Solution Exercise 3.3





Pooled *t*-tests

- Previous *t*-tests treated each probe set separately, resulting in little data used to estimate variance.
- *t*-statistics sensitive to variance estimate.
- Can improve testing using pooled probe set variances in classical linear model or in an empirical Bayesian approach.
- Both approaches tend to perform equally well with ten or more samples in each group.
- The `lmFit` and `eBayes` functions from the **limma** package perform the former and later respectively.

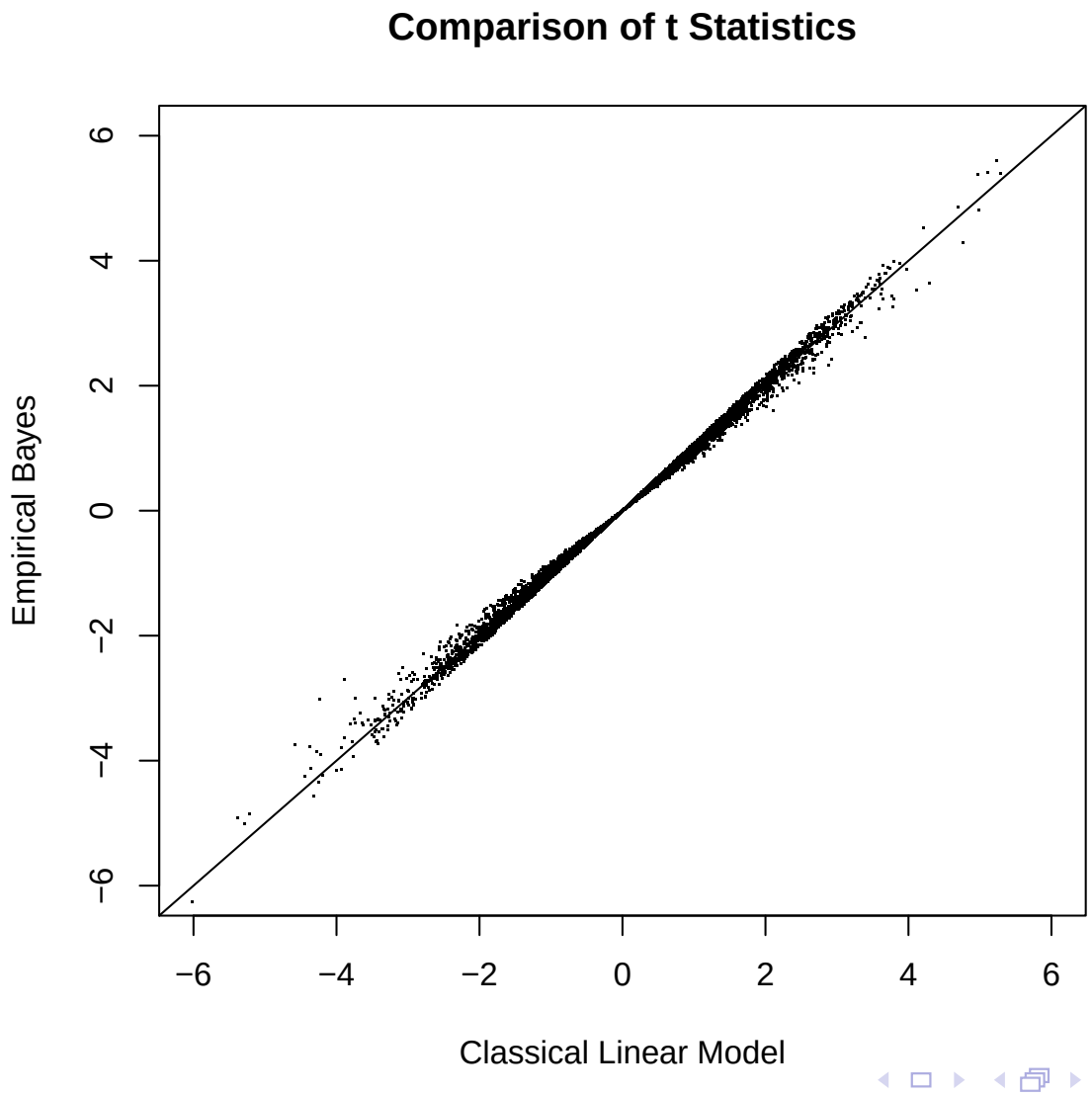
```
> library("limma")
> design <- model.matrix(~CLLf$Disease)
> CLLlim <- lmFit(CLLf, design)
> CLLeb <- eBayes(CLLlim)
```

Exercise 3.4

- Compare the t-statistics obtained under the classical linear model and (moderated) empirical Bayes approaches.

```
> plot(CLLtt$statistic, -CLLeb$t[, 2],
+      xlim = c(-6, 6), ylim = c(-6, 6),
+      xlab = "Classical Linear Model",
+      ylab = "Empirical Bayes", main = "Comparison of t Sta
+      pch = ".")
> abline(a = 0, b = 1)
```

