8. Bioconductor Intro

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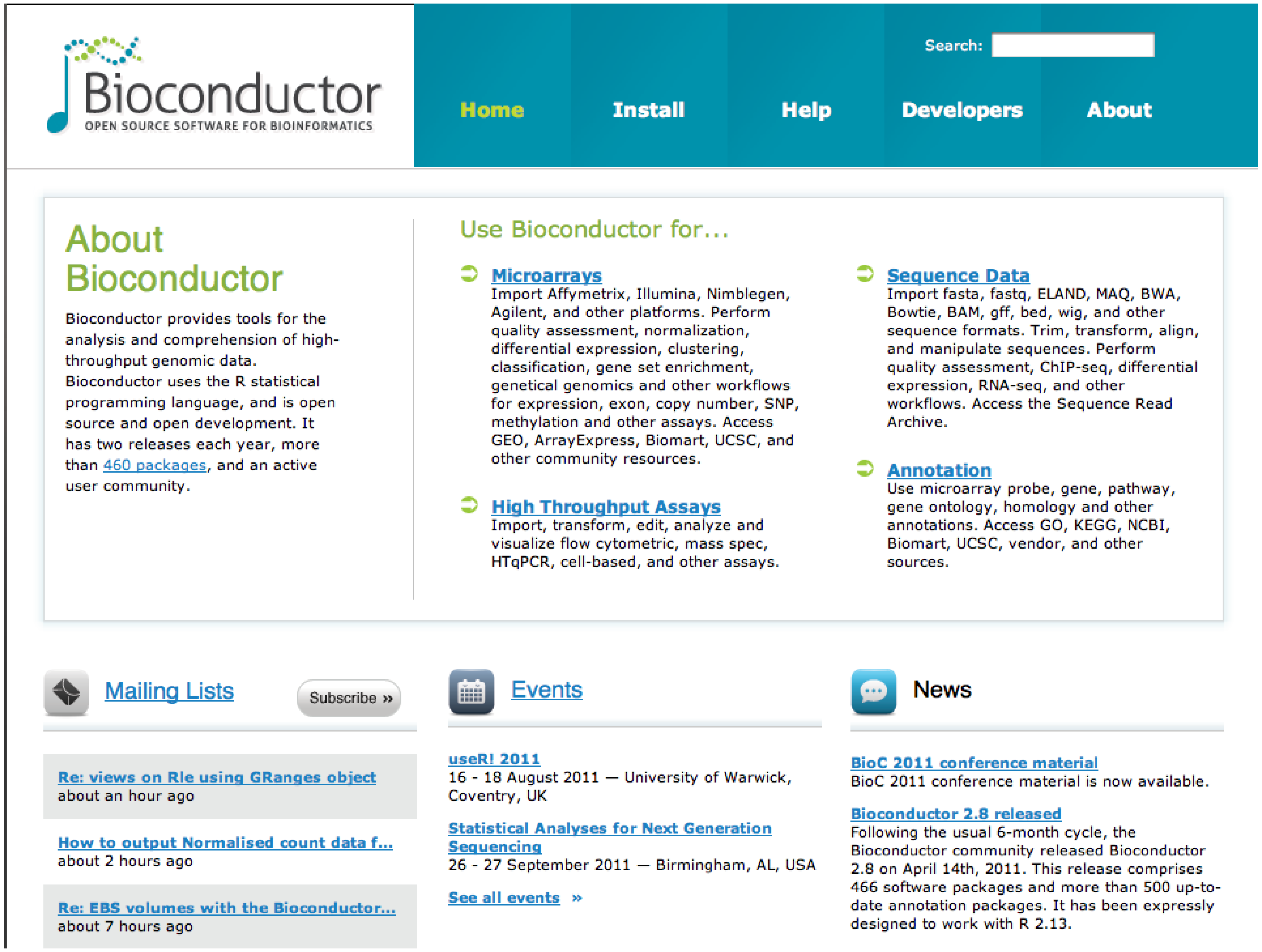
Liège, September 2011

What is Bioconductor?



About Bioconductor

Bioconductor provides tools for the analysis and comprehension of high-throughput genomic data. Bioconductor uses the R statistical programming language, and is open source and open development. It has two releases each year, more than 460 packages, and an active user community.

Use Bioconductor for...

Microarrays
Import Affymetrix, Illumina, Nimblegen, Agilent, and other platforms. Perform quality assessment, normalization, differential expression, clustering, classification, gene set enrichment, genetical genomics and other workflows for expression, exon, copy number, SNP, methylation and other assays. Access GEO, ArrayExpress, Biomart, UCSC, and other community resources.

High Throughput Assays
Import, transform, edit, analyze and visualize flow cytometric, mass spec, HTqPCR, cell-based, and other assays.

Sequence Data
Import fasta, fastq, ELAND, MAQ, BWA, Bowtie, BAM, gff, bed, wig, and other sequence formats. Trim, transform, align, and manipulate sequences. Perform quality assessment, ChIP-seq, differential expression, RNA-seq, and other workflows. Access the Sequence Read Archive.

Annotation
Use microarray probe, gene, pathway, gene ontology, homology and other annotations. Access GO, KEGG, NCBI, Biomart, UCSC, vendor, and other sources.

Mailing Lists Subscribe »

Re: views on Rle using GRanges object
about an hour ago

How to output Normalised count data f...
about 2 hours ago

Re: EBS volumes with the Bioconductor...
about 7 hours ago

Events

useR! 2011
16 - 18 August 2011 — University of Warwick, Coventry, UK

Statistical Analyses for Next Generation Sequencing
26 - 27 September 2011 — Birmingham, AL, USA

See all events »

News

BioC 2011 conference material
BioC 2011 conference material is now available.

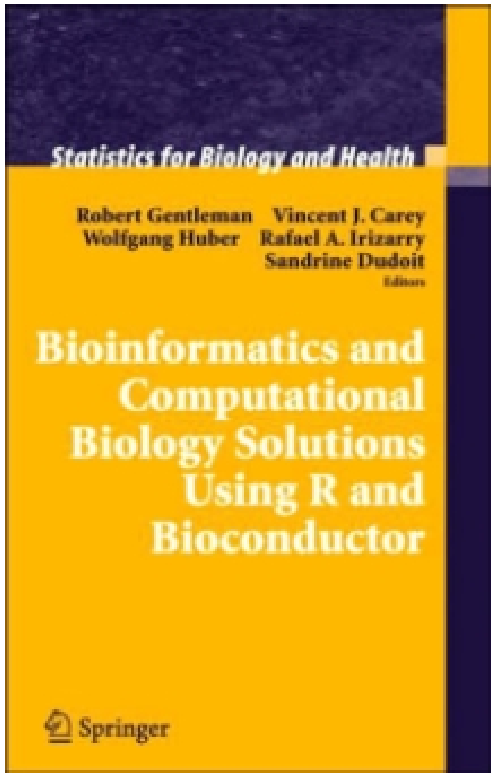
Bioconductor 2.8 released
Following the usual 6-month cycle, the Bioconductor community released Bioconductor 2.8 on April 14th, 2011. This release comprises 466 software packages and more than 500 up-to-date annotation packages. It has been expressly designed to work with R 2.13.

What is Bioconductor?

