Differential Gene Expression

Workshop on RNA-Seq

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Preparation

- Create the DESeq2 object

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

```
## class: DESeqDataSet
## dim: 10573 6
## metadata(1): version
## assays(1): counts
## rownames(10573): ENSMUSG00000098104 ENSMUSG00000033845 ...
##   ENSMUSG00000063897 ENSMUSG00000095742
## 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

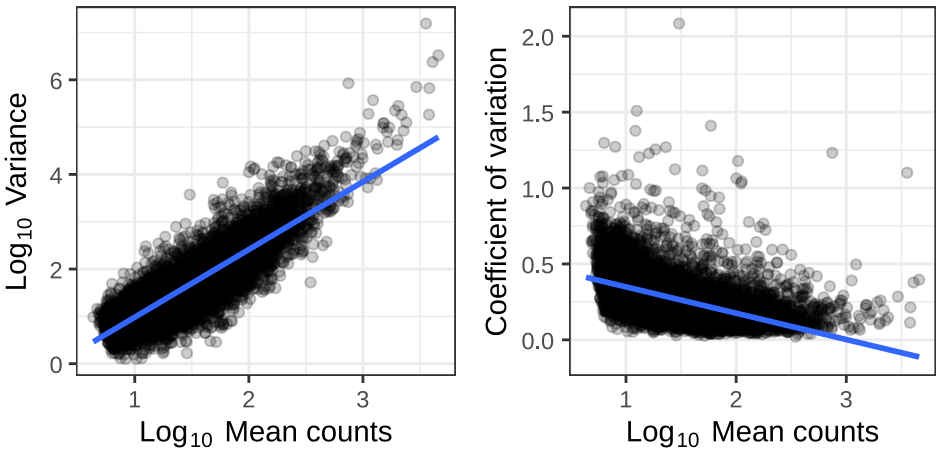
```
d <- DESeq2::estimateSizeFactors(d,type="ratio")
sizeFactors(d)
```

```
##  DSSd00_1  DSSd00_2  DSSd00_3  DSSd07_1  DSSd07_2  DSSd07_3
## 1.0136617 0.9570561 0.9965245 1.0354178 1.0780855 1.0017753
```

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)
```

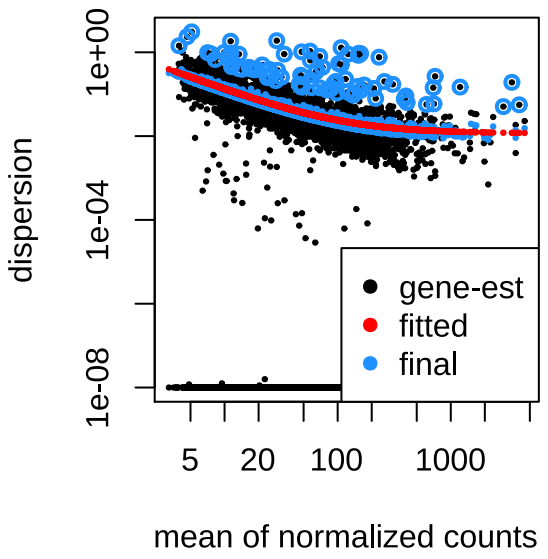


- Dispersion is a measure of variability in gene expression for a given mean

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

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

```
##
## out of 10573 with nonzero total read count
## adjusted p-value < 0.05
## LFC > 0 (up)       : 193, 1.8%
## LFC < 0 (down)     : 238, 2.3%
## outliers [1]       : 1, 0.0095%
## low counts [2]     : 4920, 47%
## (mean count < 21)
## [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
##                   <numeric>      <numeric> <numeric> <numeric> <numeric>
## ENSMUSG00000098104   18.8505       0.205656  0.401543  0.512164 0.6085362
## ENSMUSG00000033845   23.3333       0.653565  0.379627  1.721596 0.0851426
## ENSMUSG00000025903   37.1016       0.672348  0.298923  2.249232 0.0244977
## ENSMUSG00000033793   33.3673       0.144833  0.305139  0.474646 0.6350394
## ENSMUSG00000025907   22.3875       0.821006  0.376414  2.181125 0.0291742
## ENSMUSG00000051285   21.1485       0.452451  0.378725  1.194669 0.2322163
##                         padj
##                    <numeric>
## ENSMUSG00000098104        NA
## ENSMUSG00000033845  0.377432
## ENSMUSG00000025903  0.177491
## ENSMUSG00000033793  0.886264
## ENSMUSG00000025907  0.201741
## ENSMUSG00000051285        NA
```

- 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 : 30-Nov-2020 at 15:57:41

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