Solution Exercise 3.4

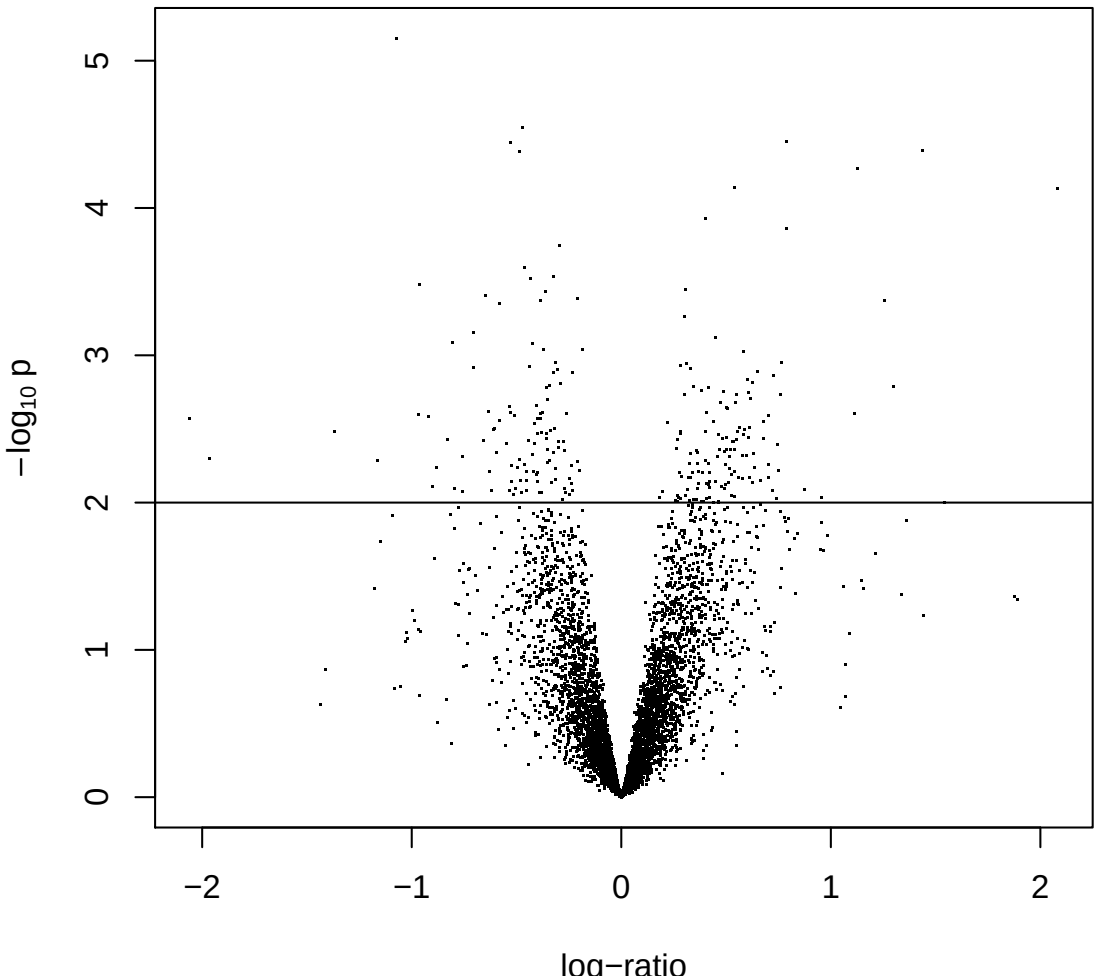


Volcano Plot (1/2)

- A volcano plot displays statistic, like t-statistic p-value, used for ranking against log-fold change.
- Reference lines are often added to plot to determine statistical and/or biological significance.

```
> plot(CLLtt$dm, -log10(CLLtt$p.value),
+     pch = ".", xlab = "log-ratio", ylab = expression(-log
+         p))
> abline(h = 2)
```

Volcano Plot (2/2)