- `www.bioconductor.org`
- Software project for analysis of genomic data – and related tools, resources/datasets
- **Open source** and **Open development**
- **Free**

You could use commercial software; but **experts** typically **write** `R` **code first**. The help manuals are **not a sales pitch** and encourage **appropriate** use

Bioconductor basics


Statistics for Biology and Health

Robert Gentleman Vincent J. Carey
Wolfgang Huber Rafael A. Irizarry
Sandrine Dudoit
Editors

Bioinformatics and Computational Biology Solutions Using R and Bioconductor

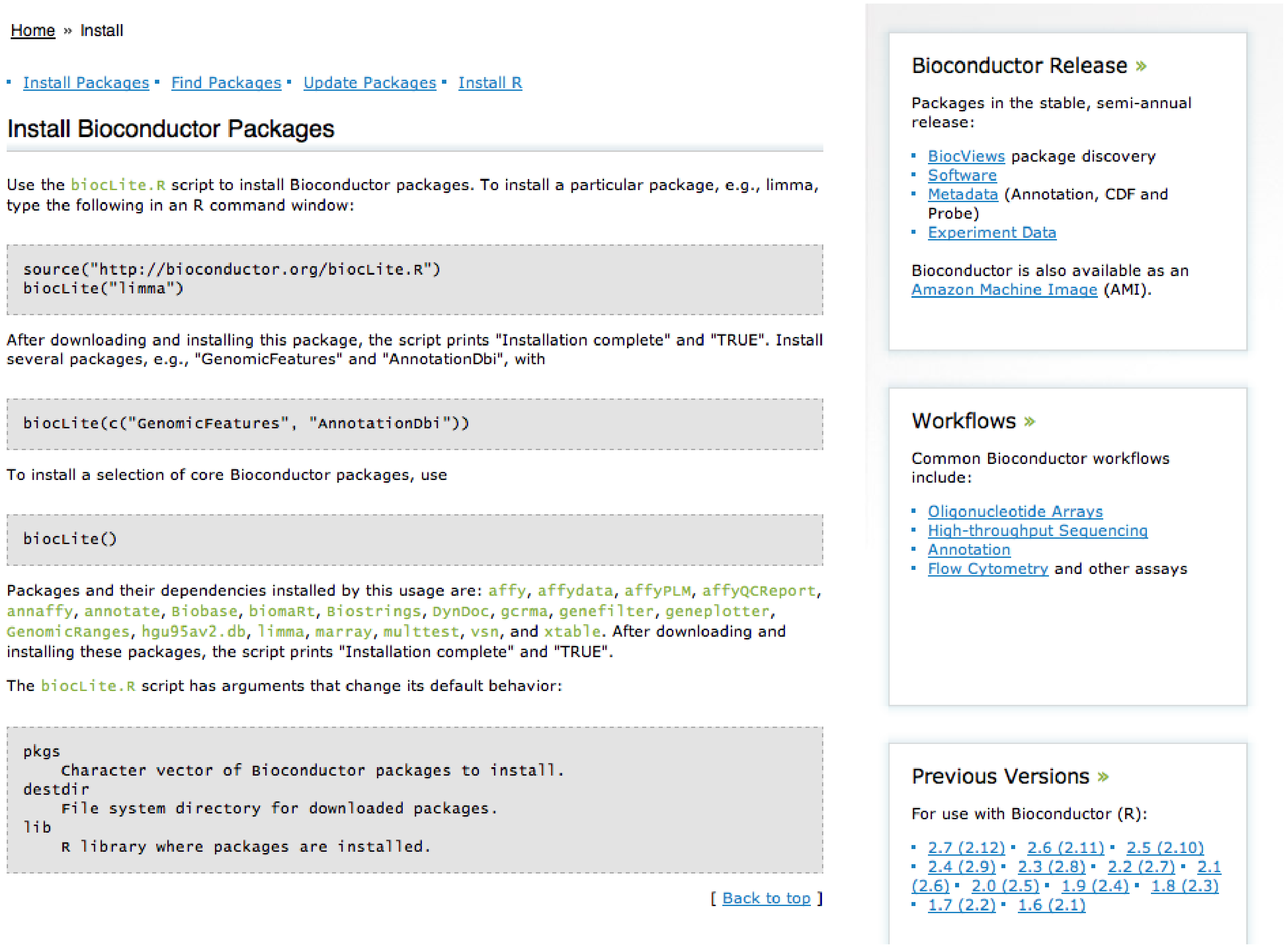
Springer

- Begun in 2001, based at Harvard and now FHCRC (Seattle)
- A large collection of `R` packages (they also convert good software to `R`)
- Far too much for our little course!

We'll give examples of what Bioconductor can do, and how to learn more. Gentleman et al (above) is a helpful reference text

Bioconductor basics

Getting started...



Home » Install

- Install Packages ▪ Find Packages ▪ Update Packages ▪ Install R

Install Bioconductor Packages

Use the `biocLite.R` script to install Bioconductor packages. To install a particular package, e.g., limma, type the following in an R command window:

```
source("http://bioconductor.org/biocLite.R")
biocLite("limma")
```

After downloading and installing this package, the script prints "Installation complete" and "TRUE". Install several packages, e.g., "GenomicFeatures" and "AnnotationDbi", with

```
biocLite(c("GenomicFeatures", "AnnotationDbi"))
```

To install a selection of core Bioconductor packages, use

```
biocLite()
```

Packages and their dependencies installed by this usage are: `affy`, `affydata`, `affyPLM`, `affyQCReport`, `annaffy`, `annotate`, `Biobase`, `biomaRt`, `Biostrings`, `DynDoc`, `gcrma`, `genefilter`, `geneplotter`, `GenomicRanges`, `hgu95av2.db`, `limma`, `marray`, `multtest`, `vsn`, and `xtable`. After downloading and installing these packages, the script prints "Installation complete" and "TRUE".

The `biocLite.R` script has arguments that change its default behavior:

```
pkgs
    Character vector of Bioconductor packages to install.
destdir
    File system directory for downloaded packages.
lib
    R library where packages are installed.
```

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Bioconductor Release »

Packages in the stable, semi-annual release:

- BiocViews package discovery
- Software
- Metadata (Annotation, CDF and Probe)
- Experiment Data

Bioconductor is also available as an Amazon Machine Image (AMI).

Workflows »

Common Bioconductor workflows include:

- Oligonucleotide Arrays
- High-throughput Sequencing
- Annotation
- Flow Cytometry and other assays

Previous Versions »

For use with Bioconductor (R):

- 2.7 (2.12) ▪ 2.6 (2.11) ▪ 2.5 (2.10)
- 2.4 (2.9) ▪ 2.3 (2.8) ▪ 2.2 (2.7) ▪ 2.1 (2.6) ▪ 2.0 (2.5) ▪ 1.9 (2.4) ▪ 1.8 (2.3)
- 1.7 (2.2) ▪ 1.6 (2.1)

Bioconductor basics

```
> source("http://bioconductor.org/biocLite.R")
> biocLite()
```

installs the following libraries;

`affy`, `affydata`, `affyPLM`, `annaffy`, `annotate`, `Biobase`, `Biostrings`, `DynDoc`, `gcrma`, `genefilter`, `geneplotter`, `hgu95av2.db`, `limma`, `marray`, `matchprobes`, `multtest`, `ROC`, `vsn`, `xtable`, `affyQCReport`

... then you use e.g. `library(ROC)` as before.

`vignette(package="ROC")` tells you to look at `vignette("ROCnotes")` for a **worked example** – a very helpful introduction. (Or use e.g. `openVignette("ROC")` from the `Biobase` package)

