9. More Bioconductor packages

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GWAS analysis

Genome-Wide Association Studies (GWAS) provide a good example as high-throughput work – typically, these studies genotype e.g. 1M-10M SNPs on several thousand subjects in large, already-established studies.

- Usually on 1000's of subjects
- Analysis uses 'simple' t-tests or regressions, for each SNP (like microarrays)
- Millions of *anything* takes a long time! (up to 72 hours)
- Just **loading** big datasets is non-trivial – but some tools are available, suggesting other ways to speed up analysis

GWAS analysis

snpStats is a Bioconductor package for GWAS analysis – written by David Clayton (analysis lead on Wellcome Trust)

```
> biocLite("snpStats") #in a new session
> library(snpStats)
> data(for.exercise)
> ls()
[1] "snp.support" "snps.10" "subject.support"
```

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 SnpMatrix 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`)
- This is *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;

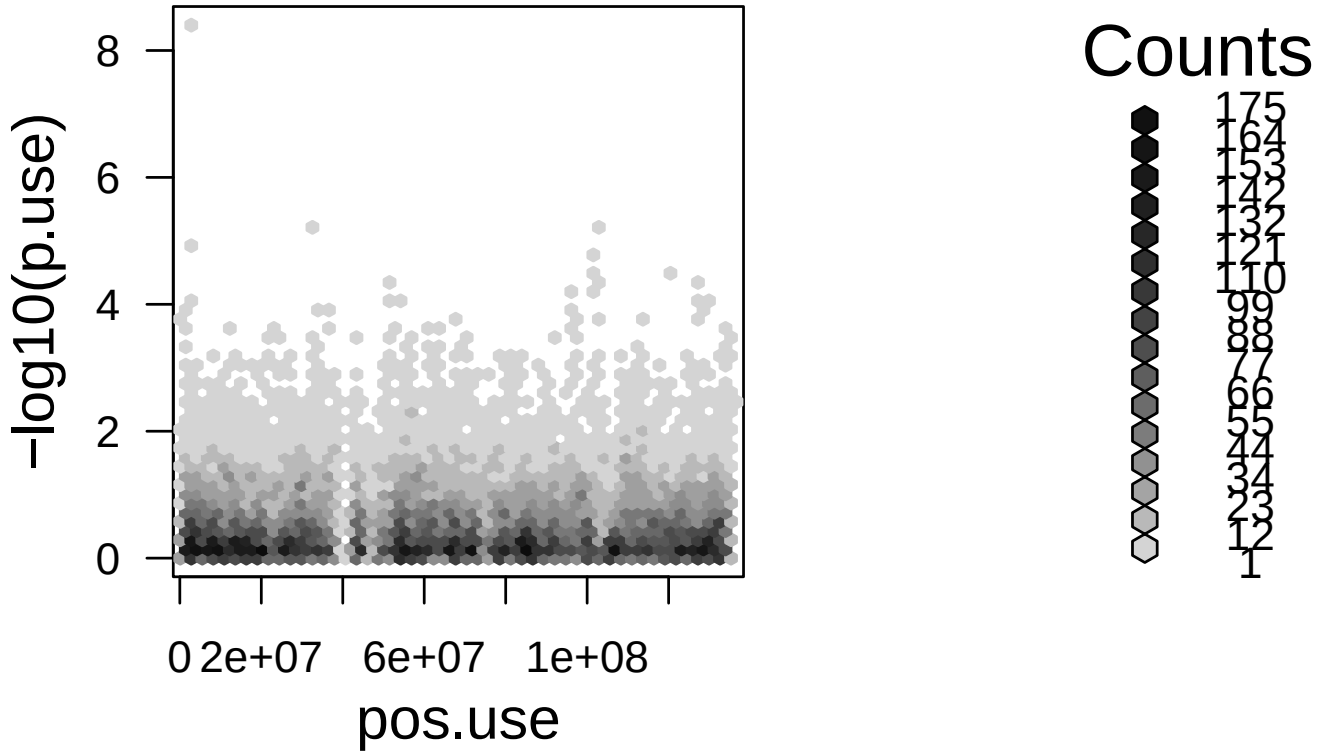
```
tests <- single.snp.tests(cc, data = subject.support,
+ snp.data = snps.10)

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

A (long) table of 'top hits' will keep investigators busy. For a graphical summary...

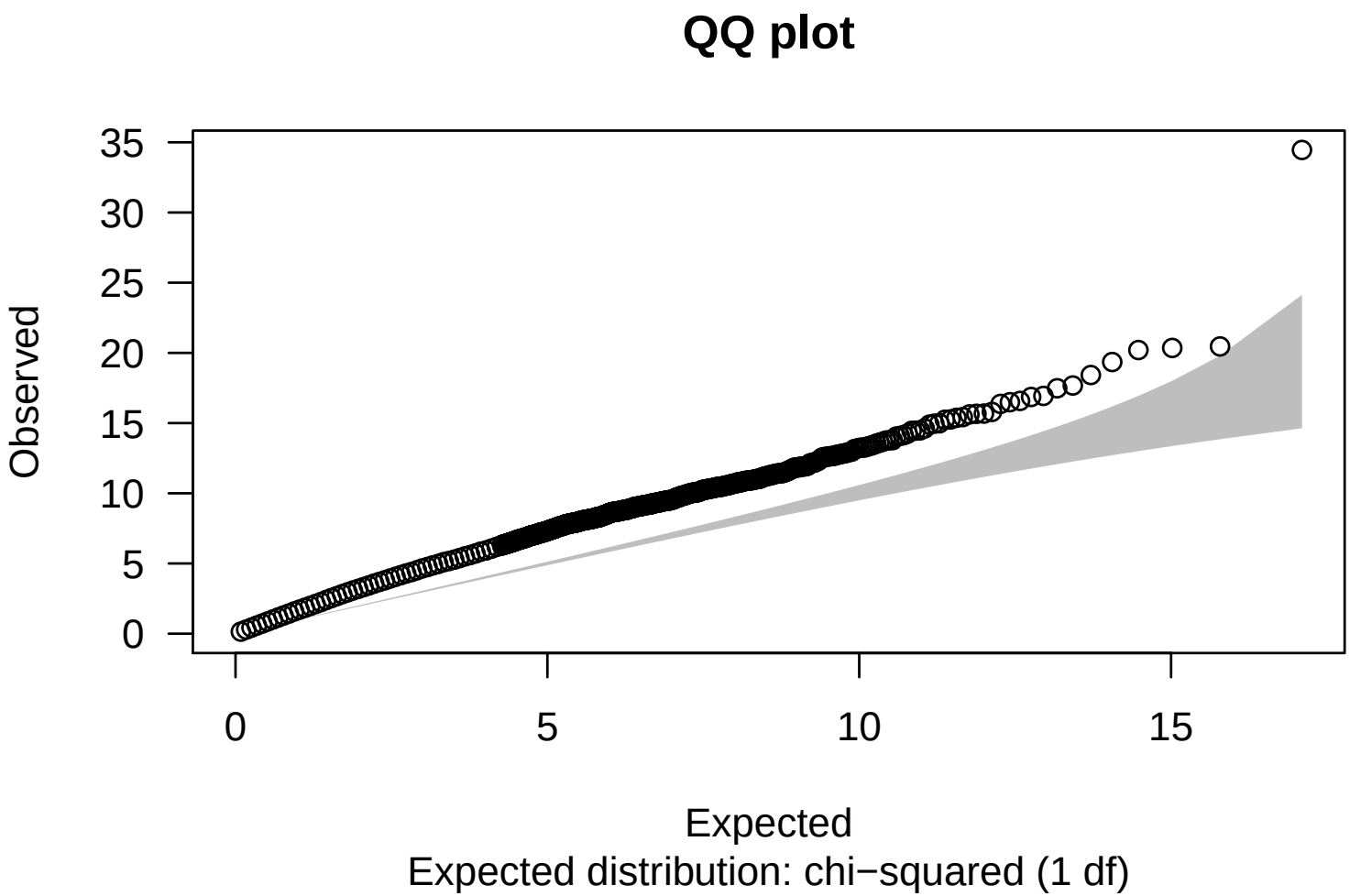
GWAS analysis

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



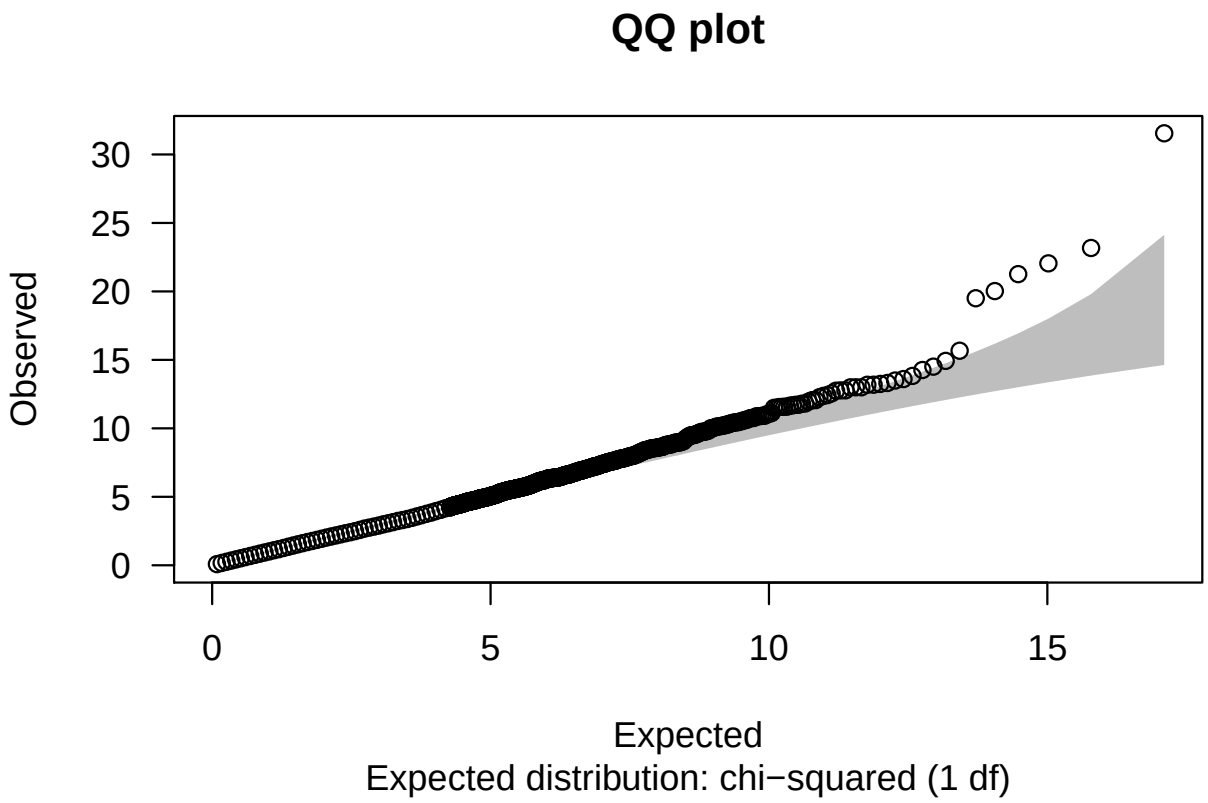
GWAS analysis

