RNA-seq differential expression analysis

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What we will cover

We will cover differential expression analysis of RNA-seq data in R/Bioconductor.

We will start from a matrix of gene-level read counts.

We will cover the two most popular packages, `DESeq2` and `edgeR`.

I will also show you how to deal with unwanted variation using the `RUVSeq` package.

What we will not cover

I will not talk about the preprocessing of RNA-seq data, i.e., what we do to obtain the gene-level read counts.

These steps are usually done with stand-alone software outside R.

I will not talk about isoform-level analysis and alternative splicing.

We will focus on gene-level differential expression.

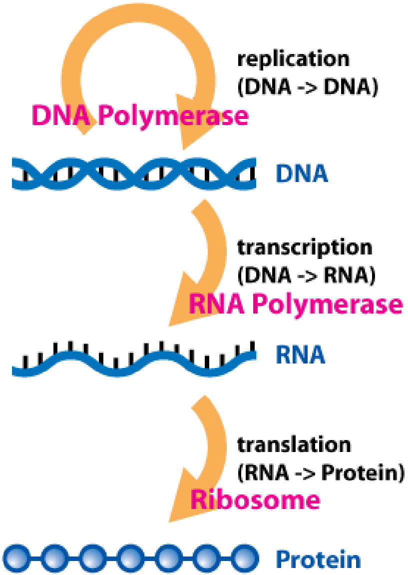
Where to find these slides

https://github.com/drisso/rnaseq_meetup

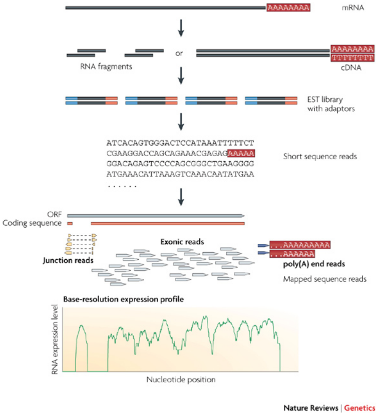
Where to find additional resources

- The edgeR user guide
  https://bioconductor.org/packages/edgeR
- The DESeq2 vignette
  https://bioconductor.org/packages/DESeq2
- The F1000 Research Bioconductor gateway
  https://f1000research.com/gateways/bioconductor
- https://support.bioconductor.org

From RNA to gene-level read counts



From RNA to gene-level read counts



From RNA to gene-level read counts

```
##                     CC3  CC5  CC6  CC7  CC8  FC3  FC5  FC6  FC7  FC8  RT3
## ENSMUSG00000000001 2034 2232 1253 2024 1510  994 1703 1796 1502 2145 1600
## ENSMUSG00000000028   81   93   77   91   85  106   81   84   70   95  121
## ENSMUSG00000000037   52   59   28   52   36   12   40   34   41   56   26
## ENSMUSG00000000049   15   32   15   18   14   49   10   18   11   24   21
## ENSMUSG00000000056 3125 3256 2175 3283 2553 1638 2276 2900 2223 3179 2504
## ENSMUSG00000000058 1412 1324  819 1243  668  446  821  815  786 1646  817
##                     RT5  RT6  RT7  RT8
## ENSMUSG00000000001 1734 1834 1982 1316
## ENSMUSG00000000028   92  102  102   60
## ENSMUSG00000000037   44   46   40   45
## ENSMUSG00000000049   22   17   11    9
## ENSMUSG00000000056 3045 3106 3441 1940
## ENSMUSG00000000058  945 1031 1170  990
```

The Poisson Model

When statisticians see counts, they immediately think about Simeon Poisson.

The Poisson Model

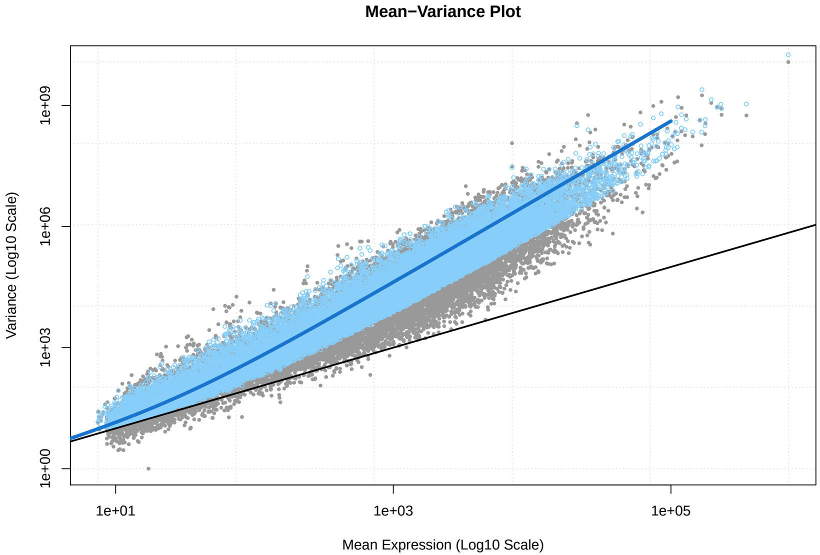
The Poisson distribution naturally arises from binomial calculations, with a large number of trials and a small probability.

It has a rather stringent assumption: **the variance is equal to the mean**!

$$Var(Y_{ij}) = \mu_{ij}$$

In real datasets the variance is greater than the mean, a condition known as **overdispersion**.

A real example



The Negative Binomial Model

A generalization of the Poisson model is the negative binomial, that assumes that the variance is a quadratic function of the mean.

$$Var(Y_{ij}) = \mu_{ij} + \phi_j \mu_{ij}^2$$

where ϕ is called the **dispersion parameter**.

Both `edgeR` and `DESeq2` assume that the data is distributed as a negative binomial.