Bioconductor basics

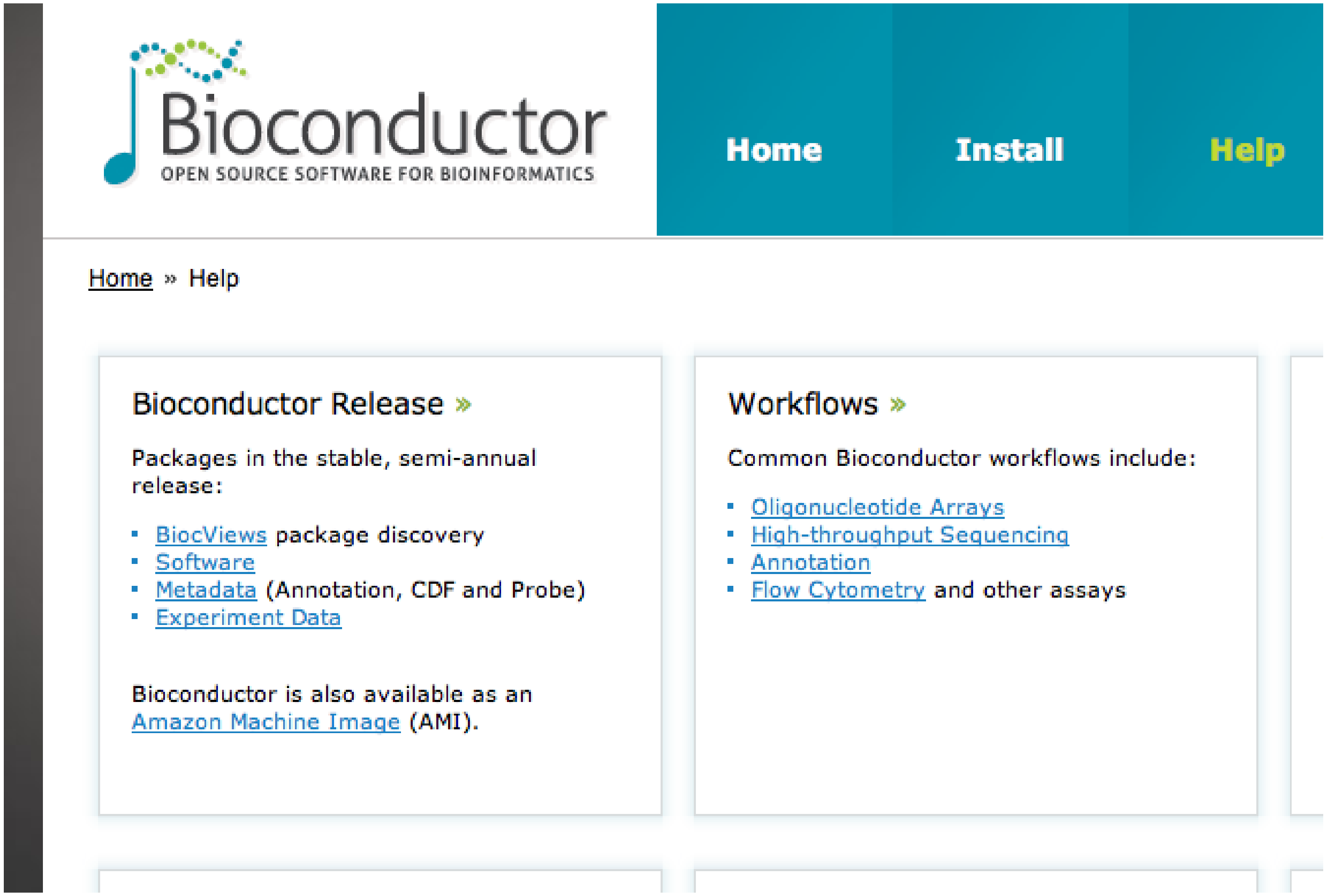
To get other packages, use e.g. `biocLite("SNPchip")`

Do not need to type `biocLite()` after you install (even in a new R session).

This would install everything again – which is harmless, but slow.

What to install?

Back to the front page – click ‘Help’



Home » Help

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Workflows »

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What to install?

- **Software** – probably what you want
- **Annotation data** – e.g. probe sequence data for microarrays of different types
- **Experiment data** – e.g. datasets from `hapmap.org`, some expression datasets

Simple QC graphics

The ”splots” package plots values from 96 or 384-well plates, for QC purposes

First, install it

```
biocLite("splots")
```

Then load into R

```
library("splots")
```

There is a single function: `plotScreen()` for displaying the results

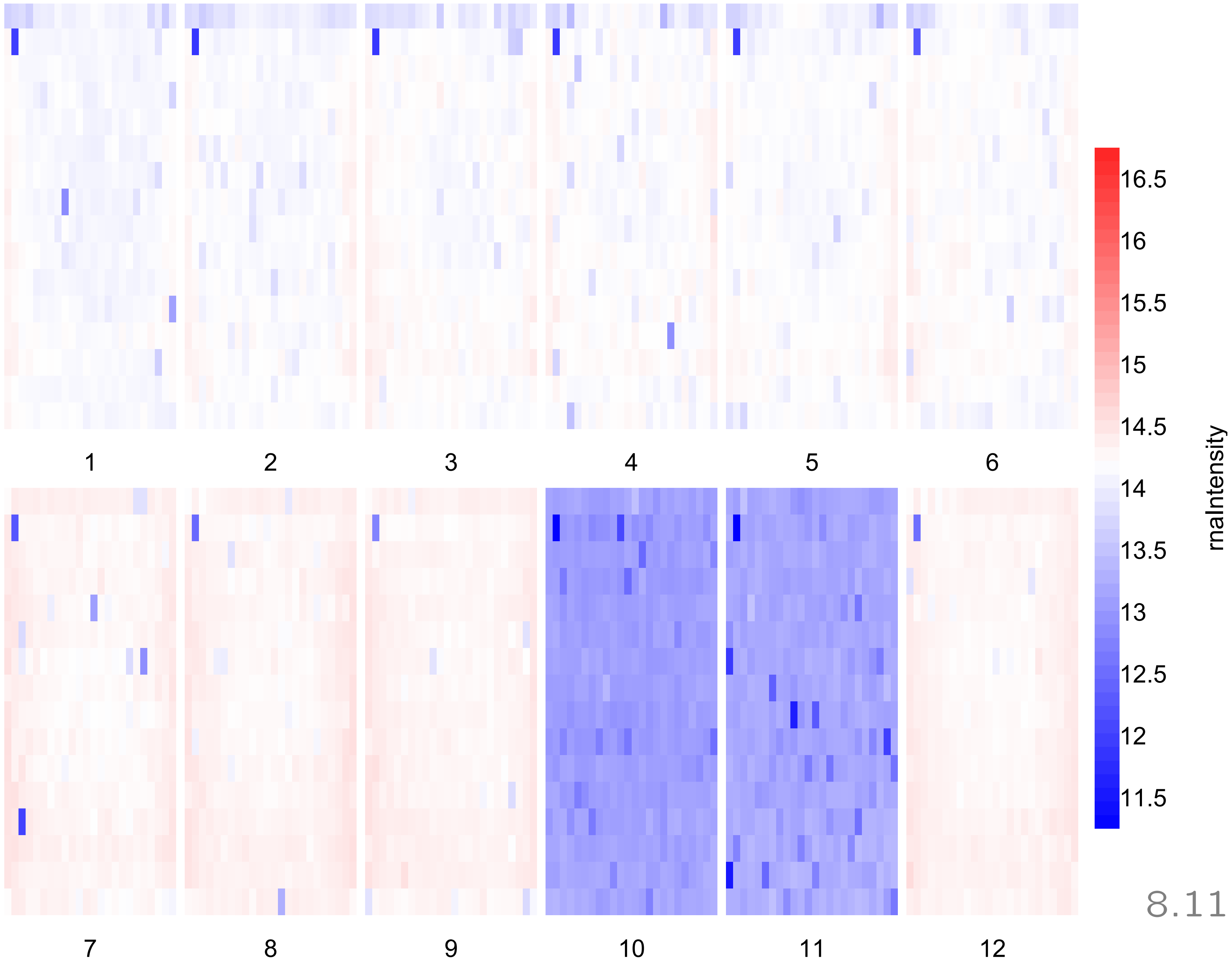
Example

The file "drosophila.rda" contains 12 of 114 plates from a RNAi gene-knockout study in fruit flies. Each spot represents a gene, and the intensity is low if knockout of that gene is lethal (data from the "RNAither" package)

```
load("drosophila.rda")
plotScreen(rnai)
```

The positive controls in the same position each plate are clear, and there are obvious plate effects that you might need to correct by normalization.