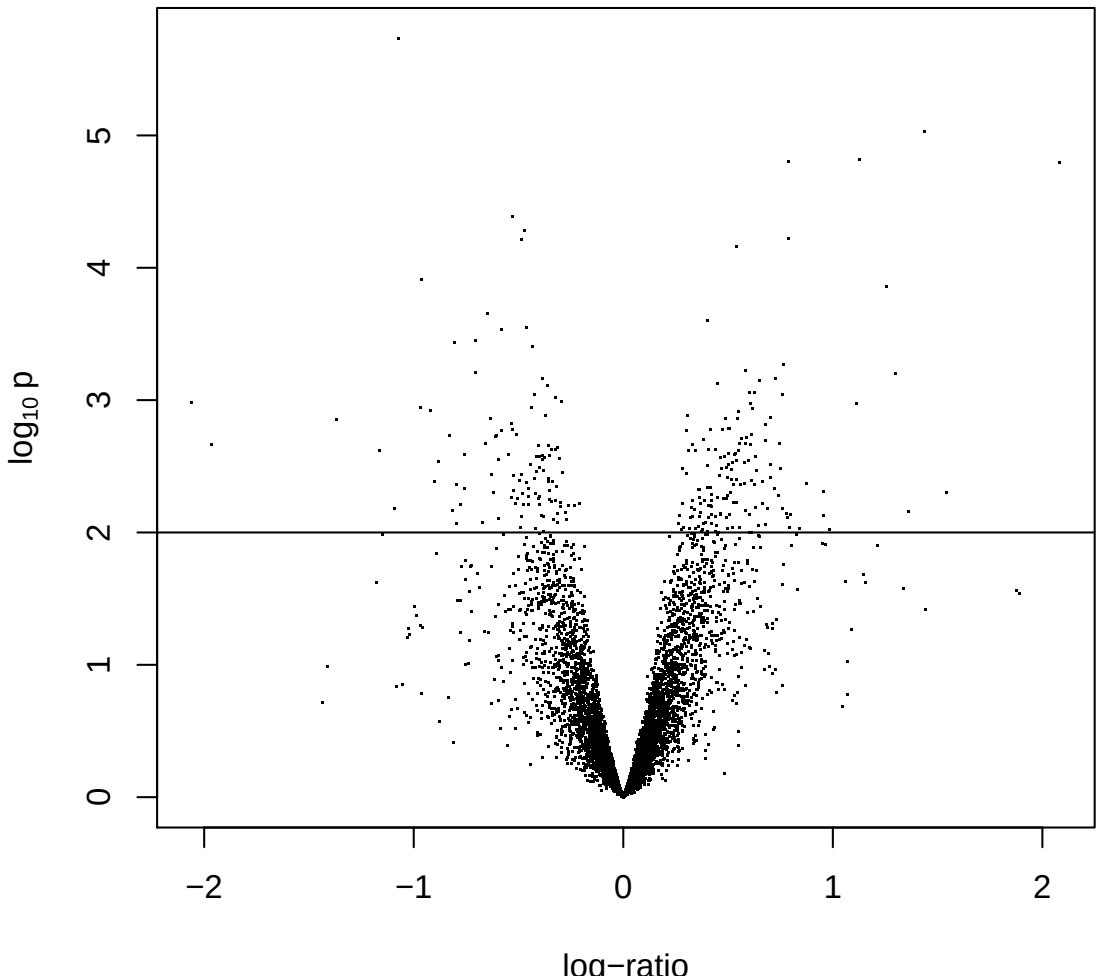
Exercise 3.5

- Generate a volcano plot using a moderated empirical Bayesian t-statistic.
- Does it look similar to the plot using the classical t-statistic?

Solution Exercise 3.5 (1/2)

```
> plot(CLLtt$dm, -log10(CLLeb$p.value[,
+     2]), pch = ".", xlab = "log-ratio",
+     ylab = expression(log[10] ~ p))
> abline(h = 2)
```

Solution Exercise 3.5 (2/2)

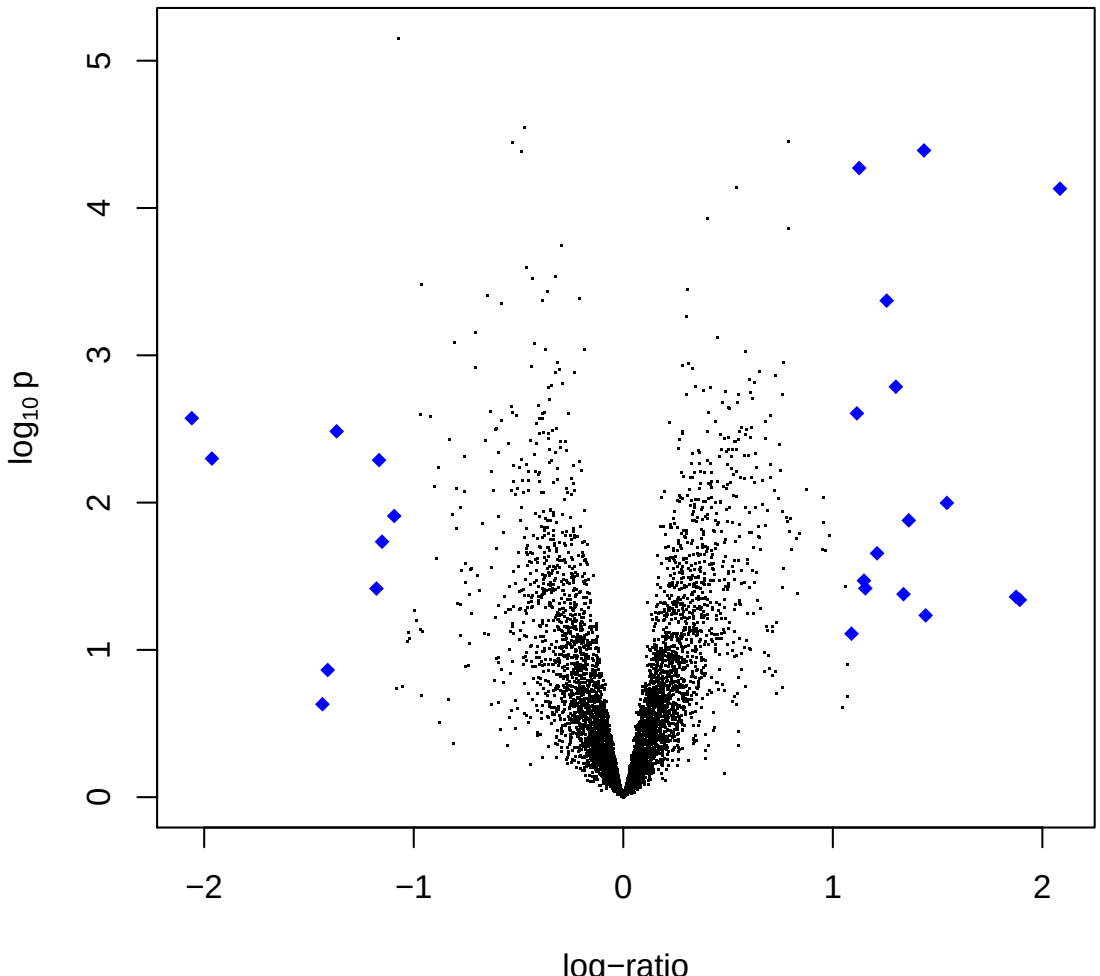


Exercise 3.6

- Use a volcano plot to highlight the top 25 genes with the smallest classical linear model p-value.

```
> plot(CLLtt$dm, -log10(CLLtt$p.value),
+      pch = ".", xlab = "log-ratio", ylab = expression(log
+          p))
> o1 <- order(abs(CLLtt$dm), decreasing = TRUE)[1:25]
> points(CLLtt$dm[o1], -log10(CLLtt$p.value)[o1],
+      pch = 18, col = "blue")
```

Solution Exercise 3.6



Multiple Testing Problem

- Due to high number of statistical tests, rule of thumb cutoffs like 0.01 for p-values yield many false positives.
- For example, using if none of the 6098 probe sets in `CLLf` were differentially expressed, the above cutoff would yield on average around 61 false positive significant test results.

Exercise 3.7

- How many probe sets with $p <= 0.01$ are there in the classical linear model t-tests?
- How many with the moderated empirical Bayesian t-tests?