```
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)
```



Handling short-read sequences

The `ShortRead` package handles short-read (a.k.a. 'next-generation') sequencing reads, mostly for QC and preprocessing.

It comes with a small subset of a Solexa sequencing run: we will look at this example.

The data are in a structured set of folders, so the first step is to specify where they can be found.

```
exptPath <- "/Users/tlumley/Library/R/2.15/library/ShortRead/extdata"
```

and then use the `SolexaPath()` function to create a simple summary of the structure, which we will pass to other functions instead of a filename

Handling short-read sequences

```
> sp <- SolexaPath(exptPath)
> sp
class: SolexaPath
experimentPath: /Users/tlumley/Library/R/2.15/library/ShortRead/extdata
dataPath: Data
scanPath: NA
imageAnalysisPath: C1-36Firecrest
baseCallPath: Bustard
analysisPath: GERALD
> imageAnalysisPath(sp)
[1] "/Users/tlumley/Library/R/2.15/library/ShortRead/extdata/
      Data/C1-36Firecrest"
> analysisPath(sp)
[1] "/Users/tlumley/Library/R/2.15/library/ShortRead/extdata/
     Data/ C1-36Firecrest/Bustard/GERALD"
```

Reading data

The `readAligned()` function reads in the aligned sequence fragments;

```
> aln <- readAligned(sp, "s_2_export.txt")
> aln
class: AlignedRead
length: 1000 reads; width: 35 cycles
chromosome: NM NM ... chr5.fa 29:255:255
position: NA NA ... 71805980 NA
strand: NA NA ... + NA
alignQuality: NumericQuality
alignData varLabels: run lane ... filtering contig
```

Examining data

The `sread()` function returns the bases read;