Example


1
2
3
4
5
6
7
8
9
10
11
12
16.5
16
15.5
15
14.5
14
13.5
13
12.5
12
11.5
rnaIntensity

GWAS analysis

Genome-Wide Association Studies (GWAS) are currently popular – typically, these genotype e.g. 1M SNPs on several thousand subjects in (large) established studies

- Usually on 1000's of subjects
- 'Simple' t-tests, regressions, for each SNP (like microarrays)
- 1M *anything* takes a long time! (up to 72 hours)
- Just **loading** big datasets is non-trivial – but some tools are available

GWAS analysis

`snpMatrix` is a Bioconductor package for GWAS analysis – maintained by David Clayton (analysis lead on Wellcome Trust)

```
biocLite("snpMatrix")
library(snpMatrix)
data(for.exercise)
```

A 'little' case-control dataset (Chr 10) based on HapMap – three objects; `snp.support`, `subject.support` and `snps.10`

GWAS analysis

```
> summary(snp.support)
   chromosome    position              A1         A2
 Min.    :10   Min.    :   101955   A:14019   C: 2349
 1st Qu.:10   1st Qu.: 28981867   C:12166   G:12254
 Median :10   Median : 67409719   G: 2316   T:13898
 Mean    :10   Mean    : 66874497
 3rd Qu.:10   3rd Qu.:101966491
 Max.    :10   Max.    :135323432
> summary(subject.support)
       cc            stratum
 Min.    :0.0    CEU     :494
 1st Qu.:0.0    JPT+CHB:506
 Median :0.5
 Mean    :0.5
 3rd Qu.:1.0
 Max.    :1.0
```

GWAS analysis

```
> show(snps.10) # show() is generic
A snp.matrix with  1000 rows and  28501 columns
Row names:  jpt.869 ... ceu.464
Col names:  rs7909677 ... rs12218790
> summary(snps.10)
$rows
   Call.rate      Heterozygosity
 Min.   :0.9879   Min.   :0.0000
 Median :0.9900   Median :0.3078
 Mean   :0.9900   Mean   :0.3074
 Max.   :0.9919   Max.   :0.3386
$cols
     Calls          Call.rate            MAF                P.AA
 Min.   : 975    Min.   :0.975    Min.   :0.0000    Min.   :0.00000
 Median : 990    Median :0.990    Median :0.2315    Median :0.26876
 Mean   : 990    Mean   :0.990    Mean   :0.2424    Mean   :0.34617
 Max.   :1000    Max.   :1.000    Max.   :0.5000    Max.   :1.00000
      P.AB              P.BB              z.HWE
 Min.   :0.0000   Min.   :0.00000   Min.   :-21.9725
 Median :0.3198   Median :0.27492   Median : -1.1910
 Mean   :0.3074   Mean   :0.34647   Mean   : -1.8610
 Max.   :0.5504   Max.   :1.00000   Max.   :  3.7085
                                    NA's   :  4.0000
```

GWAS analysis

- 28501 SNPs, all with Allele 1, Allele 2
- 1000 subjects, 500 controls (`cc=0`) and 500 cases (`cc=1`)
- **Far too much** data for a regular `summary()` of `snps.10` – even in this small example

GWAS analysis

We'll use just the column summaries, and a (mildly) ‘clean’ subset;

```
> snpsum <- col.summary(snps.10)
> use <- with(snpsum, MAF > 0.01 & z.HWE^2 < 200)

> table(use)
use
FALSE  TRUE
  317 28184
```

GWAS analysis

Now do single-SNP tests for each SNP, and extract the p-value for each SNP, along with its location;

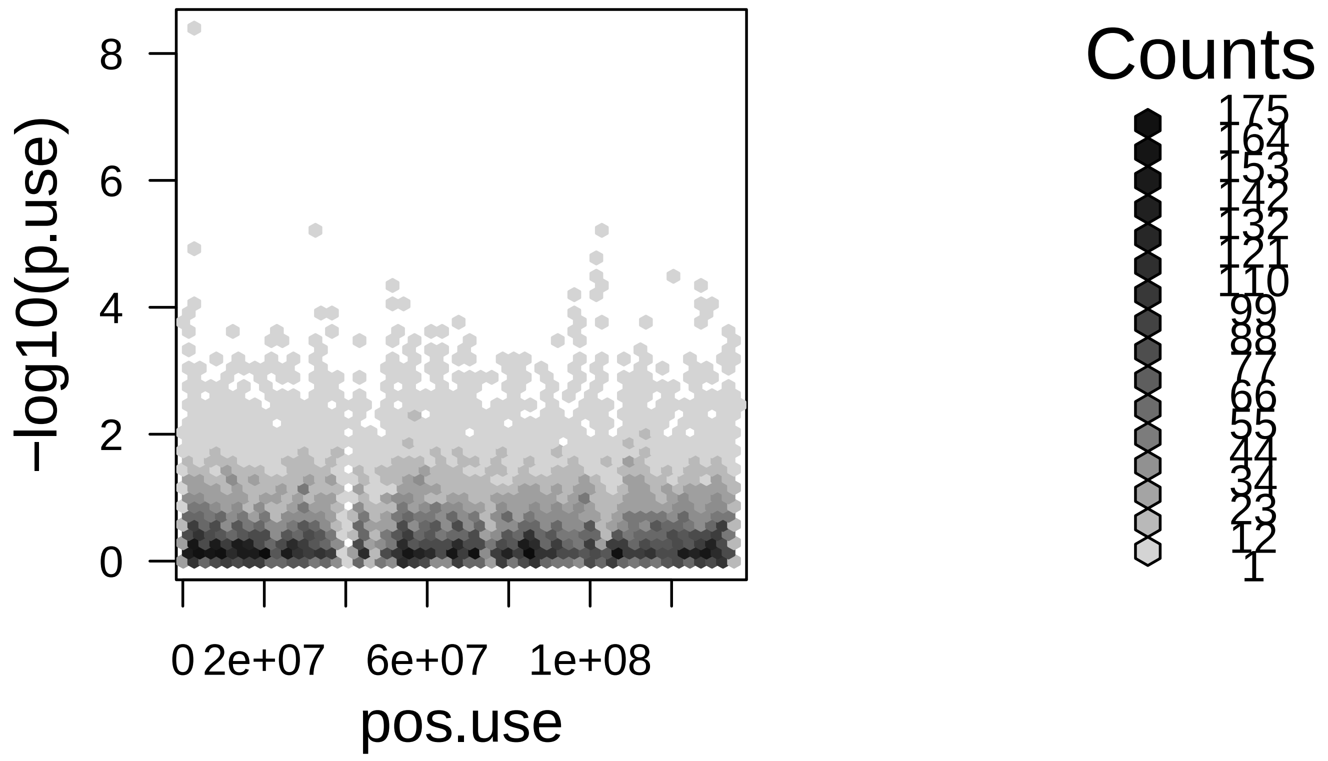
```
tests <- single.snp.tests(cc, data = subject.support,
+ snp.data = snps.10)
```

```
pos.use <- snp.support$position[use]
p.use   <- p.value(tests, df=1)[use]
```

We'd usually give a table of 'top hits,' but...