Solution Exercise 3.7

```
> sum(CLLtt$p.value <= 0.01)
[1] 242
> sum(CLLeb$p.value[, 2] <= 0.01)
[1] 260
```

Adjusting for Multiple Tests

- Many methods exist for dealing with the multiple testing problem.
- The **multtest** package provides implementation of many of these methods.
- Alternatively, the `topTable` function from the **limma** package contains multiple testing adjustment methods, including Benjamini and Hochberg's false discovery rate FDR, simple Bonferroni correction, and several others.

```
> tab <- topTable(CLLeb, coef = 2, adjust.method = "BH",
+     n = 10)
> genenames <- as.character(tab$ID)
> genenames
 [1] "1303_at"  "33791_at" "37636_at" "36131_at"
 [5] "36939_at" "36129_at" "551_at"   "41776_at"
 [9] "39400_at" "36122_at"
```

Microarry Annotations

- The **annotate** package provides facilities to navigate microarray annotations.
- Many packages like **hgu95av2.db** contain platform-specific annotations.

```
> library("annotate")
> annotation(CLLf)
```

```
[1] "hgu95av2"
```

```
> suppressMessages(library("hgu95av2.db"))
```

EntrezGene ID and Gene Symbol

- The getEG and getSYMBOL functions from the **annotate** package return the EntrezGene ID and gene symbol for a specified annotation package respectively.

```
> ll <- getEG(genenames, "hgu95av2")
 1303_at 33791_at 37636_at 36131_at 36939_at
  "6452"  "10301"   "9767"   "1192"   "2823"
36129_at   551_at 41776_at 39400_at 36122_at
  "9905"   "2033"    "475"  "23102"   "5687"
> sym <- getSYMBOL(genenames, "hgu95av2")
  1303_at  33791_at  37636_at  36131_at
 "SH3BP2"   "DLEU1"   "PHF16"   "CLIC1"
 36939_at  36129_at    551_at  41776_at
  "GPM6A"   "SGSM2"   "EP300"   "ATOX1"
 39400_at  36122_at
"TBC1D2B"   "PSMA6"
```

HTML Reporting of Top Genes (1/2)

- The `htmlpage` from the **annotate** package creates an HTML table containing both static information as well as links to various online annotation sources.

```
> tab <- data.frame(sym, signif(tab[, -1],
+     3))
> htmlpage(list(ll), othernames = tab,
+     filename = "GeneList1.html", title = "HTML report",
+     table.center = TRUE, table.head = c("Entrez ID",
+         colnames(tab)))

> browseURL("GeneList1.html")
```

HTML Reporting of Top Genes (2/2)

- The `aafTableAnn` from the **annaffy** package creates an alternative HTML table containing both static information as well as links to various online annotation sources.
- The `colnames` argument can be used to select a subset of the metadata for the specified probe IDs.

```
> library("annaffy")
> library("KEGG.db")
> atab <- aafTableAnn(genenames, "hgu95av2.db",
+     aaf.handler())
> saveHTML(atab, file = "GeneList2.html")
> atab <- aafTableAnn(genenames, "hgu95av2.db",
+     aaf.handler()[c(2, 5, 8, 12)])
> saveHTML(atab, file = "GeneList3.html")
```

```
> browseURL("GeneList2.html")
> browseURL("GeneList3.html")
```

Advanced Preprocessing

- Bioconductor provides functionality for performing custom preprocessing.
- This preprocessing task starts with the "raw" feature intensity data from the CEL files and the assignment of features to target genes information from CDF files.

Probe-Level Data

- Probe-level data from Affymetrix GeneChip®Microarrays originate from CEL files in the form of PM and MM probe intensities.
- The `pm` and `mm` functions from the **affy** package extract data from PM and MM probes respectively.

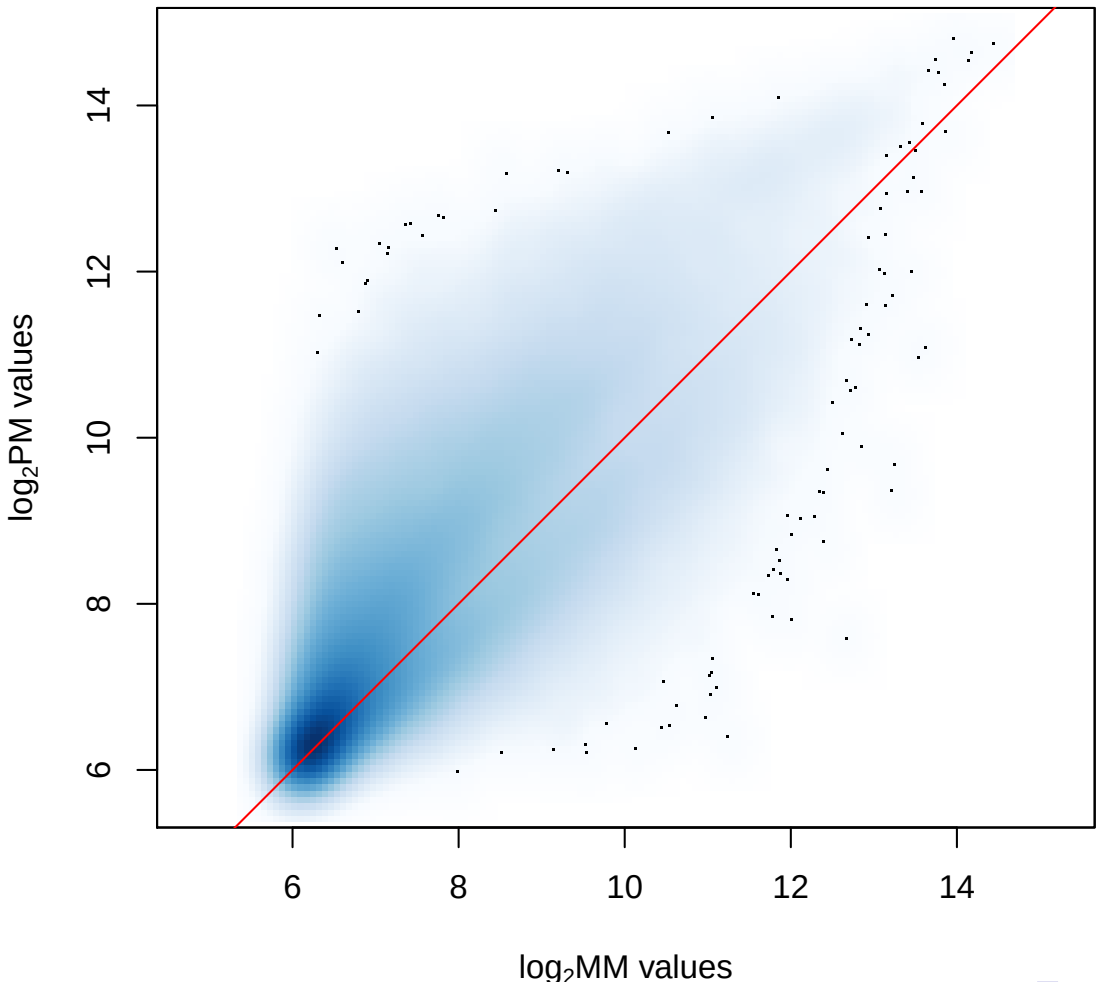
```
> pms <- pm(CLLB)
> mms <- mm(CLLB)
```