```
> sread(aln)
  A DNAStringSet instance of length 1000
      width seq
   [1]    35 CCAGAGCCCCCCGCTCACTCCTGAACCAGTCTCTC
   [2]    35 AGCCTCCCTCTTTCTGAATATACGGCAGAGCTGTT
   [3]    35 ACCAAAAACACCACATACACGAGCAACACACGTAC
   [4]    35 AATCGGAAGAGCTCGTATGCCGGCTTCTGCTTGGA
   [5]    35 AAAGATAAACTCTAGGCCACCTCCTCCTTCTTCTA
<continues>
```

...and the `quality()` function gives the read quality, coded with Z as the best.

Examining data

```
> quality(aln)
class: SFastqQuality
quality:
  A BStringSet instance of length 1000
      width seq
   [1]    35 YQMIMIMMLMMIGIGMFICMFFFIMMHIIHAAGAH
   [2]    35 ZXZUYXZQYYXUZXYZYYZZXXZZIMFHXQSUPPO
   [3]    35 LGDHLILLLLLLLLIGFLLALDIFDILLHFIAECAE
   [4]    35 JJYYIYVSYYYYYYYYSDYYWVUYYNNVSVQQELQ
   [5]    35 LLLILIIIDLLHLLLLLLLLLLALLLLHLLLLEL
   [6]    35 YYYYYYYWVVMGGUHQHQMUFMICDMCDHQHEDDD
   [7]    35 ZZZZZZZZZZYZZZZZYZZZZYYZZZZZZZUUUUU
   [8]    35 ZZZZZZZZUZZUZZZZZZZZZZZZYZYZZZUUHUH
   [9]    35 ZZZZZZZYZZYZZZZYZZZZZZZZZZZZXUNUUU
```

Note how the quality goes down later in the read.

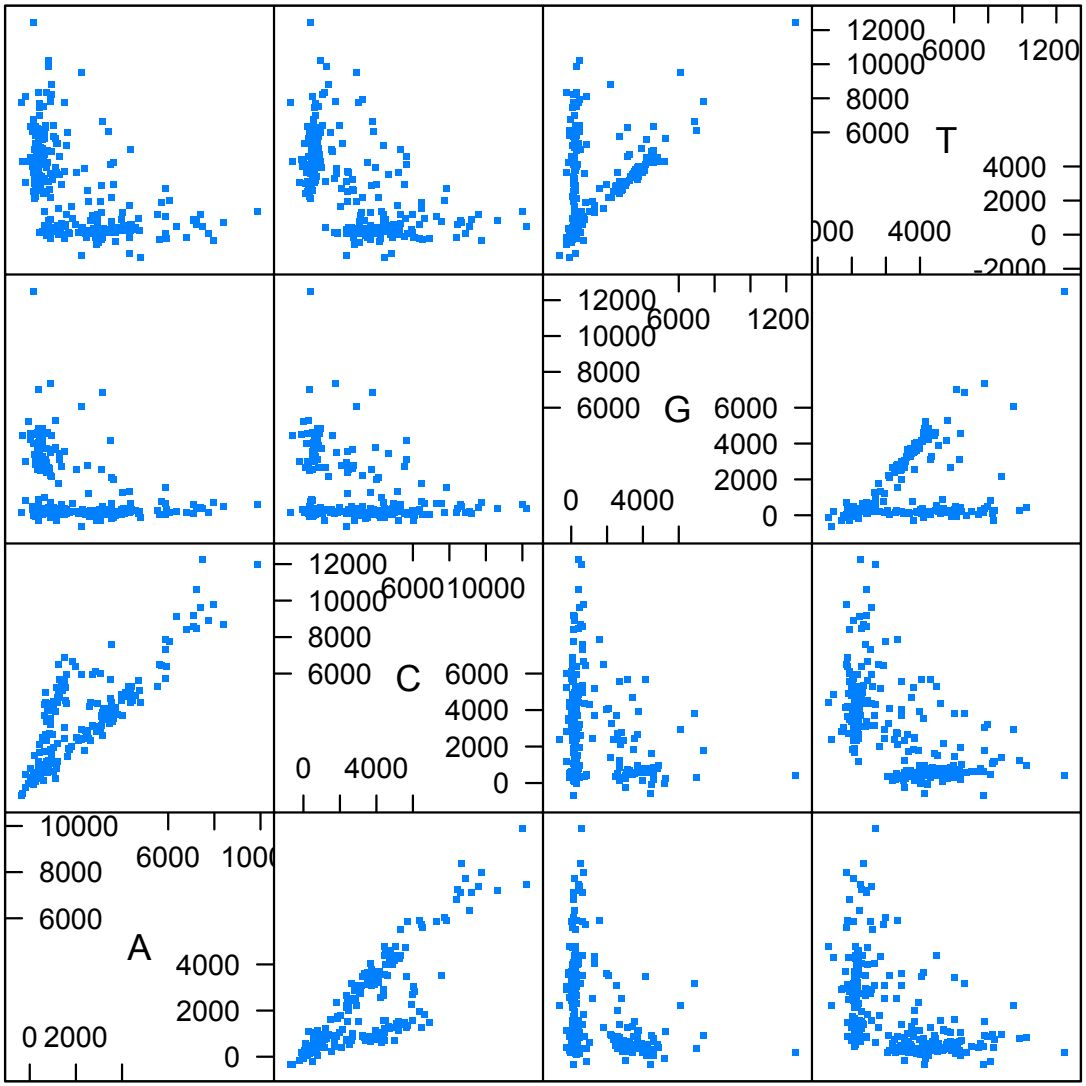
Examining data

We can read other information, such as the fluorescence intensities for each base

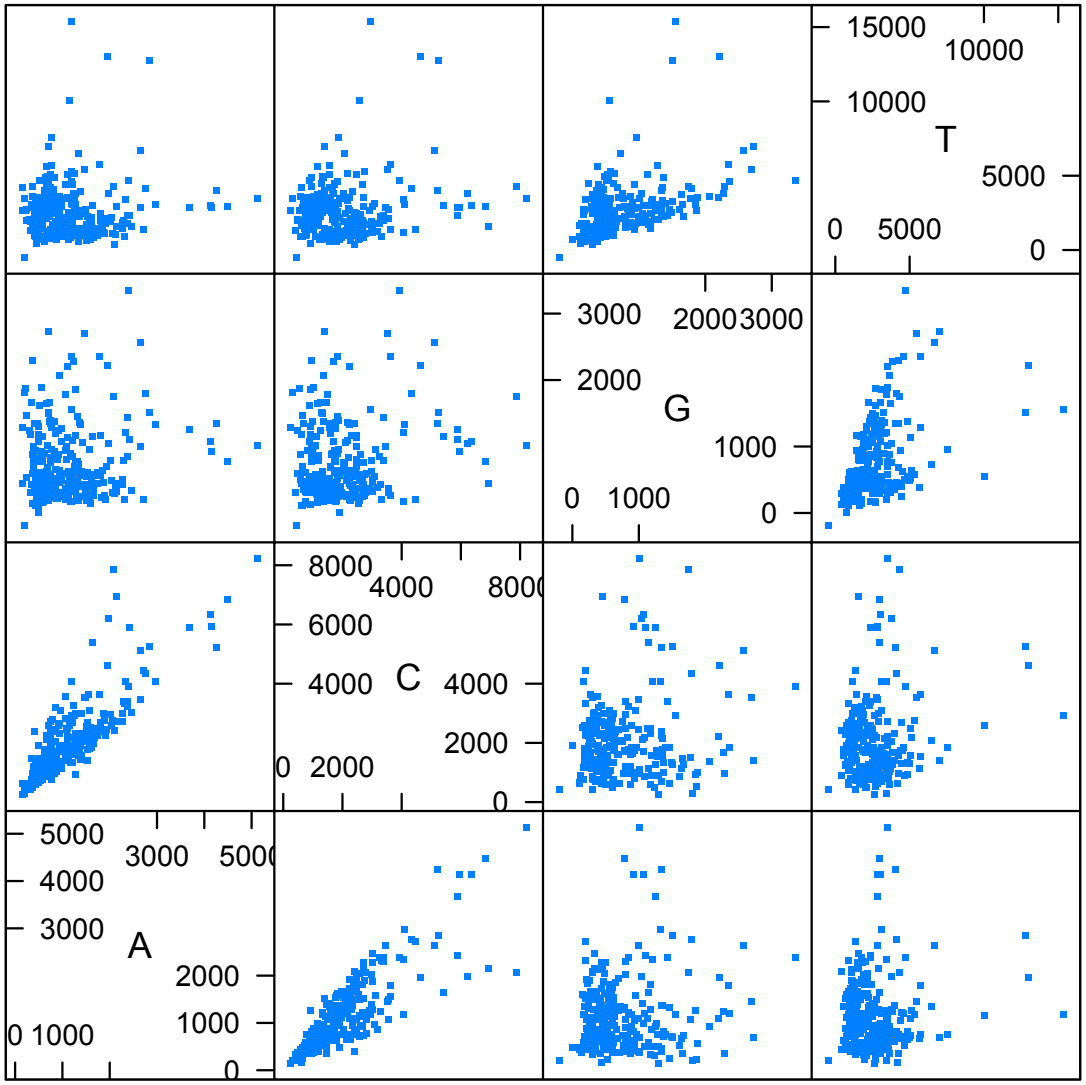
```
> int <- readIntensities(sp)
> int
class: SolexaIntensity
dim: 256 4 36
readInfo: SolexaIntensityInfo
intensity: ArrayIntensity
measurementError: ArrayIntensity
> splom(intensity(int)[[,,2]], pch=".", cex=3)
> splom(intensity(int)[[,,35]],pch=".",cex=3)
```

and then create a scatterplot matrix of the intensities for the 2nd and 35th bases. The following plots show the correlation between A-T and C-G channels, and the deterioration towards the end of the read.

Intensities: 2nd position



Intensities: 35th position



Scatter Plot Matrix

DIfferential expression by RNAseq

The `edgeR` package (among others) estimates differential RNA expression from RNAseq experiments. It's a sequel to the `limma` package for microarray gene expression data.

RNAseq produces a count for each transcript, rather than the continuous measure produced by microarray experiments, and the statistical analysis relies on models for variation in counts.

We will look at an RNAseq experiment comparing gene expression in prostate cancer cells with and without treatment with an androgen (a testosterone analogue).

The experiment had three treated samples and four control samples, sequenced in seven of the lanes of a single flow cell on an Illumina 1G sequencer.

The data have been mapped to the human genome and turned into counts of transcripts in a simple text file.

RNAseq of prostate cancer