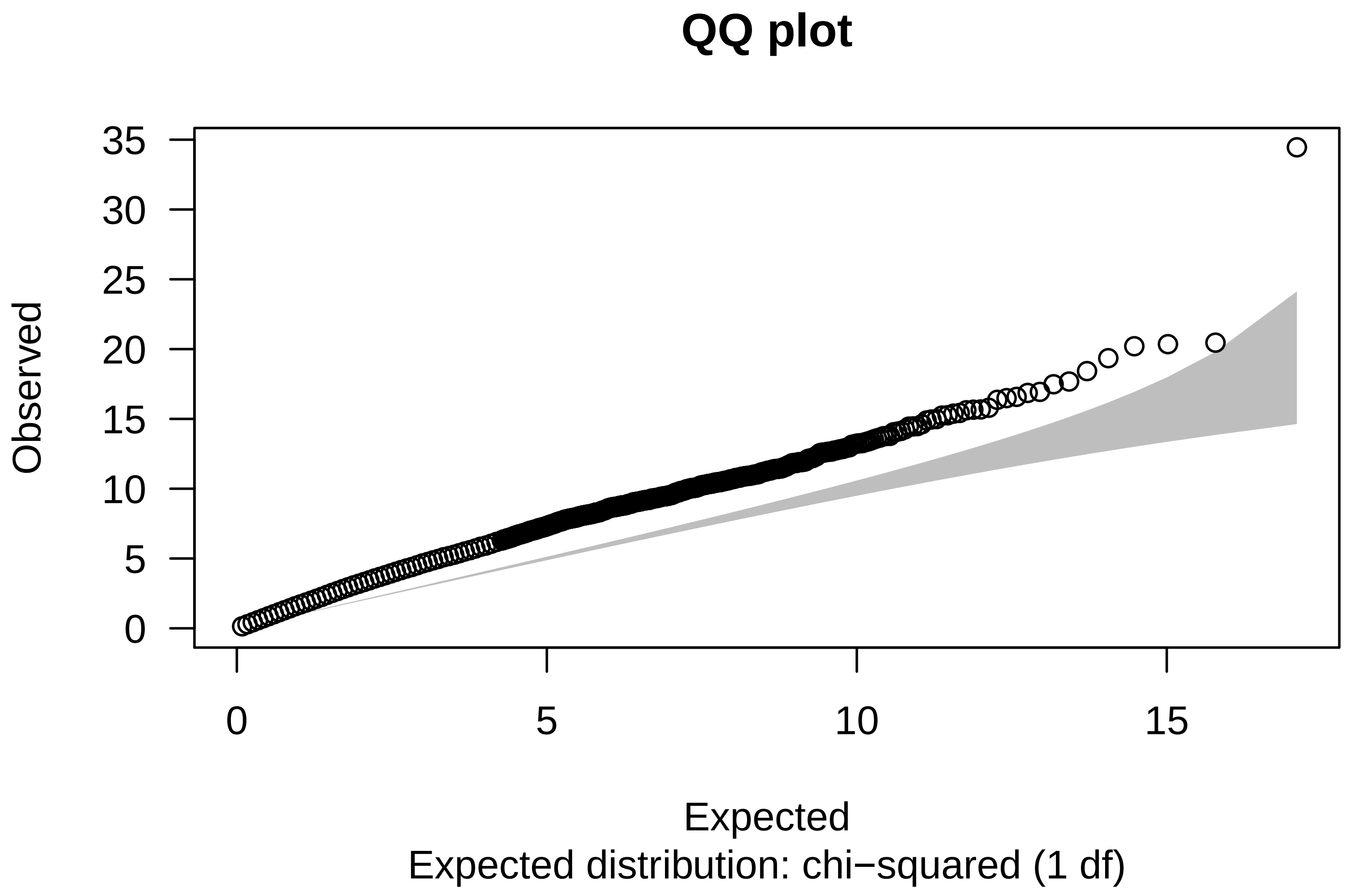
GWAS analysis

```
plot(hexbin(pos.use, -log10(p.use), xbin = 50))
```



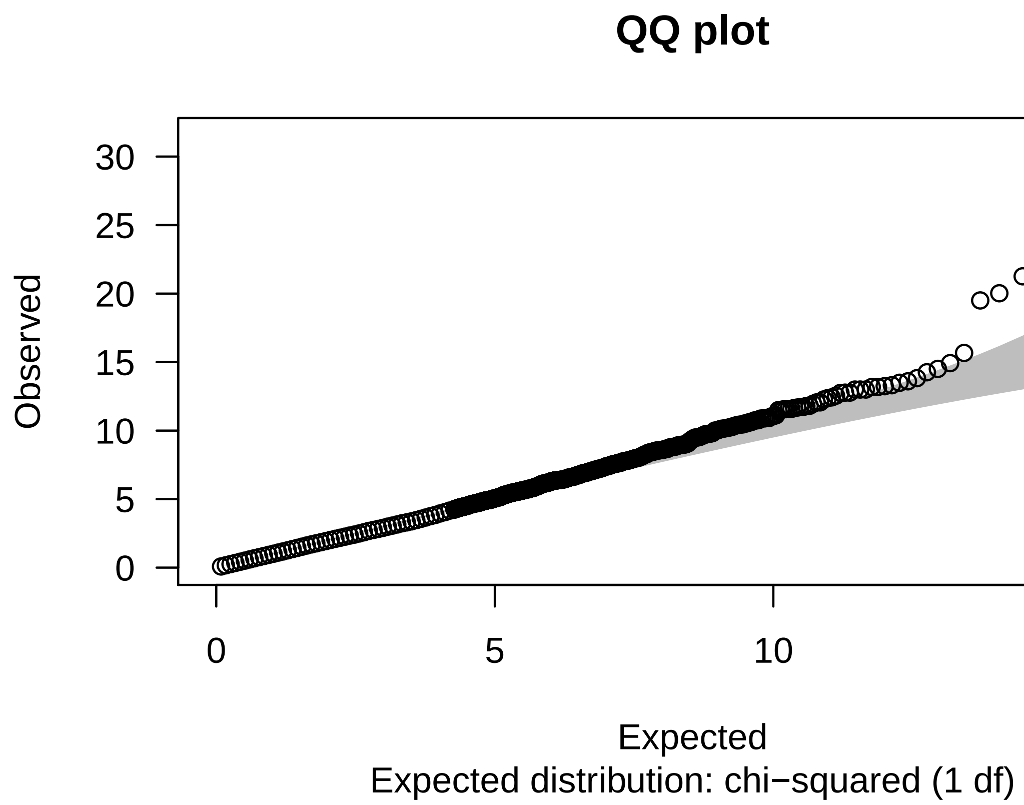
GWAS analysis

```
qq.chisq(chi.squared(tests, df=1)[use], df=1)
```



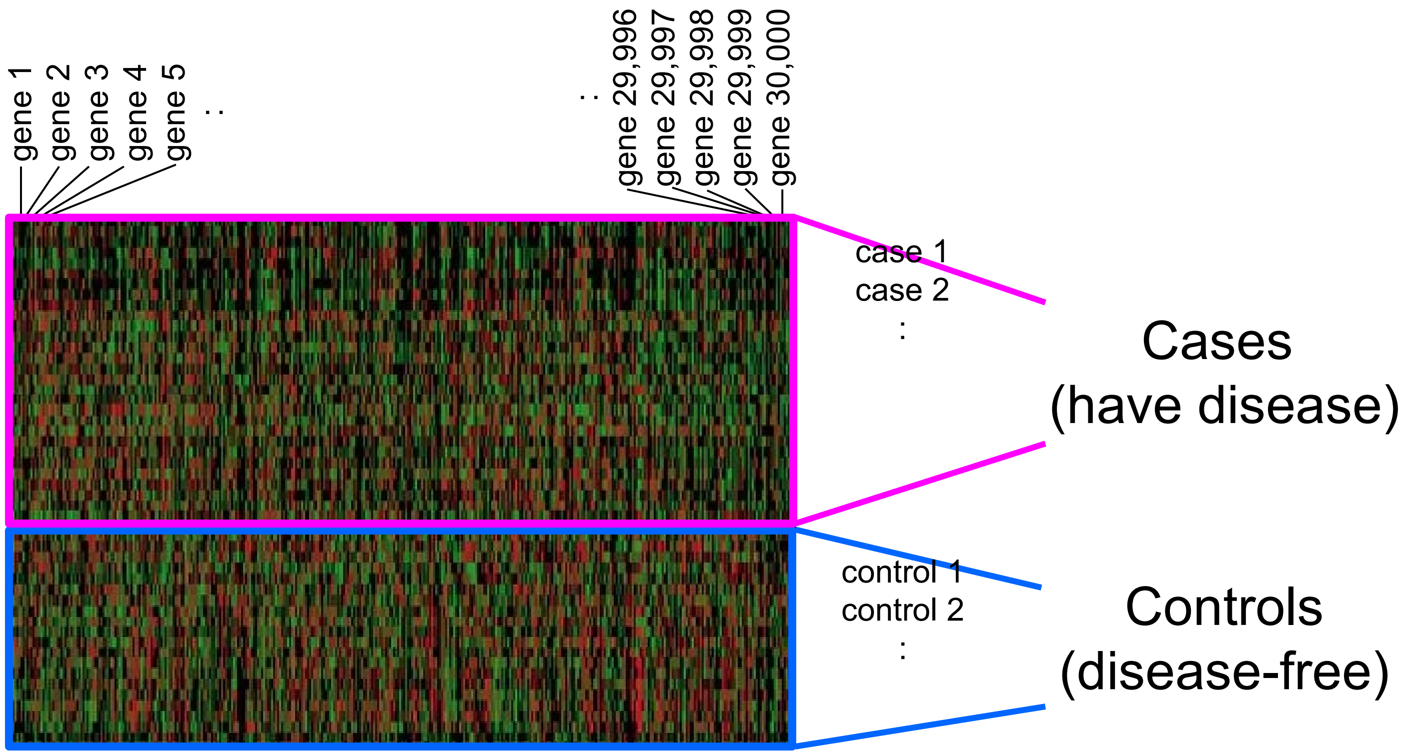
GWAS analysis

```
tests2 <- single.snp.tests(cc, stratum, data = subject.support,
+ snp.data = snps.10)
qq.chisq(chi.squared(tests2, 1)[use], 1)
```