Exercise 3.8 (1/3)

- For the first array in the CLL data, make a scatterplot of the PM values versus the MM values and interpret the results.
- How many MM probes have larger intensities than their corresponding PM probes?

```
> smoothScatter(log2(mms[, 1]), log2(pms[,
+     1]), xlab = expression(log[2] * "MM values"),
+     ylab = expression(log[2] * "PM values"),
+     asp = 1)
> abline(a = 0, b = 1, col = "red")
```

Exercise 3.8 (2/3)



Exercise 3.8 (3/3)

- In a large number of cases, the MM value is larger than the PM value.
- This complicates the simple story that hybridization to the perfect match probe exceeds that of the mismatch probe.

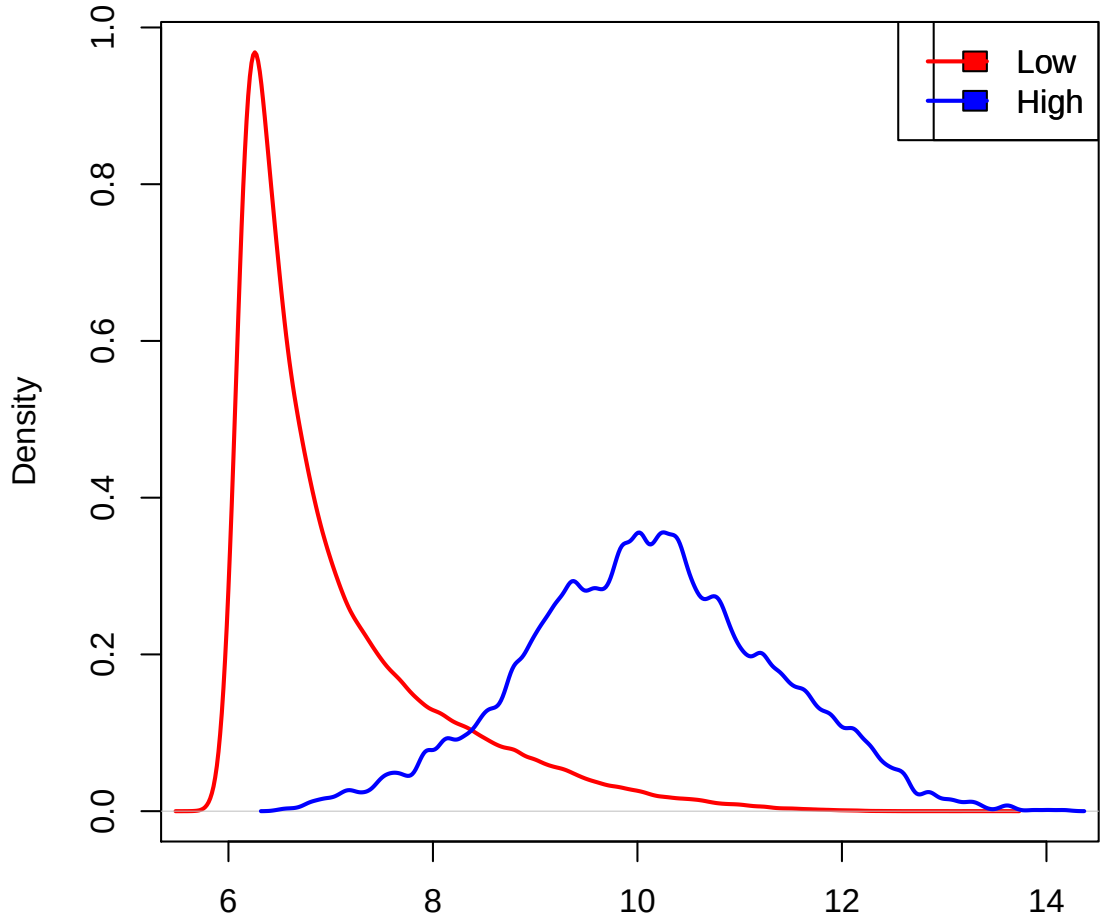
```
> table(sign(pms - mms))

     -1       0       1
1414590   31828 2993182
```

