Microarray Analysis

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Basics

Moderated *t*-tests

Using limma

p-value Correction

Resources

Introduction

- Identify differentially expressed genes associated with biological or experimental conditions.
- Primarily concerned with two-class problems.
- Data with n samples and p probes ($p >> n$).

A	A	A	A	A	B	B	B	B	B
$x_{1,1}$	$x_{1,2}$	$x_{1,3}$	$x_{1,4}$	$x_{1,5}$	$x_{1,6}$	$x_{1,7}$	$x_{1,8}$	$x_{1,9}$	$x_{1,10}$
$x_{2,1}$	$x_{2,2}$	$x_{2,3}$	$x_{2,4}$	$x_{2,5}$	$x_{2,6}$	$x_{2,7}$	$x_{2,8}$	$x_{2,9}$	$x_{2,10}$
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
$x_{p,1}$	$x_{p,2}$	$x_{p,3}$	$x_{p,4}$	$x_{p,5}$	$x_{p,6}$	$x_{p,7}$	$x_{p,8}$	$x_{p,9}$	$x_{p,10}$

Approaches

- Gene-by-gene hypothesis testing
  - Treating each gene independently of others.
  - Goal: find statistically significant associations of biological conditions.
  - Genes are deemed to be interesting if the p-value is small.
  - Method: t-tests, moderated t-tests, ROC, F-test.
- Machine learning

t-tests

$$t_g = \frac{\mu_x - \mu_y}{\sqrt{\sigma_x^2 - \sigma_y^2}}$$

Drawback:

- Parametric assumptions hard to justify with few arrays.
- The variance in small samples might be noisy.
- Genes with small fold-change might be significant from statistical, not biological point of view.

Moderated t-statistics

- Rather than estimating within-group variability for each gene, pool the global information from all other genes.
- Advantage: eliminate occurrence of accidentally large t-statistics due to accidentally small within-group variance.

Moderated t-statistics

Using empirical Bayesian approach to estimate:

- Overall estimate variation s_0^2.
- Per-gene deviation variation s_g^2.
- Shrinkage variation

$$\tilde{s}_g^2 = \frac{d_0 s_0^2 + d_g s_g^2}{d_0 + d_g}$$

- Contrast estimator $\hat{\beta}_g$ – the difference in means between two classes.
- Moderated t-statistics:

$$\tilde{t}_g = \frac{\hat{\beta}_g}{\tilde{s}_g \sqrt{\nu_g}}$$

Using limma

1. Define a design matrix to establish parameters of linear model `model.matrix`.
2. Fit a linear model for each gene based on the given design matrix (and a contrast matrix): `lmFit()`.
3. Use function `eBayes` to get moderated *t*-statistics and relevant statistics.

Deriving linear models

Suppose we define a design matrix as the following:

sample i	(intercept)	mol.biolNEG
NEG	1	1
BCR/ABL	1	0
NEG	1	1
$\vdots$	$\vdots$	$\vdots$

Each gene Y_j for all sample i, the expression level can be expressed by

$$\begin{bmatrix} Y_{NEG_i,j} \\ Y_{BCR/ABL_i,j} \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ 1 & 0 \end{bmatrix} \begin{bmatrix} \beta_{intercept} \\ \beta_{mol.biolNEG} \end{bmatrix} + \epsilon$$

$$\Rightarrow \beta_{mol.biolNEG} = Y_{BCR/ABL_i,j} - Y_{NEG_i,j} + \epsilon$$

$$y_j = \beta_{intercept} + \beta_{mol.biolNEG}\, a_{ij} + \epsilon$$

$$\Rightarrow y_j = \beta_0 + \beta_1 a_{ij} + \epsilon$$

Using limma

Step 1:

Code: define design matrix and contrast model

```
> library(limma)
> design <- model.matrix( ~mol.biol, ALLfilt_bcrneg)
>
```

Step 2:

Code: linear models and eBayes

```
> fit1 <- lmFit(exprs(ALLfilt_bcrneg), design)
> fit2 <- eBayes(fit1)
> topTable(fit2, coef=2, adjust.method="BH",
+          number=5)
```

Deriving linear models

Suppose we define a design matrix as the following:

sample i	mol.biolBCR	mol.biolNEG
BCR/ABL	1	0
BCR/ABL	1	0
BCR/ABL	1	0
⋮	⋮	⋮
NEG	0	1
NEG	0	1
NEG	0	1
⋮	⋮	⋮

$$y_i = \beta_1 a_{ij} + \beta_2 b_{ij} + \varepsilon_i$$

Using limma

Step 1:

Code: define design matrix and contrast model

```
> library(limma)
> design <- model.matrix( ~0+mol.biol, ALLfilt_bcrneg)
> colnames(design) <- c("BCR_ABL", "NEG")
> contr <- makeContrasts(BCR_ABL-NEG, levels=designs)
> # contr <- c(1, -1)
```

Step 2:

Code: linear models and eBayes

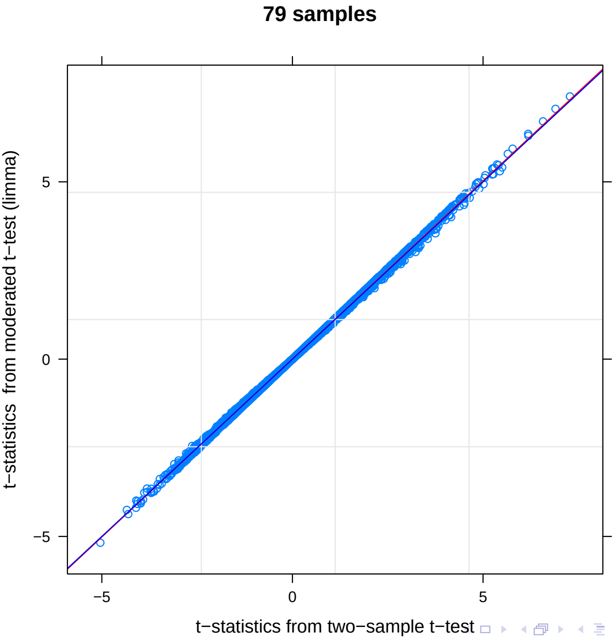
```
> fit <- lmFit(exprs(ALLfilt_bcrneg), design)
> fit1 <- contrasts.fit(fit, contr)
> fit2 <- eBayes(fit1)
> topTable(fit2,  adjust.method="BH",  number=5)
```

t-tests vs. moderated t-tests

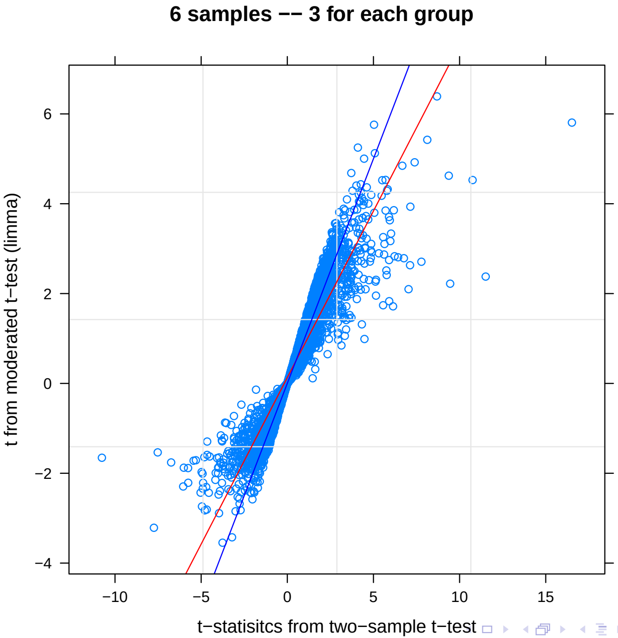
- In larger sample size, there is not big difference between the ordinary and the moderated tests.
- For smaller sample size the difference will be larger.

The empirical Bayes moderation is more useful in cases with fewer replicates.

t-tests vs. moderated *t*-tests



t-tests vs. moderated *t*-tests



p-value corrections

- Basic idea: reduce critical value used to reject.
- Trade-off between sensitivity and specificity.
- Approaches implemented in the *multtest* package:
  - criteria for error rate control include family-wise error rate (FWER) and false discovery rate (FDR).
  - Permutation-based maxT methods.

Lab activity

- Chapter 6 and 7 in *Bioconductor Case Studies*.
- Goals: get familiar with functions provided by *Bioconductor* packages to perform differential expression analysis.

Resources

- G.K. Smyth, Linear models and empirical Bayes methods for assessing differential expression in microarray experiments, *Statistical Applications in Genetics and Molecular Biology*, 3(1), 2004.
- G. K. Smyth, *limma: Linear Models for Microarray Data*, Bioconductor package vignette, 2005.
- Florian Hahne et. al., *Bioconductor Case Studies*, Springer, 2007.