Signficance Analysis of Microarrays (SAM)

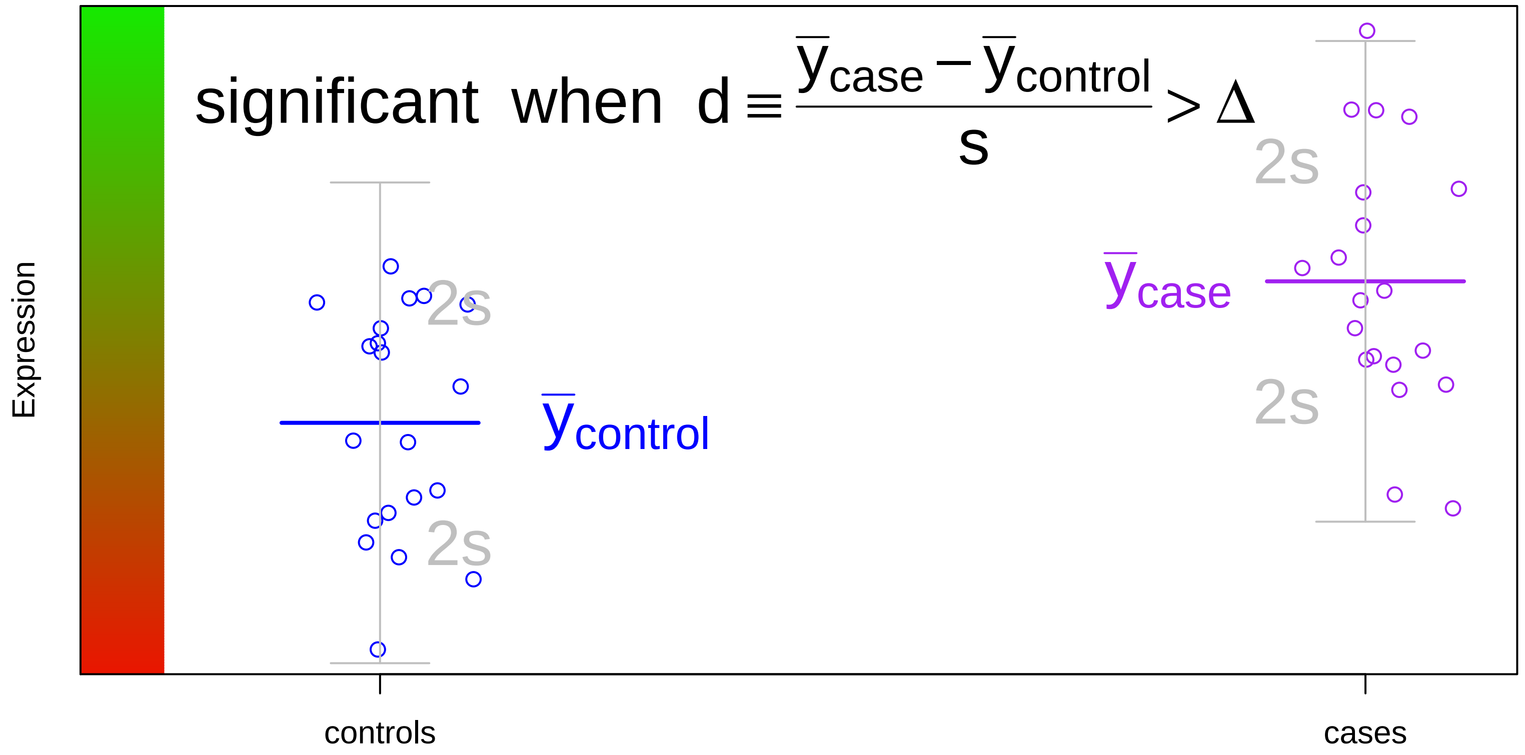
SAM is a popular new method (Tusher et al 2001) which identifies **differentially expressed genes**



i.e. large red/green difference between cases and controls

Signficance Analysis of Microarrays (SAM)

Why so popular? Here's the traditional method;



Do this $\times$30,000 genes; d in each is **quite unstable**. Small values of s give large d, which may give **false positive** results

Signficance Analysis of Microarrays (SAM)

SAM has a quick fix for this problem;

Traditional	SAM
$d_i = \dfrac{\bar{y}_{i,\text{case}} - \bar{y}_{i,\text{control}}}{s_i}$	$d_i = \dfrac{\bar{y}_{i,\text{case}} - \bar{y}_{i,\text{control}}}{s_i + s_0}$

For each gene (each i), SAM's s_0 **borrows strength** from the other genes.

SAM (and `siggenes`) then does some clever permutation testing to produce False Discovery Rates

Signficance Analysis of Microarrays (SAM)

Golub et al (1999) give differential expression for 3,051 genes, in 27 ‘controls’ (ALL) and 11 ‘cases’ (AML)

```
> library(multtest)
> data(golub)
> table(golub.cl)
 0  1
27 11
```

Now let’s do the SAM analysis; we give a **random seed** for the permutations – and tell R how many to do;

```
> sam.out <- sam(golub, golub.cl, B=100, rand = 123)
```