Exercise 3.9 (1/2)

- For the second array, make a histogram of the MMs for which $PM > 2000$?
- Compare it to the histogram where the PM values are less than 2000.

```
> library("geneplotter")
> grouping <- cut(log2(pms)[, 2], breaks = c(-Inf,
+     log2(2000), Inf), labels = c("Low",
+     "High"))
> multidensity(log2(mms)[, 2] ~ grouping,
+     main = "", xlab = "", col = c("red",
+         "blue"), lwd = 2)
> legend("topright", levels(grouping),
+     lty = 1, lwd = 2, col = c("red",
+         "blue"))
```

Exercise 3.9 (2/2)



- Optical noise and cross-hybridizations results in positive feature intensities from Affymetrix microarrays even when expected to be zero.
- Background correction is essential to obtain good sensitivity.
- We will explore robust multi-array analysis (RMA) and variance-stabilizing normalization (VSN) methods.

Background Correction using RMA

- In RMA, background modeled using a Normal-Exponential mixture.
- PM intensity values corrected by subtracting background estimate of each probe.
- Corrected PM values guaranteed to be positive.

```
> bgrma <- bg.correct.rma(CLLB)
> exprs(bgrma) <- log2(exprs(bgrma))
```

Background Correction using VSN

- In VSN, one overall background estimate is computed for the whole array.
- This estimate can be larger than some of the smaller feature intensities on the array, resulting in some of the background-subtracted values ≤ 0.
- Generalized logarithm transformation, which handles nonpositive values, applied to corrected values.
- The `justvsn` function from the **vsn** package fits the vsn model.

```
> library("vsn")
> bgvsn <- justvsn(CLLB)
```

Exercise 3.10 (1/3)

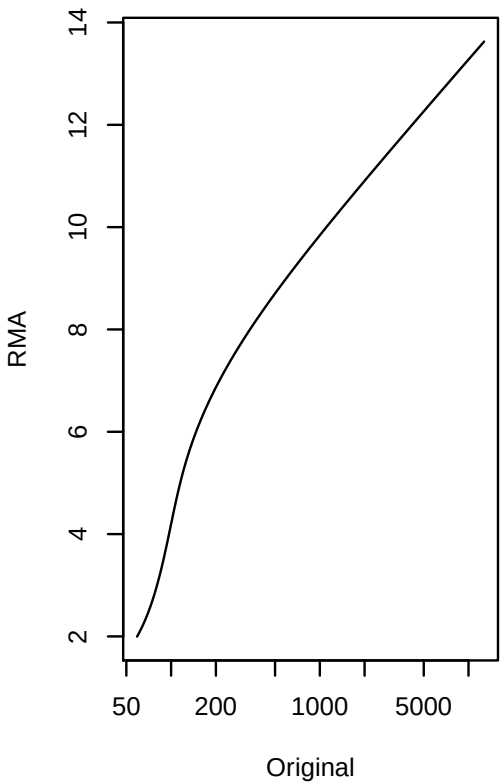
- Compare the results of the two background-correction methods to the original values and between each other. Use a subset of 500 randomly selected PM probes to speed up calculations.

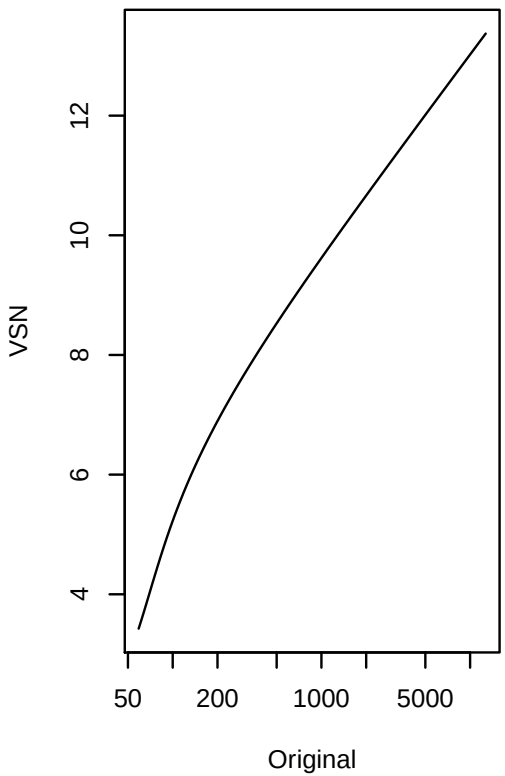
```
> sel <- sample(unlist(indexProbes(CLLB,
+     "pm")), 500)
> sel <- sel[order(exprs(CLLB)[sel, 1])]

> yo <- exprs(CLLB)[sel, 1]
> yr <- exprs(bgrma)[sel, 1]
> yv <- exprs(bgvsn)[sel, 1]
```

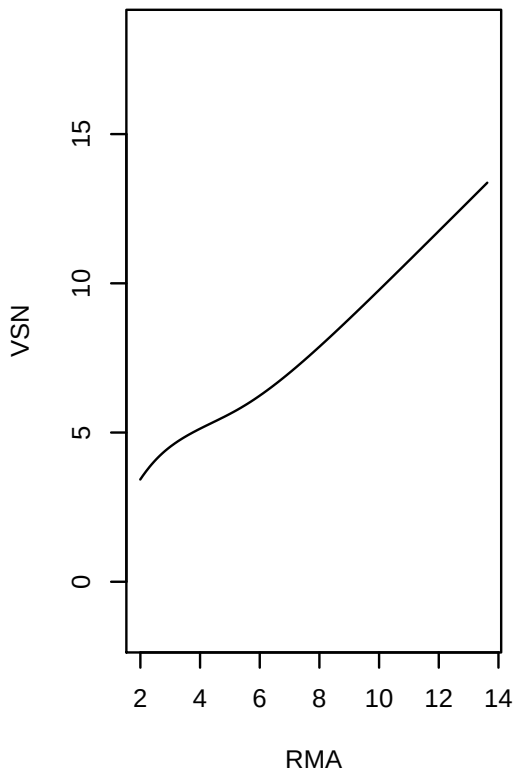
Exercise 3.10 (2/3)

```
> par(mfrow = c(1, 3))
> plot(yo, yr, xlab = "Original", ylab = "RMA",
+     log = "x", type = "l", asp = 1)
> plot(yo, yv, xlab = "Original", ylab = "VSN",
+     log = "x", type = "l", asp = 1)
> plot(yr, yv, xlab = "RMA", ylab = "VSN",
+     type = "l", asp = 1)
```