```
> x <- read.delim("pnas_expression.txt", row.names=1,
      stringsAsFactors=FALSE)
> head(x)
                 lane1 lane2 lane3 lane4 lane5 lane6 lane8  len
ENSG00000215696     0     0     0     0     0     0     0  330
ENSG00000215700     0     0     0     0     0     0     0 2370
ENSG00000215699     0     0     0     0     0     0     0 1842
ENSG00000215784     0     0     0     0     0     0     0 2393
ENSG00000212914     0     0     0     0     0     0     0  384
ENSG00000212042     0     0     0     0     0     0     0   92
```

The `stringsAsFactors` argument is needed because we want to keep the transcript names as strings, not turn them into factors.

RNAseq of prostate cancer

We also need to specify which 'lanes' are treated and which are control;

```
> targets
     Lane Treatment Label
Con1    1   Control  Con1
Con2    2   Control  Con2
Con3    3   Control  Con3
Con4    4   Control  Con4
DHT1    5       DHT  DHT1
DHT2    6       DHT  DHT2
DHT3    8       DHT  DHT3
```

RNAseq of prostate cancer

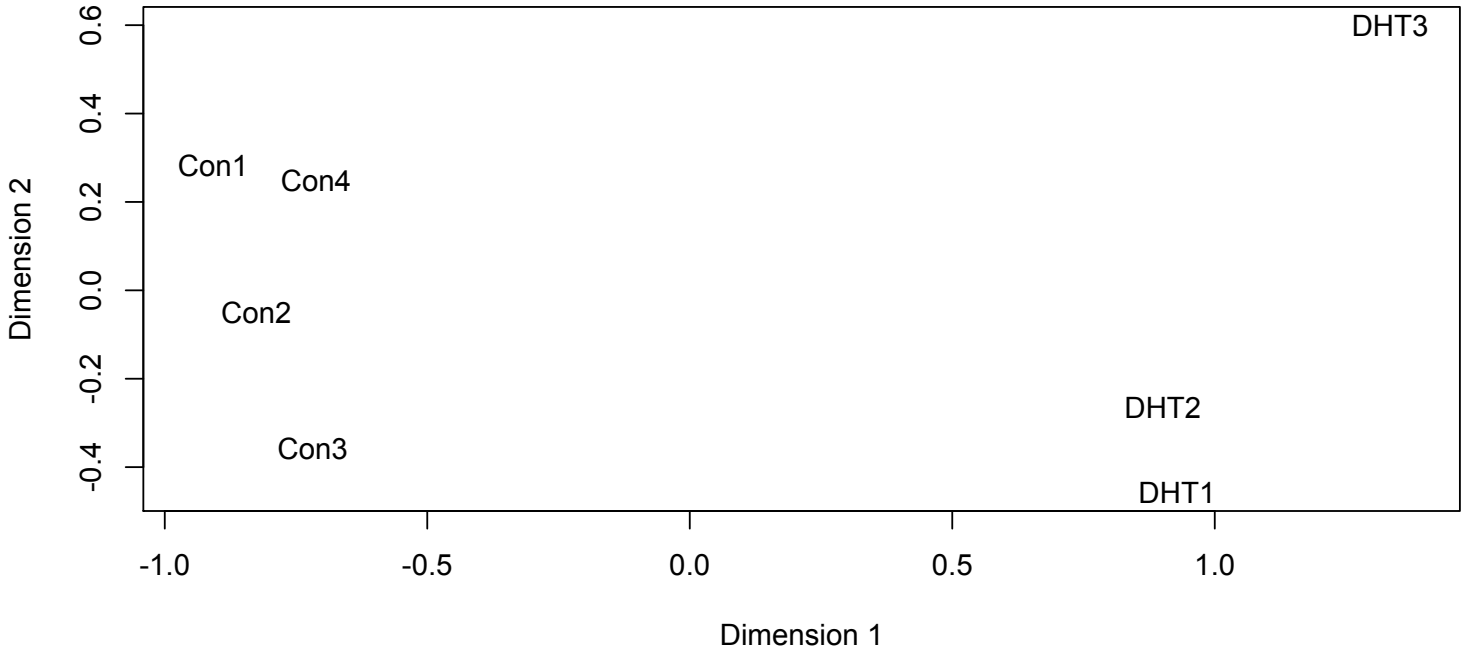
Putting the two together and filtering out low-expression transcripts gives data for analysis;

```
y <- DGEList(counts=x[,1:7], group=targets$Treatment,
             genes=data.frame(Length=x[,8]))
colnames(y) <- targets$Label
keep <- rowSums(cpm(y)>1) >= 3
y <- y[keep,]
y$samples$lib.size <- colSums(y$counts)
```

Here, `cpm()` means counts per million

Overall differences?

`plotMDS(y)` does multidimensional scaling, projecting the data into two dimensions to see how well separated the samples are. In this case there are big treated/control differences and smaller between-replicate differences;



Differential expression (at last)

The statistical analysis has two steps. First, the variability is estimated, then the treatment and control groups are compared for each gene, using a test related to Fisher's exact test.

```
y <- estimateCommonDisp(y, verbose=TRUE)
y <- estimateTagwiseDisp(y)

et <- exactTest(y)
top <- topTags(et)
```

Differential expression (at last)

The output gives the gene name the log fold-change, the overall abundance, the p-value and FDR.

```
Comparison of groups:  DHT-Control
                 Length    logFC    logCPM        PValue           FDR
ENSG00000151503   5605 5.704455  9.651026  0.000000e+00  0.000000e+00
ENSG00000096060   4093 4.881110  9.886097 1.065531e-315 8.787436e-312
ENSG00000166451   1556 4.539306  8.781549 1.168385e-217 6.423783e-214
ENSG00000127954   3919 8.054706  7.152971 3.838688e-200 1.582883e-196
ENSG00000162772   1377 3.204621  9.689874 8.819449e-170 2.909360e-166
ENSG00000113594  10078 3.960785  7.986306 1.108187e-144 3.046406e-141
ENSG00000116133   4286 3.143633  8.740383 1.291088e-138 3.042172e-135
ENSG00000115648   2920 2.513583 11.429414 6.775660e-130 1.396972e-126
ENSG00000123983   4305 3.473311  8.534717 9.250437e-129 1.695297e-125
ENSG00000116285   3076 4.104133  7.306976 1.758623e-128 2.900673e-125
```

Differential expression (at last)

We could use biomaRt or one of the other annotation packages to convert to other gene names

```
> getBM(attributes="hgnc_symbol",filters="ensembl_gene_id",
    values=rownames(top$table),mart=human)
   hgnc_symbol
1         FKBP5
2          LIFR
3          MLPH
4        DHCR24
5        ERRFI1
6         ACSL3
7        STEAP4
8        NCAPD3
9          ATF3
10        CENPN
```