... takes only a few seconds. Use `B=1000` or more if you can

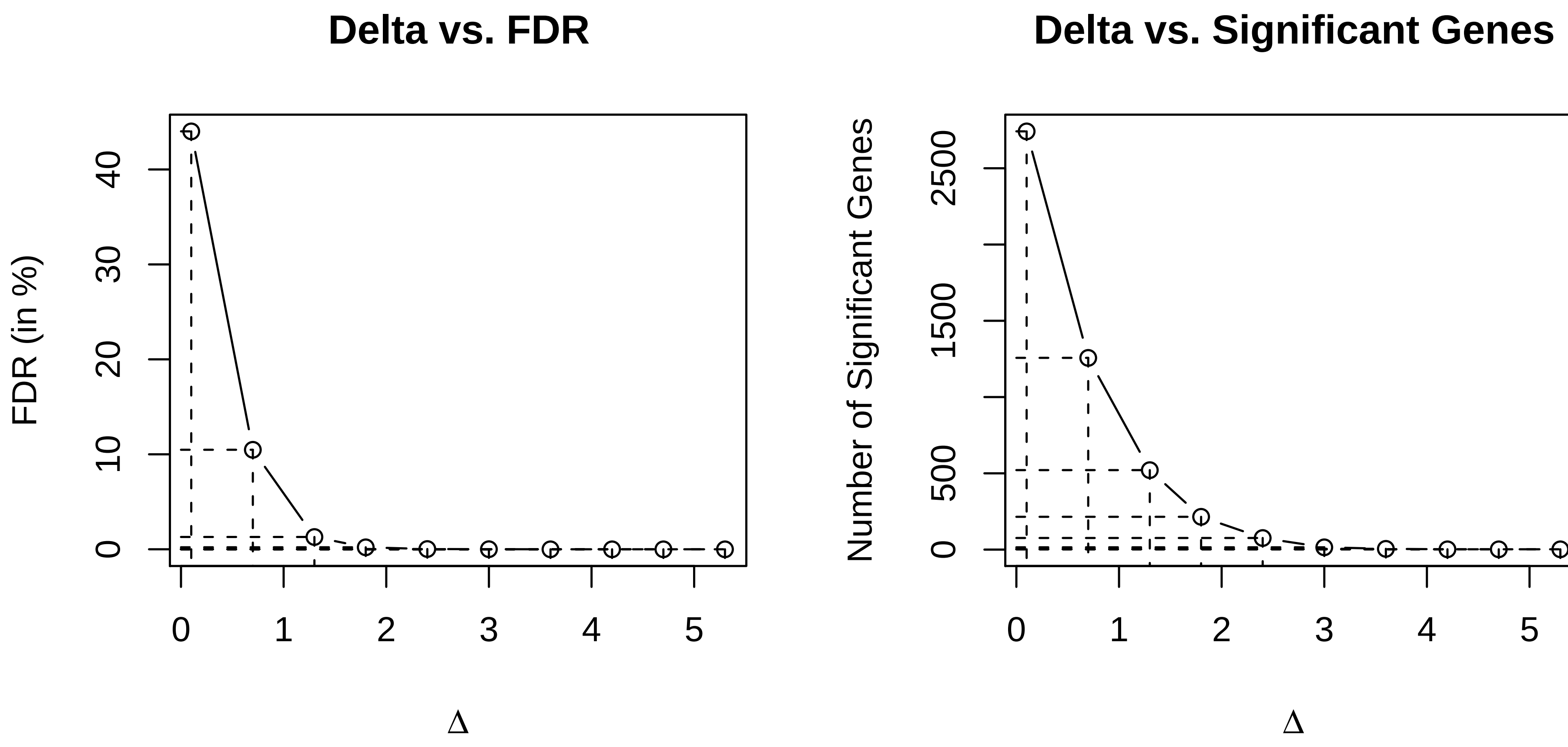
Signficance Analysis of Microarrays (SAM)

```
> summary(sam.out)
 s0 = 0.0584  (The 0 % quantile of the s values.)
 Number of permutations: 1000
   Delta    p0    False Called      FDR cutlow cutup   j2   j1
1    0.1 0.499 2420.329   2742 0.440123 -0.160 0.244 1446 1756
2    0.7 0.499  264.208   1257 0.104804 -1.247 1.438  746 2541
3    1.3 0.499   13.526    521 0.012945 -2.270 2.488  325 2856
4    1.8 0.499    0.903    215 0.002094 -3.119 3.311  139 2976
5    2.4 0.499    0.043     76 0.000282 -4.157 4.259   44 3020
6    3.0 0.499    0.003     15 9.97e-05 -5.577 5.139    4 3041
7    3.6 0.499        0      5        0   -Inf 5.971    0 3047
8    4.2 0.499        0      2        0   -Inf 7.965    0 3050
9    4.7 0.499        0      2        0   -Inf 7.965    0 3050
10   5.3 0.499        0      2        0   -Inf 7.965    0 3050
```

p0 is the **prior** probability of differential expression. Also note that the FDR values are **rounded**

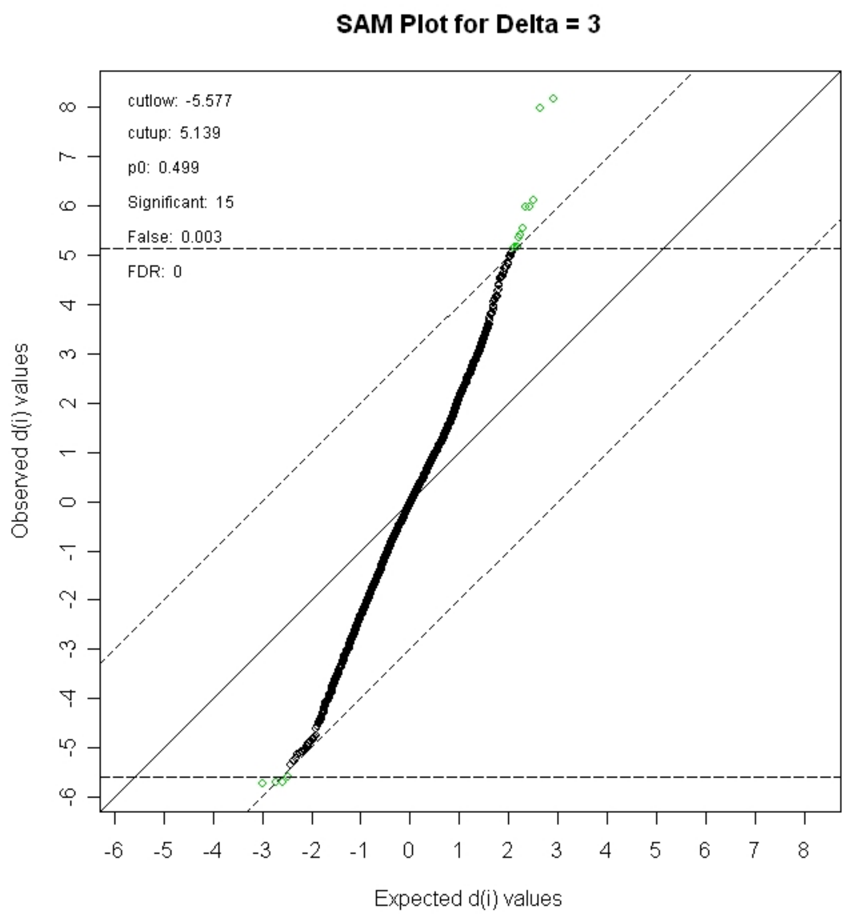
Signficance Analysis of Microarrays (SAM)

```
> plot(sam.out)
```



Signficance Analysis of Microarrays (SAM)

```
> plot(sam.out, 3) #specifies Delta
```



Microarray analysis with `limma`

The `limma` package can do **several** analyses for microarrays. It reads in **raw data**, in standard formats

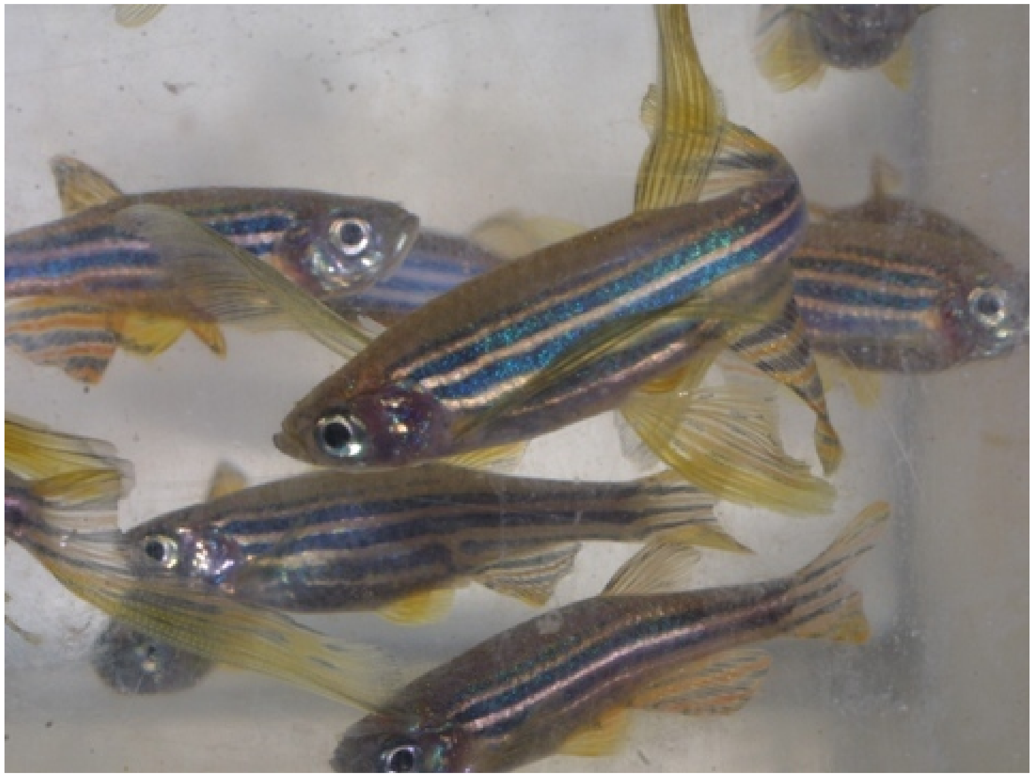
```
> library(limma)
> my.files <- dir(pattern=".spot")
> my.files
[1] "swirl.1.spot" "swirl.2.spot" "swirl.3.spot" "swirl.4.spot"
> RG <- read.maimages(my.files, source="spot")
Read swirl.1.spot
Read swirl.2.spot
Read swirl.3.spot
Read swirl.4.spot
```