Exercise 3.10 (3/3)







VSN Preprocessing

- The `vsnrma` function from the **vsn** package summarizes the probe sets after performing probe-wise background correction and between-array normalization using VSN.
- Its output is derived from the **affy**'s `rma` function.

```
> vsnrma
> CLLvsn <- vsnrma(CLLB)
```

Non-Specific Filtering After VSN

- We can repeat the non-specific filtering and testing for differential expression that was performed after RMA.

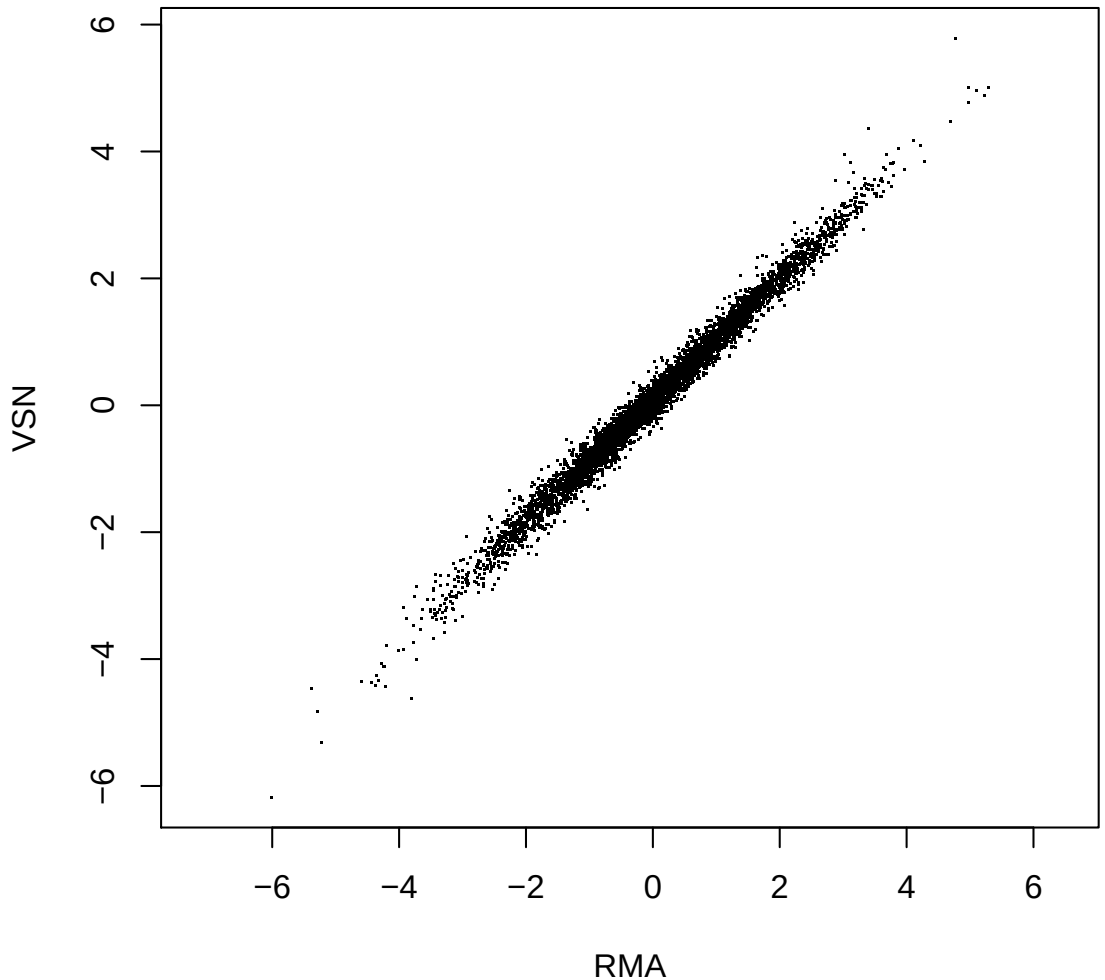
```
> CLLvsnf <- nsFilter(CLLvsn, remove.dupEntrez = FALSE,
+     var.cutoff = 0.5)$eset
> CLLvsntt <- rowttests(CLLvsnf, "Disease")
```

Exercise 3.11 (1/2)

- Compare the results in `CLLvsntt` with those obtained for the RMA derived `CLLtt`.
- Produce a scatterplot between the t-statistics obtained for both cases.

```
> inboth <- intersect(featureNames(CLLvsnf),
+     featureNames(CLLf))

> plot(CLLtt[inboth, "statistic"], CLLvsntt[inboth,
+     "statistic"], pch = ".", xlab = "RMA",
+     ylab = "VSN", asp = 1)
```

Exercise 3.11 (2/2)



Reporter Summarization

- Bioconductor provides functionality for performing custom probe set (reporter) summarization.
- This is performed using data are obtained from the CDF file.

