Differential Gene Expression

Workshop on RNA-Seq

Roy Francis | 26-Nov-2020
NBIS, SciLifeLab

Preparation

- Create the DESeq2 object

```
library(DESeq2)
mr$Group <- factor(mr$Group)
d <- DESeqDataSetFromMatrix(countData=cf,colData=mr,design=~Group)
d
```

```
## class: DESeqDataSet
## dim: 17515 6
## metadata(1): version
## assays(1): counts
## rownames(17515): mt-Cytb mt-Td ... 4930447M23Rik Gm6518
## rowData names(0):
## colnames(6): DSSd00_1 DSSd00_2 ... DSSd07_2 DSSd07_3
## colData names(7): SampleName SampleID ... Group Replicate
```

- Categorical variables must be factors
- Building GLM models: `~var`, `~covar+var`

Size factors

- Normalisation factors are computed

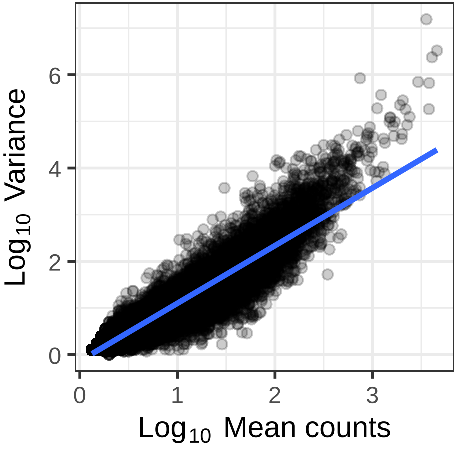
```
d <- DESeq2::estimateSizeFactors(d,type="ratio")
sizeFactors(d)
```

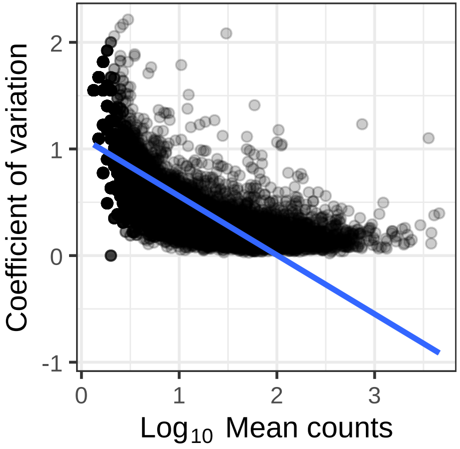
```
##  DSSd00_1  DSSd00_2  DSSd00_3  DSSd07_1  DSSd07_2  DSSd07_3
## 1.0153287 0.9597101 0.9984645 1.0358161 1.0787996 0.9988740
```

Dispersion

- We need to measure the variability of gene counts

```
dm <- apply(cf,1,mean)
dv <- apply(cf,1,var)
cva <- function(x) sd(x)/mean(x)
dc <- apply(cf,1,cva)
```



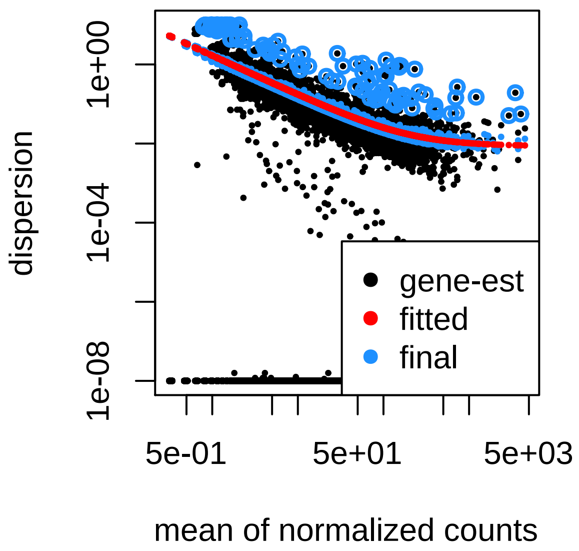


- Dispersion is a measure of variability in gene expression for a given mean

Dispersion

- Dispersion is unreliable for low mean counts
- Genes with similar mean values must have similar dispersion
- Estimate likely (ML) dispersion for each gene based on counts
- Fit a curve through the gene-wise estimates
- Shrink dispersion towards the curve

```
d <- DESeq2::estimateDispersions(d)
{par(mar=c(4,4,1,1))
plotDispEsts(d)}
```



Testing

- Log2 fold changes changes are computed after GLM fitting

```
dg <- nbinomWaldTest(d)
resultsNames(dg)
```

```
## [1] "Intercept"            "Group_day07_vs_day00"
```

- Use `results()` to customise/return results
  - Set coefficients using `contrast` or `name`
  - Filtering results by fold change using `lfcThreshold`
  - `cooksCutoff` removes outliers
  - `independentFiltering` removes low count genes
  - `pAdjustMethod` sets method for multiple testing correction
  - `alpha` set the significance threshold

Testing

```r
res1 <- results(dg,name="Group_day07_vs_day00",alpha=0.05)
summary(res1)
```

```
##
## out of 17515 with nonzero total read count
## adjusted p-value < 0.05
## LFC > 0 (up)         : 194, 1.1%
## LFC < 0 (down)       : 217, 1.2%
## outliers [1]         : 1, 0.0057%
## low counts [2]       : 9169, 52%
## (mean count < 10)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results
```

Testing

```
head(res1)
```

```
## log2 fold change (MLE): Group day07 vs day00
## Wald test p-value: Group day07 vs day00
## DataFrame with 6 rows and 6 columns
##           baseMean log2FoldChange     lfcSE      stat    pvalue      padj
##          <numeric>      <numeric> <numeric> <numeric> <numeric> <numeric>
## mt-Cytb   1.304697       0.658760  1.658716  0.397150  0.691257        NA
## mt-Td     1.492515      -1.080724  1.521562 -0.710273  0.477535        NA
## mt-Co1    0.327758       1.813649  3.064543  0.591817  0.553973        NA
## mt-Tw    18.839105       0.209559  0.425694  0.492277  0.622524  0.930584
## mt-Ti     2.649343      -1.200646  1.135727 -1.057160  0.290438        NA
## mt-Nd1   23.325014       0.657781  0.401785  1.637148  0.101599  0.529489
```

- Use `lfcShrink()` to correct fold changes for high dispersion genes

Thank you. Questions?

R version 4.0.3 (2020-10-10)

Platform: x86_64-pc-linux-gnu (64-bit)

OS: Ubuntu 18.04.5 LTS

Built on : 📅 26-Nov-2020 at 🕒 14:58:35

2020 • SciLifeLab • NBIS