Microarray analysis with `limma`

What is `swirl`? A mutation affecting **zebrafish**

Microarray analysis with `limma`

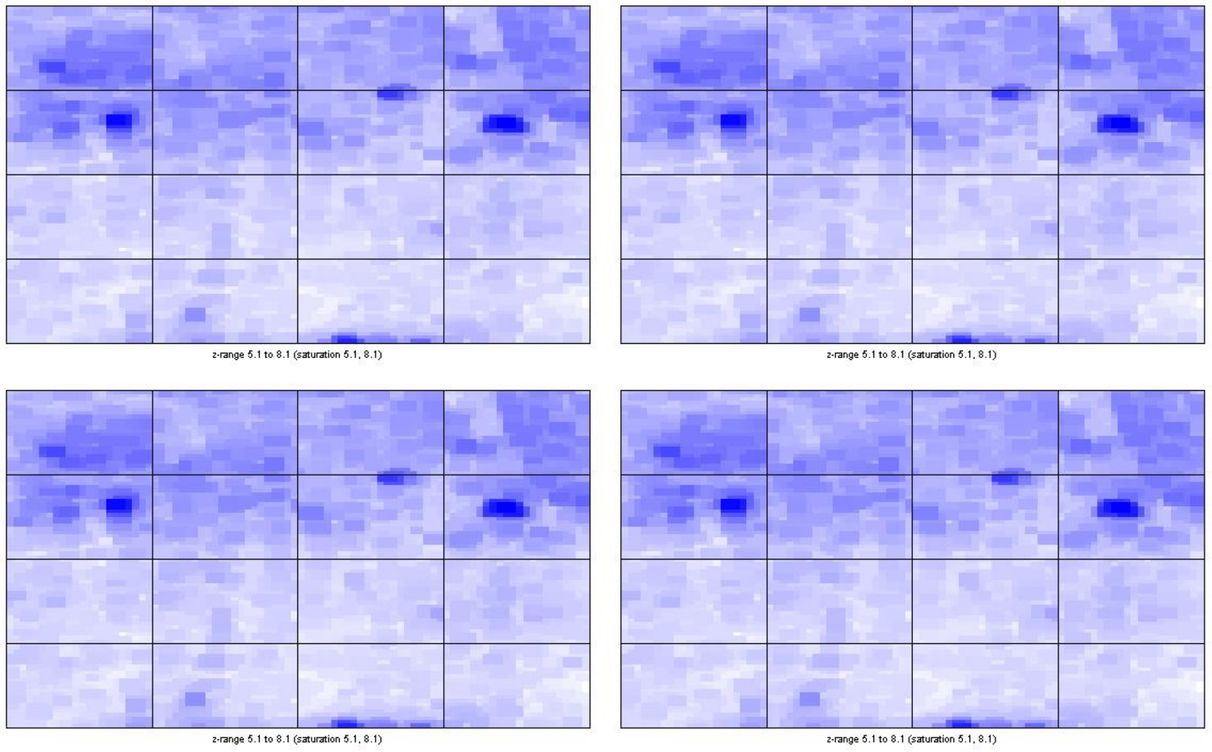
What is `swirl`? A mutation affecting **zebrafish**



We have 2 mutants, and 2 wild-type fish

Microarray analysis with `limma`

Here are the red intensities from each microarray;

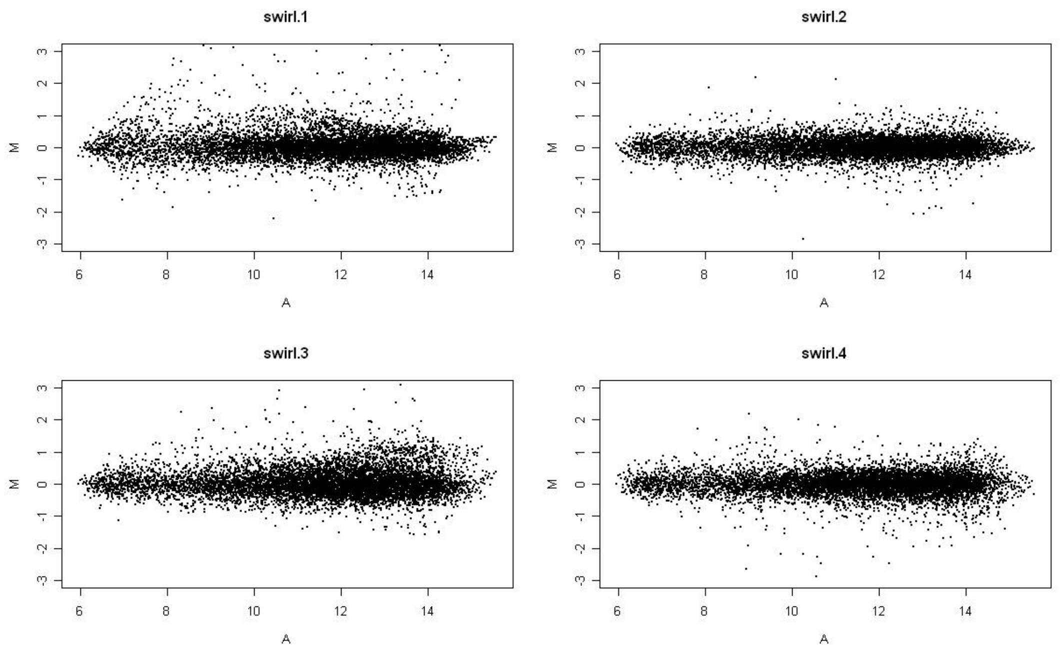


– need to **normalize** each array (or get a bigger sample!)

Microarray analysis with `limma`

`limma` has 'default' normalization techniques

```
> MA1 <- normalizeWithinArrays(RG)
> MA2 <- normalizeBetweenArrays(MA1)
```



Can you guess where the 'signals' are?

Microarray analysis with `limma`

`limma` fits ‘plain’ models to each gene, and also ‘robustifies’ them with an Empirical Bayes approach (much the same as SAM)

```
> fit1 <- lmFit(MA2, design=c(-1,1,-1,1))
> options(digits=3); toptable(fit, n=30, adjust="fdr")
         M     t  P.Value adj.P.Val    B
2961 -2.66 -20.8 1.44e-07   0.00121 7.55
3723 -2.19 -17.6 4.59e-07   0.00194 6.75
1611 -2.19 -16.1 8.44e-07   0.00238 6.29
7649 -1.60 -14.2 2.02e-06   0.00326 5.58
515   1.26  13.7 2.55e-06   0.00326 5.39

> fit2 <- eBayes(fit1)
> options(digits=3); topTable(fit2, n=30, adjust="fdr")
     Block Row Column      ID   Name     M     A     t  P.Value adj.P.Val    B
2961     6  14      9 fb85d05 18-F10 -2.66 10.33 -20.8 1.44e-07   0.00121 7.55
3723     8   2      3 control   Dlx3 -2.19 13.24 -17.6 4.59e-07   0.00194 6.75
1611     4   2      3 control   Dlx3 -2.19 13.45 -16.1 8.44e-07   0.00238 6.29
7649    15  11     17 fb58g10 11-L19 -1.60 13.49 -14.2 2.02e-06   0.00326 5.58
515      1  22     11 fc22a09 27-E17  1.26 13.19  13.7 2.55e-06   0.00326 5.39
```