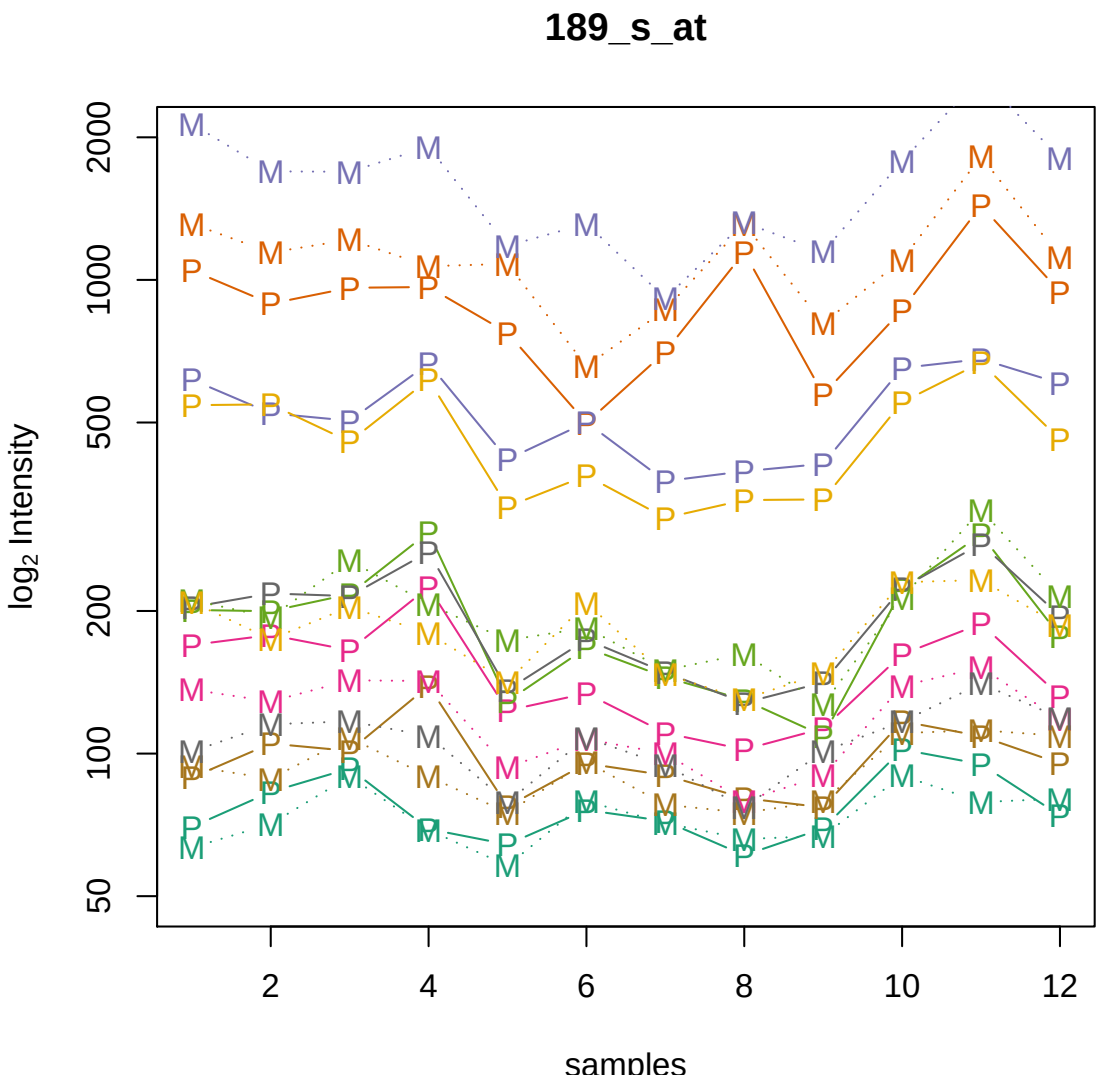
```
> pns <- probeNames(CLLB)
> indices <- split(seq(along = pns), pns)

> length(indices)

[1] 12625

> indices[["189_s_at"]]

 [1] 15714 15715 15716 15717 15718 15719 15720
 [8] 15721 15722 15723 15724 15725 15726 15727
[15] 15728 15729
```

Exercise 3.12 (1/2)

- Can you plot the PM and MM intensities for the probes of one probe set across a set of arrays?

```
> colors <- brewer.pal(8, "Dark2")
> Index <- indices[["189_s_at"]][seq(along = colors)]
> matplot(t(pms[Index, 1:12]), pch = "P",
+     log = "y", type = "b", lty = 1, main = "189_s_at",
+     xlab = "samples", ylab = expression(log[2] ~
+         Intensity), ylim = c(50, 2000),
+     col = colors)
> matplot(t(mms[Index, 1:12]), pch = "M",
+     log = "y", type = "b", lty = 3, add = TRUE,
+     col = colors)
```

Exercise 3.12 (2/2)



Naive Summary Method

- One naive (robust) summarization of the probe sets is to take the median difference between the PM and MM values for each sample.

```
> newsummary <- t(sapply(indices, function(j) rowMedians(t(
+     ] - mms[j, ])))))
> dim(newsummary)
```

```
[1] 12625    22
```

Exercise 3.13

- What percent of probe sets, for each array, yield negative values for each array? Will this concern biologist who are unhappy with negative expression estimates?

```
> colMeans(newsummary < 0) * 100

 [1] 20.19802 19.60396 19.39010 18.26535
 [5] 21.03762 22.62970 21.65545 19.63564
 [9] 21.73465 21.05347 18.85941 18.70891
[13] 20.62574 23.06535 19.56436 21.04554
[17] 18.63762 21.56040 21.43366 19.63564
[21] 19.69109 19.75446
```

Looking Forward

- Chapter 4 addresses these same issues for two-color arrays.
- Chapter 5 contains more on variance stabilization and calibration for microarray data.
- Questions?